Proteins in cardiac and skeletal muscle cells, as in other cells, are continually being synthesized and degraded back to their constituent amino acids. Protein turnover in cardiac myocytes utilizes the same proteolytic systems as other eukaryotic cells: the ubiquitin-proteasome pathway, which catalyzes the rapid degradation of misfolded and regulatory proteins, and the lysosomal-autophagic system, which degrades organelles and aggregated proteins. These systems are of major importance in determining cardiac size and functional capacity. The overall rates of proteolysis in a cell and the degradation of individual components are precisely regulated. For example, cardiac hypertrophy occurs when overall rates of protein synthesis exceed overall rates of protein degradation; conversely, cells decrease in mass when degradation rates exceed synthesis, as occurs in skeletal muscle with disuse, fasting, and many systemic diseases, including cardiac failure. In addition, the levels of individual proteins, whether they are enzymes, transcription factors, or components of the sarcomere, are determined in large part by their rates of ubiquitin-mediated degradation. In this issue of Circulation Research, Usui et al.1 demonstrate how a single ubiquitination enzyme can have major effects on cardiac growth and function.

In this pathway, proteins are targeted for degradation by the 26S proteasome by covalent attachment of a chain of ubiquitin molecules. This multistep pathway first involves the activation of the small protein ubiquitin by an enzyme, E1, which then transfers the highly reactive ubiquitin to one of the cell's many ubiquitin-conjugating enzymes, E2s. A ubiquitin protein ligase, E3, then binds the protein substrate and the ubiquitin-E2 and catalyzes the formation of a chain of ubiquitins on the protein. Different E2-E3 pairs function in the degradation of different proteins, and the specificity of the E3s for specific groups of proteins provides exquisite selectivity to this degradation process. The content of different E2s and E3s varies between tissues and with different physiological conditions (as is nicely illustrated by the findings of Usui et al.), but which E2s and E3s function normally in cardiac muscle is unknown. The human genome contains over 650 ubiquitin ligases, which makes possible precise regulation of different cell processes, and in recent years, dramatic progress has been made in elucidating the roles of different E3s in regulating metabolism, transcription, cell cycle, oncogenesis, and so forth. Although the pharmacological modulation of specific ubiquitin ligases is an attractive approach for treating many diseases, this possibility has thus far not been exploited, although an inhibitor of the proteasome (Bortezomib) is now widely used in the treatment for certain hematologic cancers.

In this regard, it is intriguing that proteasome inhibitors also attenuate or reverse cardiac hypertrophy in multiple rodent models.2

Atrogin1, Also Known as MAFbx, Is Critical for Skeletal Muscle Atrophy and Cardiac Hypertrophy

The article by Usui et al. has uncovered an important, unexpected role of the ubiquitin ligase Atrogin1/MAFbx in cardiac hypertrophy. Their finding is particularly surprising because this E3 has been shown to be crucial in skeletal muscle atrophy. In fact, because of its dramatic induction in nearly all forms of muscle atrophy, its expression is widely used as a marker of muscle wasting (especially when accompanied by induction of another atrophy-related ubiquitin ligase, MuRF1). In studies to identify the key mechanisms driving muscle atrophy, about 100 genes were identified that were named atrophy-related genes or atrogenes,3 whose expression was induced or suppressed similarly in rodent muscles undergoing various types of atrophy, including fasting, untreated diabetes, renal failure, acidosis, cancer cachexia, and denervation or disuse.3,4 They were therefore named “atrogenes,” for atrophy-related genes. Among this set of genes, Atrogin1/MAFbx was one of the most dramatically induced. It is a muscle-specific E3, present also in cardiac and smooth muscle, although in fasting and catabolic disease, it is induced. It is a muscle-specific E3, present also in cardiac and smooth muscle, although in fasting and catabolic disease, it is induced specifically in skeletal muscles, where proteolysis increases to provide the stressed organism with a supply of amino acids for gluconeogenesis and adaptive responses. A similar induction has been observed in muscles of patients with trauma, sepsis, and renal failure, in which there is marked muscle wasting. In experimental models, induction of Atrogin1 mRNA accompanies the rapid atrophy; that is, within 1 to 3 days after cutting the motor neuron, its level rises maximally and then falls after 14 days back to control level. Thus, high levels of this protein coincide with and help trigger the rapid weight loss.5

