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REVIEW ARTICLE

Signaling pathways controlling skeletal muscle mass

Marc A. Egerman and David J. Glass

Novartis Institutes for Biomedical Research, Cambridge, MA, USA

Abstract

The molecular mechanisms underlying skeletal muscle maintenance involve interplay between multiple signaling pathways. Under normal physiological conditions, a network of interconnected signals serves to control and coordinate hypertrophic and atrophic messages, culminating in a delicate balance between muscle protein synthesis and proteolysis. Loss of skeletal muscle mass, termed "atrophy", is a diagnostic feature of cachexia seen in settings of cancer, heart disease, chronic obstructive pulmonary disease, kidney disease, and burns. Cachexia increases the likelihood of death from these already serious diseases. Recent studies have further defined the pathways leading to gain and loss of skeletal muscle as well as the signaling events that induce differentiation and post-injury regeneration, which are also essential for the maintenance of skeletal muscle mass. In this review, we summarize and discuss the relevant recent literature demonstrating these previously undiscovered mediators governing anabolism and catabolism of skeletal muscle.

Keywords

Cachexia, IGF-1, muscle atrophy, muscle hypertrophy, MuRF1, myostatin, sarcopenia, skeletal muscle

History

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The IGF1/PI3K/Akt hypertrophy signaling pathways

Skeletal muscle hypertrophy, characterized in the adult mammal by an increase in the size of pre-existing myofibers (rather than hyperplasia, or an increase in the number of myofibers), involves a shift towards protein synthesis and away from protein degradation. Hypertrophy can be induced by multiple anabolic stimuli – among the most studied of which is insulin-like growth factor 1 (IGF1) (Bodine *et al*., 2001b; Rommel *et al*., 2001). Signaling via IGF1 is mediated first by IGF1 ligand binding to its receptor, the tyrosine kinase IGF1 receptor (IGF1R). This binding induces transphosphorylation of the dimeric receptor, and the resultant phosphorylated tyrosines create a docking site for the recruitment of the Insulin Receptor Substrate 1 (IRS1) (Bohni *et al*., 1999). IRS1 phosphorylation is required for most of the downstream signalings, and its phosphorylation is thus a key, highly regulated step in the modulation of IGF1 signaling. The IGF1 pathway can be inactivated by targeting IRS1 for ubiquitin-mediated degradation – ubiquitination of IRS1 has been reported after prolonged insulin stimulation, in which IRS1 was degraded in a phosphatidylinositol-3 kinase (PI3K)-sensitive fashion, in various cell lines (Haruta *et al*., 2000; Lee *et al*., 2000; Tzatsos and Kandror, 2006; Xu *et al*., 2008; Zhande *et al*., 2002). However, these multiple reports differed as to the pathways downstream of PI3K that mediate IRS1 turnover (Haruta *et al*., 2000; Tzatsos & Kandror, 2006; Xu *et al*., 2008; Zhande *et al*., 2002). Xu *et al*. (2008) and Tzatsos & Kandror (2006) suggest that the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway activates IRS1 degradation, while others reported that the activation of PI3K independent of mTOR signaling is required for IRS1 degradation (Zhande *et al*., 2002).

A variety of E3 ubiquitin ligases have been demonstrated to serve as IRS1 ligases, although under distinct conditions. The first pair was SOCS1 and SOCS3, which were shown to promote degradation of IRS1 and IRS2, and which may mediate inflammation-induced insulin resistance (Rui *et al*., 2002). The cullin 7 complex, containing the E3 ligase Fbxw8, was shown to be activated by an mTOR-dependent negative feedback loop after which it could degrade IRS1 (Xu *et al*., 2008). Cbl-b was then reported to degrade IRS1 in settings of muscle atrophy (Nakao *et al*., 2009).

