

Regulation of Muscle Protein Synthesis and Degradation in Normal and Pathophysiological States

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Skeletal muscle is the most abundant tissue in the human body. Its mass is controlled through a delicate balance of signaling pathways that stimulate anabolism or hypertrophy of muscle cells through the protein translation machinery or control catabolism or atrophy by inducing protein breakdown. The main regulator of hypertrophy is the Akt/mammalian target of rapamycin (mTOR) pathway, which both promotes protein synthesis through the activity of p70S6K, and inhibits the activities of Forkhead-O (FoxO) transcription factors that induce the expression of E3 ubiquitin ligases, muscle-specific RING finger-1 (MuRF-1), and atrogin1. In contrast, muscle atrophy is controlled by FoxO and other transcription factors such as NF- κ B that stimulate the expression of the E3 ubiquitin ligase genes, as well as other pathways that activate calpain and lysosomal enzymes. Under physiological conditions, hypertrophy and atrophy responses contribute equally to maintain a proper balance of muscle cell size and tissue homeostasis. However, in the event of an atrophy condition, such as that obtained from prolonged inactivity or chronic disease, this delicate balance is compromised, leading to reduced muscle cell size and eventual weakness and fatigue.

Much of what we know regarding the mechanisms regulating protein synthesis and the hypertrophy response in skeletal muscle cells comes from the work of Glass and his colleagues.¹ These researchers elucidated that in response to insulin and insulin-like growth factor (IGF), PI 3-kinase becomes activated, which in turn leads to further

activation of Akt to stimulate the protein synthetic machinery. This notion is supported by studies showing that cells from organisms lacking Akt are reduced in size, most likely because of a general decline in protein output. Formal demonstration that Akt is relevant in promoting muscle cell hypertrophy comes from in vitro and in vivo studies from the Glass laboratory.² In these studies, investigators showed that transduction of a constitutively active form of Akt in C2C12 myotubes could increase cell size. This finding was confirmed by showing that expression of a dominant negative form of glycogen synthase kinase 3 beta (GSK3 β), whose wild-type form is negatively regulated by Akt through phosphorylation, also resulted in larger cultured myotubes. Significantly, expression of the active form of Akt in intact muscles can also rescue against denervated-induced atrophy, establishing the relevance of Akt in vivo.

These initial findings led to the realization that several factors known to promote muscle atrophy, such as angiotensin II, inflammatory cytokines, and tumor factors, mediated their activities in part through the inhibition of the Akt pathway. Important contributions from the Tisdale laboratory³ showed that these factors signal in common through the double-stranded RNA protein kinase R (PKR), which functions to inhibit the protein synthesis machinery downstream of Akt by phosphorylating and inactivating the translation initiating factor, eIF4B. Thus, their work implies that chronic activation of these factors can tip the balance and induce atrophy by abrogating Akt and the hypertrophic response.

In addition, these same factors promote atrophy through their stimulation of regulatory processes that control muscle protein turnover.⁴ The major pathway thought to regulate the catabolism of muscle protein is the ubiquitin proteasome system, defined by the marking of proteins with ubiquitin moieties that signal the 26S proteasome to degrade proteins into small peptides and individual amino acids.

An important question about Akt and the proteasome is whether these muscle protein regulatory factors are mutually exclusive. Independent reports from the groups of Glass and Goldberg^{5,6} reveal that these pathways intersect at a critical signaling junction through the activities of FoxO transcription factors. Previous studies in non-muscle cells demonstrate that FoxO activity is tightly regulated by Akt phosphorylation. Upon Akt activation in response to insulin and IGF, protein synthesis is stimulated, but proteasome activity is also suppressed by direct Akt phosphorylation of FoxO protein. In turn, FoxO1 and FoxO3 are inactivated by their inability to translocate to the nucleus. However, in cachectic conditions in which IGF levels are reduced, Akt activity also declines, leading to FoxO1 and FoxO3 nuclear translocation and binding to MuRF-1 and atrogin1/MAFBx promoters, where they function to stimulate proteasome activation.

More recent studies from the laboratories of Goldberg and Sandri also show that FoxO proteins transcriptionally regulate autophagy genes to activate the lysosomal system and promote muscle atrophy.^{7,8} Therefore, aside from Akt-stimulating protein synthesis and the hypertrophy response, this kinase also functions to suppress muscle catabolism by blocking proteasome and lysosome activity through the phosphorylation of FoxO.

Another important point that had not been thoroughly investigated until recently are the proteins that are targeted by the proteasome system, leading to a catabolic state.

Previously, the assumption was that such targets represented myofibrillar proteins, likely because of their abundance in mature skeletal muscle cells. However, little evidence supported this claim. Using cultured C2C12 myotubes treated with cytokines tumor necrosis factor alpha (TNF α) and interferon gamma (IFN γ), our laboratory demonstrated that the combination of these factors downregulated expression of myosin heavy chain IIa and IIb, but not the other highly abundant contractile proteins, α -skeletal actin, troponin T, or tropomyosin α/β , myosin light chain, and actinin.^{9,10} Furthermore, selectivity of myosin heavy chain was observed in skeletal muscles from mice bearing colon-26 tumors known to secrete high levels of IL-6. Similar results were found from mice containing CHO tumors expressing a combination of TNF α and IFN γ , or G361 melanoma tumors expressing proteolysis-inducing factor (PIF).⁹

Since this report was published, the Glass laboratory confirmed that myosin heavy chain (myHC) is a bona fide target of MuRF-1.¹¹ That study nicely showed that in response to dexamethasone treatment, myosin is a predominant muscle protein that is downregulated and can be immunoprecipitated with a MuRF-1 antibody. Importantly, the researchers also provided genetic evidence that silencing MuRF-1 with siRNA or the complete deletion from MuRF-1-deficient mice restored myosin levels in response to dexamethasone treatment in vitro and in vivo, respectively.