This gene contains an F-box, a hallmark of a large family of multicomponent ubiquitin ligases. The F-box protein is the substrate-binding subunit that functions in a larger complex together with 3 other subunits (Skp1, Rbx1, and Cullin1) that also function with other F-box proteins in the ubiquitination
of other proteins. This ubiquitin ligase was discovered simultaneously by Goldberg’s laboratory, who named it Atrogin1 because of its dramatic induction in diverse types of atrophy, and by Glass and Yancopoulos’s group, who named it MAFbx, because it was a muscle F-box protein. Both names, though widely used, now seem inappropriate because a number of other muscle F-box proteins are now known and because it is not specific to atrophy, as shown by Usui et al. They report that Atrogin1/MAFbx is also induced during compensatory hypertrophy of the heart, where it is important for the large increase in cardiac mass after aortic constriction and especially for the growth of cardiac myocytes induced with phenylephrine. In fact, had the present studies appeared 11 years ago, Atrogin1 might have been named Hypertrog1. Moreover, this E3 appears to contribute particularly to the development or pathological hypertrophy and cardiac failure because MAFbx-knockout animals showed a mild reduction in hypertrophy and a large decrease in various pathological sequelae such as pulmonary congestion and fibrosis and cardiomyocyte apoptosis. This surprising induction in hypertrophy is consistent with the prior finding that its level decreases during cardiac atrophy induced by unloading.

Such a dual role in hypertrophy and atrophy is indeed surprising because these processes have opposite consequences and appear to involve quite different signaling mechanisms. Muscle hypertrophy proceeds through increased activity of the PI3K-AKT-FoxO pathway, which enhances protein synthesis and suppresses proteolysis, whereas in atrophy, PI3K-AKT signaling is reduced below normal levels, which causes synthesis to fall and proteolysis to rise (Figure in Sandri et al.).

To clarify MAFbx function in skeletal muscle, Bodine et al. generated mice lacking MAFbx and found a decreased loss of weight and fiber diameter on denervation or glucocorticoid treatment. Reduced atrophy was also seen on deletion of the coinduced ubiquitin ligase MuRF1. During atrophy, these two enzymes appear to promote the breakdown of different cell proteins; for example, MuRF1 (unlike Atrogin1) specifically ubiquitinates components of the thick filament. Therefore, it will be interesting to learn if expression of MuRF1 also rises during cardiac hypertrophy.

In addition, Atrogin1 has been shown to be important in other conditions, including the muscle pain and weakness that can result from treatment with all known statins. A recent elegant study has demonstrated that Atrogin1 is essential for statin-induced myopathy, where knockdown of Atrogin1 in zebra fish prevented the muscle damage induced with statins, although its exact role in causing these symptoms is unclear.

Non-standard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>eIF3-f</td>
<td>eukaryotic initiation factor 3-f</td>
</tr>
<tr>
<td>NF</td>
<td>nuclear factor</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>peroxisome proliferator-activated receptor gamma coactivator 1-alpha</td>
</tr>
<tr>
<td>PI3K</td>
<td>phosphatidylinositol 3-kinase</td>
</tr>
</tbody>
</table>

Figure. In atrophying skeletal muscles, diverse catabolic stimuli activate FoxO3 and NF-κB transcription factors and induce expression of Atrogin1/MAFbx and MuRF1, which in turn decrease overall protein synthesis and increase protein degradation. Although similar regulation of Atrogin1/MAFbx by FoxO3 is conserved in cardiac muscles, in response to pressure overload or catecholamines, Atrogin1/MAFbx expression in heart increases and degrades IxBα, the inhibitor of NF-κB. It is unclear how the resulting activation of NF-κB triggers the changes in protein turnover that lead to physiological or pathological hypertrophy.