As for IGF1 induced phosphorylation of IRS1, the E3 ligase that modulates IRS1 levels after IGF1R phosphorylation of IRS1 is the Fbxo40–SCF complex (Shi *et al*., 2011) (Figure 1). IRS1 is rapidly degraded after IGF1 stimulation, and this limits downstream activation of the IRS1/PI3K/Akt pathway. A screen for the causative mechanism demonstrated that knockdown of the Fbox-containing protein Fbxo40 resulted in a sparing of IRS1 even after IGF1 stimulation of the pathway (Shi *et al*., 2011). Fbxo40 null mice experience a more rapid increase in mass during their growth phase where IGF1 levels are high, and these animals have enhanced muscle size. Fbxo40, thus, is a physiologic regulator of IGF1 signaling; it is noteworthy that the Fbxo40–SCF complex can induce rapid turnoff of the IGF1 pathway only if IRS1 cannot be replenished by new protein synthesis. Thus, the mechanism seems to be a checkpoint to stop IGF1 signaling under those conditions where the muscle is incapable of responding to an

Address for correspondence: David J. Glass, Novartis Institutes for Biomedical Research, 100 Technology Square, RM 4210, Cambridge, MA 02139, USA. E-mail: david.glass@novartis.com

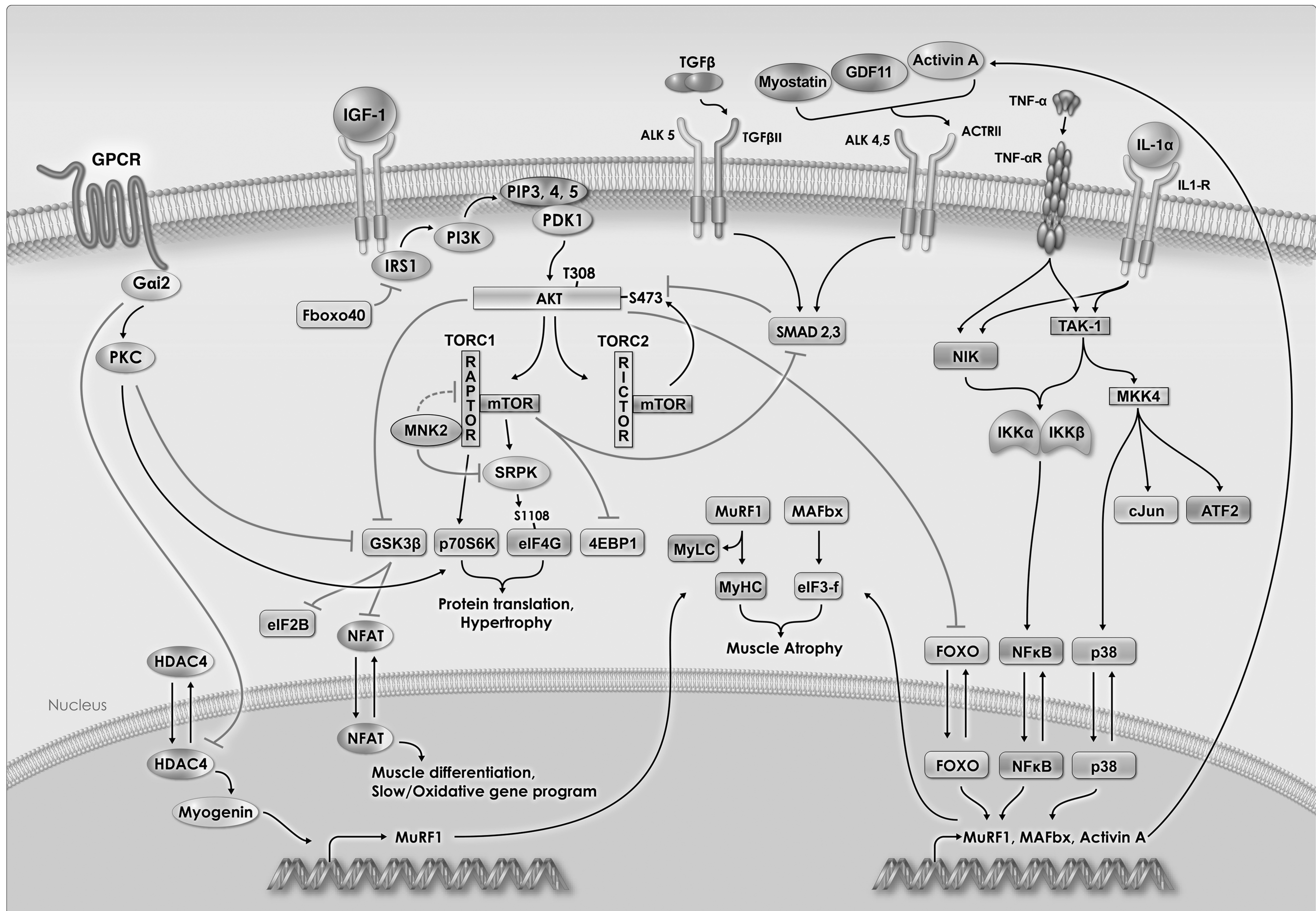

Figure 1. The signaling pathways involved in the control of skeletal muscle atrophy and hypertrophy. Signaling activated by insulin-like growth factor-1 (IGF1) positively regulates muscle mass, primarily via induction of protein synthesis, downstream of Akt and mTOR. The myostatin/GDF11/activin pathway negatively regulates muscle size, as a result of the phosphorylation of SMAD2/3 – primarily by inhibiting Akt. IGF1 acts via the IGF receptor (IGFR), and the insulin receptor substrate 1 (IRS1), – activating Akt. Akt activates mTOR complex 1 (mTORC1). mTORC1 is a multiprotein complex that requires the protein raptor for its function and is acutely inhibited by FKBP/rapamycin. mTORC1 controls protein synthesis by phosphorylating S6 kinase 1 (S6K) and eIF4E-binding protein (4E-BP). The multiprotein complex mTORC2 includes the protein rictor and contributes to the activation of Akt. Downstream targets of Akt include glycogen synthase kinase 3β (GSK3β) and Forkhead box O (FOXO) transcription factors. Inhibition of GSK3β by Akt relieves inhibition onto the initiation factor eIF2B, and thereby increases protein synthesis. Activation of Akt also inhibits FOXO and decreases expression of the E3 ubiquitin ligases Muscle Atrophy Fbox (MAFbx) and Muscle Ring Finger1 (MuRF1). Substrates of MAFbx and MuRF1 are the initiation factor eIF3-f and myosin chains, respectively. Another more recently discovered E3 ligase is Fbxo40, which can ubiquitinate IRS1 upon IGF1 