Cell, Vol. 119, 907–910, December 29, 2004, Copyright ©2004 by Cell Press

Molecular Mechanisms of Muscle Atrophy

Minireview

Iain W. McKinnell and Michael A. Rudnicki*
Ottawa Health Research Institute
501 Smyth Road
Ottawa
Ontario K1H 8L6
Canada

Skeletal muscle atrophy has extreme adverse consequences. Molecular mechanisms that mediate the process of atrophy are not well defined. Recent studies have focused on diverse molecular cascades that control the activation of ubiquitin ligases, indicating that the involvement of the ubiquitin proteasome may be common to a range of atrophic stimuli.

Skeletal muscle atrophy can be defined as a wasting or decrease in muscle mass owing to injury, lack of use, or disease. As is clear from this definition, the etiology of atrophy can be diverse. Within the general population, atrophy most commonly results from disuse, which can be described as acute atrophy and is readily reversible following exercise. Sarcopenia, an age-related loss of muscle mass and strength, is considered to be chronic atrophy and is determined by initial muscle mass and its subsequent rate of decline. Muscle atrophy resulting from chronic disease rather than disuse is described as cachexia and generally arises either from damage to the nerves that supply the muscles or from disease of the muscle itself. Both causes can be the result of either underlying genetic abnormality, such as amyotrophic lateral sclerosis (nerve damage) or muscular dystrophy, myotonia congenita (muscle damage); or systemic disease pathology, such as poliomyelitis (nerve damage) inflammatory or metabolic myopathies, diabetes, cancer, renal failure, or pulmonary obstruction (resulting in muscle damage) (Marcell, 2003; Glass, 2003).

The maintenance of skeletal muscle depends upon the balance of dynamic anabolic and catabolic reactions to determine the level of muscle protein. Essentially, all atrophic conditions share the commonality of an imbalance in this system, resulting in reduced protein synthesis and increased protein breakdown/proteolysis, which in turn results in reduced muscle mass and muscle fiber size. Given the similarity of the endpoint of diverse atrophic stimuli, one would expect that common mechanisms exist via which these stimuli are able to affect protein breakdown. Indeed, the process of atrophy is characterized by the activation of distinct pathways, in particular the ATP-dependent ubiquitin-proteasome proteolysis pathway. In genetic screens aimed at identifying markers of muscle atrophy, two genes in particular, atrogin-1/MAFbx and MuRF1, were dramatically upregulated prior to the onset of muscle loss in multiple experimental models (see Bodine et al. [2001a], Gomes et al. [2001], Stevenson et al. [2003]). Both these genes encode proteins that are referred to as E3 ubiquitin ligases, which are responsible for substrate specificity of ubiquitin conjugation. Furthermore, the observation that genetic deletion of either atrogin-1/MAFbx or MuRF1 results in a partial protection against muscle wasting demonstrates that dissection of the molecular regulation of these two proteins is of great significance with regard to their resulting effects on muscle atrophy (Bodine et al., 2001a).

The presence of distinct atrophic processes described above raises questions regarding whether muscle atrophy (the result of increased protein catabolism) as the converse process of muscle hypertrophy (resulting from increased protein anabolism) occurs via entirely independent processes or if these two mechanisms are in some way coregulated. A significant advance in our understanding of the molecular control of muscle has arisen from the recent work of two groups that focuses on the role of insulin growth factor (IGF) signaling cascades that converge to control the balance between hypertrophy and atrophy. When released, IGF binds to the IGF receptor, resulting in the activation of a number of signaling cascades. One involves stimulation of the Ras-Raf-Erk pathway, which affects muscle fiber-type composition but not size, while another pathway, of great significance to the control of muscle size, involves activation of the lipid kinase PI3K. IGF-1 is released from muscle in response to increased load/exercise and plays a role in subsequent hypertrophy of muscle through the accumulation of cellular protein. IGF-1 achieves this by stimulating translation via a hierarchy of activation that begins with PI3K; this leads to the phosphorylation of the serine/threonine kinase Akt1, which subsequently results in the regulation of GSK3β and mTOR kinases—ultimately leading to increased protein synthesis and muscle hypertrophy (Bodine et al., 2001b; Rommel et al., 2001; for review, see Glass [2003]).