This finding was followed up more recently by the Goldberg group, which used a knock in mouse containing a mutant form of MuRF-1 where the RING domain had been deleted.¹² In comparing this to a knock in of a wild-type version of MuRF-1, the group was able to demonstrate conclusively that MuRF-1 activity is required for muscle atrophy in response to denervation. They also elucidated by using a combination of their mouse model and proteomic analysis that in addition to MyHC, other heavy filament proteins such as myosin light chain (MyLC) and myosin binding protein C (MyBP-C) are downregulated during denervation, and in fact loss of MyLC and MyBP-C precedes the degradation of MyHC.

It had been argued that actinomyosin complexes are insensitive to the ubiquitin proteasome system but can be cleaved by calcium-activated calpain activity, which in turn allows accessibility of the individual filaments to the ubiquitin ligases to mediate their proteasomal degradation. In a revised model, Goldberg and colleagues propose that MuRF-1 is itself capable of ubiquitinating thick filament proteins, MyLC and MyBP-C, thus causing their degradation and leading to the release of the actinmyosin complex and subsequent destruction of MyHC.¹² Since little in vivo evidence indicates that troponin and tropomyosin are susceptible to a similar mechanism of protein turnover, the question remains whether loss of thick filament proteins is sufficient to promote muscle loss in response to an atrophic condition.

The involvement of the NF- κ B signaling pathway in skeletal muscle wasting conditions also should be addressed. NF- κ B has been increasingly associated with a variety of

skeletal muscle disorders, including cachexia, muscular dystrophy, and pediatric muscle cancer rhabdomyosarcoma. Past studies have alluded to the involvement of NF- κ B by demonstrating its activation in response to several factors that are themselves tightly linked to muscle wasting. Inflammatory cytokines and tumor factors activate NF- κ B through the I κ B kinase (IKK) complex that functions to phosphorylate and induce I κ B degradation to release NF- κ B to the nucleus where it binds its cognate DNA sequences to regulate gene expression. One gene related to the ubiquitin proteasome system that it is thought to be regulated by NF- κ B is MuRF-1. Work from the Shoelson group demonstrated that muscle-specific overexpression of a constitutively active form of IKK α leads to severe muscle atrophy.¹³ Muscle atrophy was specific to NF- κ B because muscles expressing a dominant negative inhibitor of NF- κ B were resistant to denervation-induced atrophy. Furthermore, Shoelson and colleagues showed that MuRF-1 was a downstream target of NF- κ B since muscle size was restored in IKK β transgenic mice crossed with MuRF-1 knock out mice.

This work revealed that the classical pathway of NF- κ B is a contributing factor in muscle atrophy. In addition to classical signaling, different dimer complexes of NF- κ B can be regulated by what is referred to as the noncanonical or alternative pathway. Here, IKK α dimers are activated by different upstream factors (usually related to lymphoid cells), which in turn phosphorylate p100 to cause its proteasomal processing to the mature p52 protein. The resulting p52/RelB complex translocates to the nucleus to regulate a presumably different series of genes than what is controlled through the classical pathway. Deciphering how these respective pathways function in skeletal myogenesis may provide

insight for how NF- κ B might be involved in various skeletal muscle disorders such as cachexia and muscular dystrophy.^{14,15}

Our laboratory recently confirmed by genetic means that the classical pathway functions as a negative regulator of myogenesis.^{16,17} Thus, in muscular dystrophy and rhabdomyosarcoma, classical signaling is thought to repress differentiation, thereby limiting the regenerative capacity of satellite cells, or facilitating tumor progression, respectively. In contrast, we find that the alternative pathway is not required for myotube formation, but instead helps maintain homeostasis of mature myotubes. We made this discovery by observing that myotubes expressing the alternative signaling component, IKK α were more resistant to nutrient deprivation than vector control cells. Because myotubes regulate their energy capacity via oxidative phosphorylation, we speculated that the alternative pathway might be a regulator of mitochondria. Indeed, in recently published findings,¹⁷ we describe that mitochondrial content was increased in C2C12 myotubes expressing IKK α , and remarkably, microarray analysis determined that nearly 50% of the genes regulated by IKK α are related to mitochondrial and metabolic processes.

In more recent unpublished work, we addressed whether alternative pathway regulation of mitochondria is relevant in vivo. First, we found that myogenesis was not affected in differentiating IKK α knockout primary fetal myoblasts, corroborating our results in C2C12 cells. Second, in either primary cells or limb muscles lacking IKK α , we confirmed that mitochondrial markers were markedly reduced. Consistent with these results, we observed that overexpression of IKK α in intact muscles led to an increase in

mitochondria content. Taken together, these data support the notion that alternative NF- κ B signaling functions in muscle cells in a distinctly different manner from the classical pathway. We believe such information will be vital for increasing our understanding of the mechanisms underlying the regulation of muscle size and balance between the protein synthetic and degradation pathways.

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