stimulation, short-circuiting this pathway unless the muscle is capable of synthesizing new IGF1, via maintenance of TORC1/protein synthesis signaling. To induce hypertrophy, in addition to the classical IGF-1/Akt pathway, more recently the Galpha-i2 pathway has been shown to induce hypertrophy via PKC, bypassing Akt. In addition to the PKC pathway downstream of Galpha-i2, there is a PKC-independent pathway which involves inhibition of HDAC4.The myostatin/TGFβ pathway acts via several receptors and results in the activation of Smad 2,3. Activation of Smad proteins inhibits the function of Akt and the expression of MAFbx and MuRF1 by FOXO transcription factors. The function of Smad 2,3 is also inhibited by mTORC1. (see colour version of this figure at www.informahealthcare.com/bmg).

anabolic signal due to a lack of protein. In those settings, where the protein synthesis is turned off, IRS1 cannot be regenerated after Fbxo40-mediated degradation, and the IGF1 pathway is thus short-circuited (Figure 1). A distinct protein, MG53, has recently been shown to mediate the degradation of IRS1 when it is bound to the Insulin Receptor (IR), but not when bound to the IGF1 Receptor (IGF1R) (Song *et al*., 2013). As such, the primary substrate of MG53 appears to be the IR itself (Song *et al*., 2013). By mediating the turnover of the IR and the associated IRS1, MG53 can, therefore, inhibit myogenesis (Yi *et al*., 2013).

In skeletal muscle, downstream of IGF1 induced IRS1 phosphorylation, there is a subsequent stimulation of the PI3K/Akt pathway, resulting in the parallel phosphorylation and activation of TORC1 signaling, in one distinct pathway, and inhibition of glycogen synthase kinase 3β (GSK3β) in the other pathway (Rommel *et al*., 2001) (Figure 1). Genetic activation of IGF1 (Coleman *et al*., 1995; Musaro *et al*., 2001) or Akt (Lai *et al*., 2004) has been shown to be sufficient in inducing muscle hypertrophy, as demonstrated by tissue-specific transgenic mouse models. Lai *et al*. produced an inducible constitutively active Akt model, which allowed for the demonstration that even in an adult animal, relatively short-term activation of Akt resulted in doubling of skeletal muscle mass (Lai *et al*., 2004). In a distinct transgenic mouse model, Akt was inducibly activated only in fast glycolytic muscle, and this more restricted activation was nevertheless shown to be sufficient to reduce fat mass (Izumiya *et al*., 2008). This effect was either due to a simple tradeoff in calories, given the enhanced requirements of the hypertrophic

muscle, or due to the actual secretion of ''myokines'' from the skeletal muscle, resulting in the loss of white adipose tissue.