Sandri et al. (2004) and Stitt et al. (2004) both focused on this pathway and the possibility that an alternative target of the IGF-1/PI3K/Akt signaling cascade, the Forkhead box O (Foxo) family of transcription factors, may be a possible mediator of atrophy. In two different models of muscle cultures, a clear atrophy of the muscle fibers was induced together with a decrease in IGF-1/PI3K/Akt pathway activity. Concurrent with this decrease was a rapid and robust induction of ubiquitin ligases, most strongly atrogin-1/MAFbx, suggesting the mechanism of atrophy was increased protein catabolism via ubiquitin-ligase mediated proteolysis. Conversely, addition of IGF-1 or constitutive activation of the PI3K/Akt pathway was able to prevent muscle loss in these models, apparently by suppressing atrogin-1/MAFbx induction. Significantly, in addition to its role as an activator, Akt is also able to functionally inhibit Foxo transcription factors by phosphorylation, which results in them being sequestered in the cytoplasm. As would be predicted, the reduction in PI3K/Akt pathway activity observed in the models of muscle loss resulted in decreased levels of phosphorylated Foxo in the cytoplasm and a marked increase of nuclear Foxo protein. When constitutively active Foxo1 proteins were introduced

*Correspondence: mrudnicki@ohri.ca

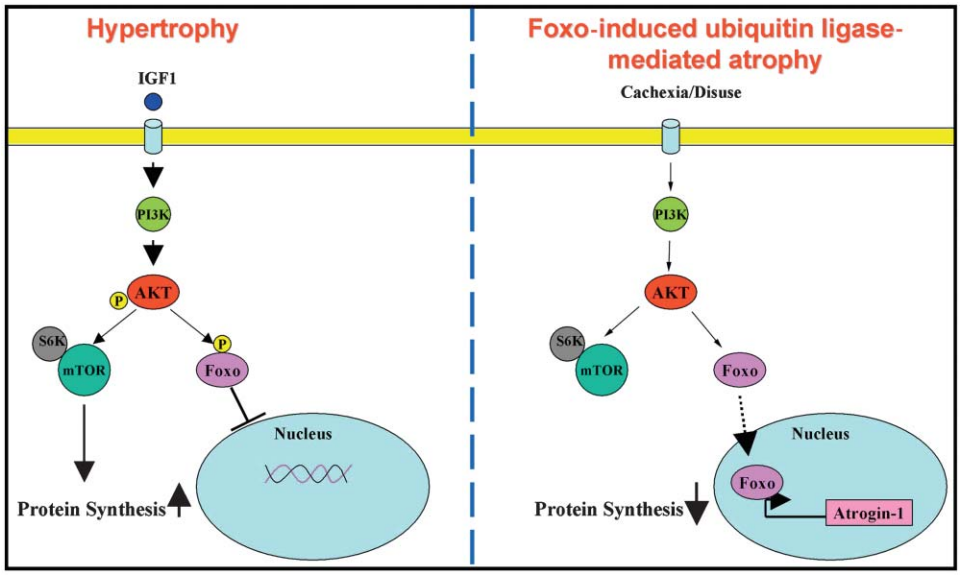

Figure 1. IGF1-Akt Signaling and the Balance between Hypertrophy and Atrophy

into myotubes, Stitt et al. (2004) did not observe any appreciable change in ubiquitin ligase mRNA levels, leading them to suggest that active Foxo is not sufficient for transcription. However, the introduction of constitutively active Foxo3 by Sandri et al. (2004) was observed to cause a dramatic increase in the mRNA of atrogin-1/MAFbx, indicating that this family of transcription factors may play a direct transcriptional role in the process of atrophy. Indeed, analysis of the 5′ region of the atrogin-1/MAFbx gene revealed the presence of a number of Forkhead binding sites, which, when taken with the preceding data, suggests that Foxo binds and activates the atrogin-1/MAFbx promoter to affect muscle atrophy.

The significance of these findings is that they demonstrate for the first time a molecular link between the mediators of hypertrophy and atrophy, showing a direct anabolic/catabolic balance mechanism involving IGF-1 signaling, whereby the activation of the PI3K/Akt pathway not only promotes muscle growth but also directly inhibits breakdown via the inhibition of the Foxo family. In turn, a reduction in PI3K/Akt pathway activity, which may result from disease or disuse, releases the Foxo proteins. The working hypothesis, then, is that Foxo is free to activate transcriptional targets, in particular atrogin-1/MAFbx, which results in muscle atrophy (Figure 1).