In addition, smooth muscle cells can undergo compensatory hypertrophy and atrophy. Interestingly, during involution of the uterus, Atrogin1/MAFbx and MuRF1 were induced in smooth muscle cells. Despite these crucial effects, only a few proteins have thus far been identified as substrates for Atrogin1/MAFbx, and they all appear to be involved in growth-related processes. When overexpressed in myoblasts, this enzyme inhibits cell differentiation and promotes degradation of the key muscle transcription factor MyoD and the key activator of protein synthesis, eIF3-f. When Atrogin1 expression was increased, eIF3-f was polyubiquitinated and degraded by proteasomes. eIF3-f stimulates the initiation of polypeptide synthesis at the ribosome and is important for the growth of mature muscles. Thus, when eIF3-f was overexpressed in cultured muscle cells, it induced growth, whereas knockdown of eIF3-f reduced myotube size. Therefore, although overexpression of MAFbx in mice alone does not cause muscle atrophy, the accelerated degradation of eIF3-f should certainly reduce growth capacity. Whether a similar effect on eIF3-f occurs on Atrogin1 induction during cardiac hypertrophy is an interesting and important issue that was not addressed by Usui et al. In the heart, Atrogin1 also ubiquinates and reduces the levels of calcineurin A, which is an important factor triggering cardiac hypertrophy in response to pressure overload. In cardiac myocytes, overproduction of Atrogin1 also inhibits phenylephrine-induced hypertrophy by decreasing calcineurin A levels. The finding that Atrogin1 degrades proteins primarily involved in growth is clearly difficult to reconcile with this protein’s newly discovered role in promoting hypertrophy, although it could be important in the induction of cardiac failure.

Regulation of Atrogin1/MAFbx Expression

In skeletal muscle, the expression of Atrogin1 and MuRF1 are tightly regulated and rise in response to diverse stimuli that promote muscle wasting, including low insulin states,
glucocorticoids, acidosis, inactivity, and myostatin. These
catabolic stimuli all activate the FoxO family of transcription
factors, which mediate transcription of many atrogens,
including Atrogin1, MuRF1, and many genes for autophagy.
Consequently, activated FoxO3 by itself induces profound
atrophy of skeletal and cardiac muscles.5,15 Conversely, this
atrophy program is suppressed by growth factors (eg, insulin-
like growth factor-1) that stimulate the PI3K-AKT pathway
and thus promote protein synthesis, and by inactivating
FoxOs reduce proteolysis.16,17 These two actions together
synergize to cause rapid hypertrophy. In addition, the
exercise-induced transcription cofactor PGC-1α, which pro-
motes production of mitochondria, also inhibits the activation
of FoxO3, and this action helps explain the capacity of
exercise to prevent muscle atrophy.18 Despite its importance
in regulating Atrogin1 and MuRF1 in catabolic conditions
(including cardiac myocytes deprived of growth factors),
FoxO3 was not found by Usui et al to be activated during
cardiac hypertrophy when Atrogin1 was induced. Thus, it is
highly likely that an as-yet unidentified transcription factor
responds to pressure overload and catecholamines to trigger
transcription of MAFbx. Therefore, the mechanisms signal-
ing Atrogin1 (MAFbx) induction in atrophy and hypertrophy
appear to be fundamentally different, although the differences
observed thus far might still be explained by differences
between heart and skeletal muscles. So, analogous studies of
compensatory hypertrophy in skeletal muscle appear impor-
tant to clarify whether they also express Atrogin1 in response
to increased load by a FoxO-independent mechanism.

Initially, hearts of mice lacking Atrogin1/MAFbx were
reported to exhibit no clear abnormality; however, their
ability to respond to physiological challenges had not been
tested until Usui et al made the important finding that
Atrogin1/MAFbx is required for maximal hypertrophy, and
especially for its pathological consequences (ie, increase in
lung congestion, apoptosis of myocardial cells, and decreased
left ventricular production of ventricular myosin). Among the
important, unexpected findings in this report was that deletion
of Atrogin1/MAFbx decreased the degradation of IkBα, the
inhibitor of nuclear factor (NF)-κB, which is the transcription
factor triggering many pathological responses. Conversely,
overexpression of Atrogin1/MAFbx promoted the degrada-
tion of IkBα and activation of NF-κB. In other cells, these
responses are the hallmarks of inflammation and production
of inflammatory mediators. In hypertrophied heart, NF-κB
had been previously found to rise, and the discovery that
Atrogin1/MAFbx ubiquitinitates IkBα and thereby is a posi-
tive regulator of cardiac NF-κB is an important discovery
whose physiological and pathological consequences remain
to be defined. In skeletal muscle, NF-κB is necessary for
atrophy and is activated together with FoxOs when Atrogin1/
MAFbx is induced.19 Perhaps Atrogin1/MAFbx helps trigger
its activation in the atrophying muscles.