TORC1 signaling and its regulation by MNK2 in skeletal muscle

As mentioned earlier, one of the branches of the Akt pathway that mediates skeletal muscle hypertrophy is the activation of mTOR signaling (Figure 1). Once activated, mTOR exists as two distinct complexes, TORC1 and TORC2. TORC1 is characterized by the presence of regulatory-associated protein of mTOR (RAPTOR) (Kim do *et al*., 2002), whereas TORC2 binds rapamycin-insensitive companion of mTOR (RICTOR) instead (Guertin *et al*., 2006; Sarbassov *et al*., 2005). TORC2, mostly insensitive to the pharmacologic agent rapamycin with effects observed only after long-term treatment, phosphorylates Akt on serine 473 as a part of a required feedback loop (Lamming *et al*., 2012; Schalm *et al*., 2003). TORC1, sensitive to inhibition by rapamycin treatment, propagates downstream signaling through the phosphorylation and activation of p70S6K, and inhibition of 4E-BP1 (also called PHAS-1), with downstream targets including the ribosomal protein S6 (RPS6) and the eukaryotic translation initiator eIF4E (Sarbassov & Sabatini, 2005; Sarbassov *et al*., 2005) (Figure 1). The effect of rapamycin treatment on TORC1 targets does, however, vary significantly with some substrates, such as 4E-BP1, being largely resistant. Of note, in contrast, ATP-competitive mTORC1 inhibitors block the phosphorylation of all mTORC1 phosphorylation sites, regardless of their rapamycin sensitivity (Kang *et al*., 2013).

Phosphorylation and inhibition of 4E-BP1 is tightly controlled by TORC1 (Gingras *et al*., 1998), and results in the release of 4E-BP1-dependent inhibition of the translation initiator eIF4E (Brunn *et al*., 1997; Hara *et al*., 1997; Lawrence *et al*., 1997). Following dissociation from 4E-BP1, eIF4E binds with eIF4G and eIF4A forming the eIF4F complex, a key first step required for translation. Both the formation and the activity of the eIF4F complex are dependent on free eIF4E and the phosphorylation state of eIF4G. The amount of free eIF4E is correlated to the relative degree of 4E-BP1 phosphorylation; when it is complexed with the non-phosphorylated form of 4E-BP1, then eIF4E cannot bind eIF4G (Berset *et al*., 1998; Gingras *et al*., 2001). eIF4G, acting as a scaffold, links eIF4E with other members of the eIF4F complex, including mitogen-activated protein kinase-interacting kinases (or MNKs), which are responsible for directly phosphorylating and activating eIF4E at serine 209; however, it is not clear whether this phosphorylation event is necessary for the assembly of the translational initiation complex, or indeed what the effect of MNK phosphorylation is on eIF4E activity (Prevot *et al*., 2003a,b; Pyronnet *et al*., 1999; Scheper *et al*., 2001). Previous research indicated that both MNK1 and MNK2 bind eIF4G near serine 1108, a key residue whose phosphorylation is increased in an mTOR-dependent manner following IGF1 expression (Scheper *et al*., 2001).

A novel function for MNK2 on this phosphorylation site was only recently shown (Hu *et al*., 2012). Hu *et al*. reported an inverse relationship between the activity of MNK2 and eIF4G Ser^{1108} phosphorylation both *in vitro* and *in vivo* (Hu *et al*., 2012). In the presence of IGF1, overexpression of MNK2, but not MNK1, blocked eIF4G Ser^{1108} phosphorylation independent of Akt activation while siRNA knockdown of MNK2 overcame rapamycin-mediated inhibition of the phosphorylation event, suggesting that MNK2 negatively influences IGF1-Akt signaling downstream of mTOR (Hu *et al*., 2012). Similarly, in MNK2 knockout mice, but not in those lacking MNK1, phosphorylation of eIF4G Ser^{1108} was elevated. Since MNK2 is a kinase, and since its activation resulted in a decrease as opposed to an increase in eIF4G phosphorylation, the implication was that there was a kinase substrate that was inhibited by MNK2, which in turn was responsible for phosphorylating eIF4G. The serine–arginine-rich protein kinases (SRPKs) were identified through an siRNA screen as a possible link between MNK2 and eIF4G phosphorylation (Hu *et al*., 2012). MNK2 expression was shown to specifically block SPRK-mediated phosphorylation of eIF4G on Ser^{1108} via an allosteric effect.