Previously, the transcription factor NF-κB has been implicated in the process of muscle wasting as a mediator of the cytokine TNFα in the inflammatory response, which in turn triggers both apoptosis of muscle cells and specific transcriptional mechanisms that inhibit IGF-1-induced anabolism (Li et al., 2003; Hunter et al., 2002; for review, see Spate and Schulze [2004]). In addition, the idea that NF-κB may be responsible for mediating the skeletal muscle loss observed in cachexia was first proposed by Guttridge et al. (2000) in response to their observation that TNFα-induced activation of NF-κB could suppress the mRNA of the muscle-regulatory factor MyoD at the posttranscriptional level.

While this was an understandable proposal, the ability to inhibit muscle differentiation or enact a generalized inflammatory response is a different process from the precise molecular mechanisms of muscle atrophy of which we are now becoming aware. NF-κB signaling can be activated by a number of stimuli; in an inactive state, NF-κB is sequestered in the cytoplasm by a family of inhibitor proteins called the IκB. Upon activation of NF-κB, these proteins are phosphorylated by IκB kinase (IKK) complexes, resulting in their ubiquitination and degradation. This exposes the NF-κB nuclear localization signal, resulting in translocation to the nucleus, binding of consensus sequence, and subsequent gene transcription (Baeuerle and Baltimore, 1996). Recently, Cai et al. (2004) have directly demonstrated an in vivo role for the transcription factor NF-κB in severe muscle wasting reminiscent of that observed in clinical cachexia, via a previously undescribed noncytokine mechanism: the activation of the E3 ubiquitin ligase MuRF1.

The authors demonstrated this by constructing two separate mouse models designed to manipulate NF-κB levels. The NF-κB activator IKK (MIKK) and the NF-κB inhibitor IκBα (MISR) were placed under the control of a promoter that drives their expression selectively in skeletal muscle. Although inhibition of NF-κB apparently failed to reveal a phenotype, activation of NF-κB resulted in a specific increase in protein catabolism resulting in muscle atrophy. Significantly, crossing the MISR mouse with the MIKK mouse resulted in a rescue of the MIKK mouse cachexia phenotype. Furthermore, NF-κB levels were also assessed in models of nerve damage and cancer cachexia. Accordingly, levels of NF-κB activation and subsequent muscle wasting were observed to rise in both models when using wild-type animals; however, the same experiments performed in MISR mice yielded a greatly reduced effect, suggesting that the effects observed are the result of specific manipulation of NF-κB.

Most strikingly, however, the activation of NF-κB did not result in mRNA upregulation of the array of genes