Outstanding Questions
Puzzling observations and apparent paradoxes in research can
be valuable in stimulating additional experimental studies and
further progress. Beyond its novel findings, the article by
Usui et al raises several perplexing points that certainly merit
future in-depth study. Because knockout of Atrogin1/MAFbx
diminished hypertrophy, overexpression of this gene would
be expected to promote cardiac growth. However, muscle
size was decreased by overexpressing MAFbx. Thus, a major
puzzle is that both its deletion and overexpression attenuate
cardiac hypertrophy. One possible explanation could be that
Atrogin1/MAFbx plays a specific role in an initial step
promoting hypertrophy (eg, degradation of IkBα), but, subse-
sequently, when Atrogin1/MAFbx levels markedly increase,
it may then attenuate muscle growth, as these authors found
on its overexpression, and as Atrogin1/MAFbx does in
atrophying muscles. Perhaps there are distinct substrates for
this E3 in the early and later phases, where pathological
consequences and failure become evident that may also result
from the buildup of NF-κB.

Based on the apparent importance of NF-κB in hypertro-
phying hearts and in multiple other diseases, it will be
illuminating to define its precise effects on cardiac size and
function—specifically to learn whether selective downregu-
lation of NF-κB diminishes hypertrophy or overexpression of
NF-κB can increase heart size and failure. Such studies could
have therapeutic applications because inhibitors of NF-κB are
available for clinical study. In skeletal muscle, this transcrip-
tion factor synergizes with FoxOs in promoting overall
proteolysis and atrophy, which should not be of benefit in a
compromised heart. Probably, the best studied function of
NF-κB in disease is in enhancing the production of inflam-
matory mediators, including interleukin-1, tumor necrosis
factor-α, and interleukin-6, which was found to increase in
these hypertrophied hearts by a MAFbx-dependent mecha-
nism. These potent mediators appear to be likely contributors
to the development of pathological hypertrophy and cardiac
failure. However, another important action of NF-κB in most
cells, and especially in many cancers, is in reducing apopto-
sis. Such an effect in cardiac hypertrophy appears to be likely
because it would account for the finding of Usui et al of less
apoptosis and less production of the NF-κB-dependent anti-
apoptotic mediator Bcl-3 in hearts of mice lacking MAFbx.
Such effects would be expected to protect myocytes and be
beneficial. Thus, NF-κB activation can have both toxic and
helpful consequences in the overloaded heart. Finally, these
observations emphasize the importance of identifying the
major transcription factor triggering Atrogin1/MAFbx ex-
pression because it clearly plays a crucial role in cardiac
disease and because it is neither FoxO3 nor NF-κB. More-
ever, this unknown factor and its activation mechanisms
would be very attractive new therapeutic targets.

Disclosures
None.

References
1. Usui S, Maejima Y, Pain J, Hong C, Cho J, Park JY, Zablocki D, Tian B,
Glass DJ, Sadoshima J. Endogenous muscle atrophy f-box mediates
pressure overload-induced cardiac hypertrophy through regulation of
2. Stansfield WE, Tang RH, Moss NC, Baldwin AS, Willis MS, Selzman
CH. Proteasome inhibition promotes regression of left ventricular hyper-


**KEY WORDS:** Atrogin1/MAFbx • atrophy • hypertrophy • NF-κB, ubiquitin • protein degradation
Atrogin1/MAFbx: What Atrophy, Hypertrophy, and Cardiac Failure Have in Common
Donghoon Lee and Alfred Goldberg

doi: 10.1161/CIRCRESAHA.111.248872
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/109/2/123

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/