MNK2 was further shown to bind RAPTOR, giving a mechanism by which it inhibits TORC1 mediated phosphorylation p70S6K (Hu *et al*., 2012). As such, MNK2 appears to exert distinct, antagonistic effects, not shared by its closest paralog, MNK1, on both eIF4G Ser^{1108} phosphorylation and p70S6K activation, thereby acting as a downstream inhibitor of IGF1/Akt/mTOR hypertrophy signaling. It is worth noting that while both MNK1 and MNK2 are expressed in skeletal muscle, only MNK2 was induced in two animal models of atrophy, denervation-induced atrophy and dexamethasone-induced atrophy (Figure 1).

In addition and independent of its activation of mTOR, Akt phosphorylates and inactivates GSK3β (Alessi *et al*., 1996; Cross *et al*., 1995; Morisco *et al*., 2000; Rommel *et al*., 2001), which in turn promotes the activity of the translation initiator eIF2B (Hardt *et al*., 2004). Together, the pathways converge to enhance translation initiation and elongation and, thus, protein synthesis. GSK3β has other inhibitory effects on skeletal muscle, which are thus also reversed by phosphorylation of Akt. These include GSK3β mediated phosphorylation of the transcription factor NFAT (Crabtree & Olson, 2002; Rommel *et al*., 2001). When GSK3β is inhibited by Akt signaling, the dephosphorylated NFATc1 and c3 proteins can translocate to the nucleus, where their activity enhances myoblast differentiation and fiber-type switching to the slow/oxidative phenotype (Friday *et al*., 2000; Horsley & Pavlath, 2002; Horsley *et al*., 2001). Akt-mediated inhibition of GSK3β does not, however, necessitate fiber-type switching, given that adult activation of Akt is not accompanied by a change in fiber-type composition (Blaauw *et al*., 2009).

MuRF1 and MAFbx mediated induction of muscle atrophy

In contrast to hypertrophy, skeletal muscle atrophy is characterized by a shift toward protein degradation, resulting from cachectic stimuli such as inflammatory cytokines and glucocorticoids. Proteolysis, as observed in atrophy, has been shown to occur in part due to the activation of ubiquitin-mediated proteasomal degradation (Mitch & Goldberg, 1996). Multiple models of muscle atrophy, including denervation, high-dose dexamethasone treatment, treatment with

inflammatory cytokines, and simple immobilization, all induce transcriptional upregulation of *MuRF1* and *MAFbx* (also called *atrogin-1*), genes that encode for E3 ubiquitin ligases (Bodine *et al*., 2001a; Gomes *et al*., 2001). Supporting their prominent roles, under atrophic conditions mice null for either gene (MuRF1 −/− or MAFbx −/−) exhibit a resistance to muscle mass loss as compared to wild-type control littermates (Bodine *et al*., 2001a).

Muscle RING finger-containing protein 1 (MuRF1) encodes a protein that contains four domains. The most NH3-terminal domain is a RING-finger (Borden & Freemont, 1996; Saurin *et al*., 1996), which is required for MuRF1's ubiquitin ligase activity, since this is the domain which binds to an E2 protein, which in turn mediates transfer of ubiquitin to the substrate (Joazeiro *et al*., 1999). The next domain, downstream of the RING, is a ''B-box'', which can mediate self-association – the B-box in MuRF1 self-associates into dimers with high affinity (Mrosek *et al*., 2008). Next in MuRF1, there is a ''coiled-coil domain'', which may be required for the formation of heterodimers between MuRF1 and itself, in addition to the related MuRF2 protein (Witt *et al*., 2008). Additional evidence that MuRF1 and MuRF2 work together was provided by a double-knockout, which showed actual hypertrophy of cardiac muscle (Witt *et al*., 2008), and not just blockade of atrophy. Further evidence for MuRF1/MuRF2 interactions came from a double knockout (DKO) study, which demonstrated that in these animals there was a profound loss of type II fibers (Moriscot *et al*., 2010). Proteins that have a RING domain, a B-box, and a coiled-coil domain are now known as TRIM proteins (for tripartite motif; Meroni & Diez-Roux, 2005). The fourth, less recognized domain of MuRF1, is a ''MuRF domain'', which is shared by all three MuRF proteins, MuRF1, MuRF2, and the less-studied MuRF3 (Gregorio *et al*., 2005).