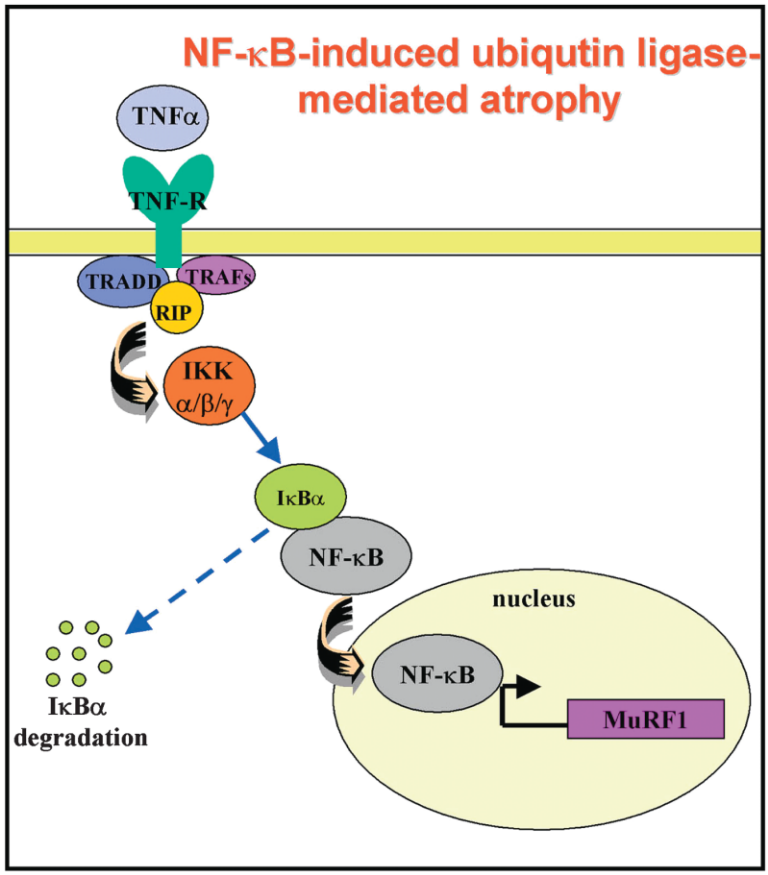

Figure 2. NF-kB Signaling and Atrophy via MuRF-1 Induction

typically associated with an NF-κB-induced cytokine response such as that seen in the immune system (Baeuerle and Baltimore, 1996). Activation of NF-κB leading to muscle wasting via a noncytokine-related pathway has been previously proposed in mouse hindlimb disuse and in vitro cell culture models. Prototypical markers of TNF-α activation of NF-κB were conspicuous by their absence, and, instead, the effects of NF-κB appeared to be mediated by components of the proteosomal degradation pathway (Hunter et al., 2002; Ladner et al., 2003). However, the study by Cai et al. (2004) is the first report to identify in vivo the link between NF-κB and noncytokine-mediated muscle wasting in cachexic conditions. Cai et al. (2004) observed an increased and specific induction of MuRF1 in response to increased NF-κB activation, thus indicating a role for NF-κB in cachexia via the activation of proteolysis (Figure 2).

When the activated NF-κB mouse was crossed with a MuRF1$^{-/-}$ knockout mouse, this resulted in a 50% rescue of the atrophic phenotype. The partial rescue of the phenotype in the compound animals would suggest that NF-κB is concurrently activating alternative, as yet undefined pathways. It will be of great importance to try and further identify these alternative pathways, as they may represent other levels of atrophy control that would be under consideration as therapeutic targets. For example, a recent report by Hunter and Kandarian (2004) has shown that genetic deletion of two further members of the NF-κB family of transcriptional regulators (Nfkb1 and Bcl3) is able to inhibit skeletal muscle atrophy caused by disuse. Given the wide range of inputs and downstream effects mediated via NF-κB signaling, these alternate pathways may well represent further functions of NF-κB that directly affect muscle maintenance.

The observation that multiple models of muscle atrophy resulted in the consistent induction of E3 ubiquitin ligases indicated the great significance of understanding the molecular mediators that converge upon their activation. Previously, very little could be stated definitively regarding the molecular cascades that transmit extracellular signals into atrophic responses. The recent discovery that defined NF-κB and IGF-1/PI3K/Akt pathway activation directly stimulated or attenuated muscle atrophy via proteolysis, apparently with a degree of ubiquitin-ligase specificity, represents a significant step toward the goal of countering the debilitating effects of muscle atrophy. However, the identification of these mechanisms is only the initial step, and translating this information into new modalities for therapeutic intervention will require considerable effort. For example, stimulation of myotubes with IGF-1 is able to induce myotube hypertrophy (Rommel et al., 2001) and protect against atrophy via activation of the PI3K-Akt pathway (see Bodine et al. [2001b] and models discussed above); furthermore, IGF-1 can attenuate the muscle atrophy observed in the mdx mouse model of muscular dystrophy when expressed in a muscle-specific manner (Barton et al., 2002); taken together, these data lend themselves to the suggestion that delivery of IGF-1 may be a viable

counter to muscle atrophy. However, systemic delivery of IGF-1 would present considerable complications with regard to its effects upon tissues other than muscle, particularly given its ability to stimulate proliferation in particular cell types. While the concept of muscle-targeted delivery is an attractive one, the absence of a safe and effective delivery method in humans remains a significant stumbling block. The next logical targets would be to use pharmacological agents directed against components of the relevant signaling pathways. For example, given that acute constitutive activation of Akt can induce rapid hypertrophy (Lai et al., 2004), pharmacological mimetics that activate Akt may represent an attractive and viable strategy. Indeed, this approach has been used with some success in the mouse models created by Cai et al. (2004), who used salicylate to inhibit the NF-κB-MuRF1-induced atrophy. However, as the authors note, the high doses of salicylate required in the mouse studies are not well tolerated in humans due to the side effects and its broad range of targets. Regardless, the identification of precise signaling cascades that direct muscle atrophy has significantly expanded the number of possible targets and strategies to be explored in clinical interventions aimed at alleviating muscle atrophy.

Selected Reading

Baeuerle, P.A., and Baltimore, D. (1996). Cell *87*, 13–20.

Barton, E.R., Morris, L., Musaro, A., Rosenthal, N., and Sweeney, H.L. (2002). J. Cell Biol. *157*, 137–148.

Bodine, S.C., Latres, E., Baumhueter, S., Lai, V.K., Nunez, L., Clarke, B.A., Poueymirou, W.T., Panaro, F.J., Na, E., Dharmarajan, K., et al. (2001a). Science *294*, 1704–1708.

Bodine, S.C., Stitt, T.N., Gonzalez, M., Kline, W.O., Stover, G.L., Bauerlein, R., Zlotchenko, E., Scrimgeour, A., Lawrence, J.C., Glass, D.J., and Yancopoulos, G.D. (2001b). Nat. Cell Biol. *3*, 1014–1019.

Cai, D., Frantz, J.D., Tawa, N.E., Jr., Melendez, P.A., Oh, B.C., Lidov, H.G., Hasselgren, P.O., Frontera, W.R., Lee, J., Glass, D.J., and Shoelson, S.E. (2004). Cell *119*, 285–298.

Glass, D.J. (2003). Nat. Cell Biol. *5*, 87–90.

Gomes, M.D., Lecker, S.H., Jagoe, R.T., Navon, A., and Goldberg, A.L. (2001). Proc. Natl. Acad. Sci. USA *98*, 14440–14445.

Guttridge, D.C., Mayo, M.W., Madrid, L.V., Wang, C.Y., and Baldwin, A.S., Jr. (2000). Science *289*, 2363–2366.

Hunter, R.B., and Kandarian, S.C. (2004). J. Clin. Invest. *114*, 1504–1511.

Hunter, R.B., Stevenson, E., Koncarevic, A., Mitchell-Felton, H., Essig, D.A., and Kandarian, S.C. (2002). FASEB J. *16*, 529–538.

Ladner, K.J., Caligiuri, M.A., and Guttridge, D.C. (2003). J. Biol. Chem. *278*, 2294–2303.

Lai, K.M., Gonzalez, M., Poueymirou, W.T., Kline, W.O., Na, E., Zlotchenko, E., Stitt, T.N., Economides, A.N., Yancopoulos, G.D., and Glass, D.J. (2004). Mol. Cell. Biol. *24*, 9295–9304.

Li, Y.P., Lecker, S.H., Chen, Y., Waddell, I.D., Goldberg, A.L., and Reid, M.B. (2003). FASEB J. *17*, 1048–1057.

Marcell, T.J. (2003). J. Gerontol. A Biol. Sci. Med. Sci. *58*, M911–M916.

Rommel, C., Bodine, S.C., Clarke, B.A., Rossman, R., Nunez, L., Stitt, T.N., Yancopoulos, G.D., and Glass, D.J. (2001). Nat. Cell. Biol. *3*, 1009–[INSERT shppict]1013.

Sandri, M., Sandri, C., Gilbert, A., Skurk, C., Calabria, E., Picard, A., Walsh, K., Schiaffino, S., Lecker, S.H., and Goldberg, A.L. (2004). Cell *117*, 399–412.

Spate, U., and Schulze, P.C. (2004). Curr. Opin. Clin. Nutr. Metab. Care *7*, 265–269.

Stevenson, E.J., Giresi, P.G., Koncarevic, A., and Kandarian, S.C. (2003). J. Physiol. *551*, 33–48.

Stitt, T.N., Drujan, D., Clarke, B.A., Panaro, F., Timofeyva, Y., Kline, W.O., Gonzalez, M., Yancopoulos, G.D., and Glass, D.J. (2004). Mol. Cell *14*, 395–403.