MuRF1 is localized to the sarcomere. This was originally suggested by virtue of its binding to the very large myofibrillar protein titin, at the M line (Centner *et al*., 2001; McElhinny *et al*., 2002; Pizon *et al*., 2002). However, there is no evidence that titin is actually a substrate for MuRF1. MuRF1 also physically binds myosin heavy chain (MyHC), as demonstrated by immunoprecipitation of epitope-tagged MuRF1 protein, which brings down the MyHC protein (Clarke *et al*., 2007). Unlike the case of titin, which also binds MuRF1, MyHC is also a substrate of MuRF1, demonstrated by the finding that MuRF1 can directly ubiquitinate MyHC (Clarke *et al*., 2007). Furthermore, MuRF1 null mice demonstrate sparing of MyHC during atrophy, and knockdown of MuRF1 results in an increase of MyHC (Clarke *et al*., 2007).

Later, it was shown that additional myosin domain-containing proteins in the thick filament of muscle were also degraded by MuRF1, including myosin light chain and myosin binding protein C (Cohen *et al*., 2009). Therefore, MuRF1 induces muscle atrophy, at least in part, by directly attacking the thick filament of the sarcomere and causing the proteolysis of myosin proteins.

Muscle atrophy Fbox protein (MAFbx) contains an Fbox domain, a motif seen in a family of E3 ubiquitin ligases called SCFs (for Skp1, Cullin, and Fbox). The Fbox containing proteins are not enzymes themselves, but instead bring substrates to the E2 by virtue of the Fbox binding to the Skp1–Cullin complex. A RING-containing protein, Rbx1, is also a part of this complex, which is responsible for activating the E2 (Kamura *et al*., 1999). Fbox containing proteins usually bind a substrate only after that substrate has first been posttranslationally modified – for example, by serine or tyrosine phosphorylation (Winston *et al*., 1999).

Substrates have been suggested for MAFbx, including MyoD (Lagirand-Cantaloube *et al*., 2009; Tintignac *et al*., 2005) and calcineurin (Li *et al*., 2004). However, it has not yet been shown whether protein is ubiquitinated either by MAFbx in skeletal muscle or under atrophy conditions. MAFbx has been convincingly shown to be an E3 ligase for eIF3-f, a protein initiation factor (Li, 2007). This finding suggests that MAFbx activity results in muscle atrophy through the downregulation of protein synthesis.

Proinflammatory cytokines, such as TNFα, TWEAK, or IL-1, signal into two established pathways: the NF-κB pathway and the p38 MAP kinase. These two signaling mediators are required to upregulate the expression of the key E3 ligases, MuRF1, which mediate sarcomeric breakdown and inhibition of protein synthesis (Clarke *et al*., 2007; Cohen *et al*., 2009), and MAFbx, which control protein synthesis by ubiquitination of eIF3c (Csibi *et al*., 2009; Lagirand-Cantaloube *et al*., 2008; Sanchez *et al*., 2013). MuRF1 is upregulated in multiple settings of muscle atrophy (Bodine *et al*., 2001a) and is responsible for mediating the ubiquitination of the thick filament of the sarcomere – MyHC (Clarke *et al*., 2007) – and other thick filament components (Cohen *et al*., 2009). The cytokine TWEAK, in particular, induces MuRF1 upregulation via NF-κB, resulting in MyHC loss (Mittal *et al*., 2010). Inhibition of classical NF-κB is sufficient to significantly decrease tumor-induced muscle loss, at least in mice, in part, by inhibiting the upregulation of MuRF1 (Cai *et al*., 2004; Moore-Carrasco *et al*., 2007). This inflammatory pathway is activated in the setting of inflammatory cachexia, with examples including pulmonary cachexia, where the inflammation is present (Langen *et al*., 2012), and joint inflammation (Ramírez *et al*., 2011).

Activation of Akt can in turn inhibit the transcriptional upregulation of MAFbx and MuRF1 normally seen during atrophy (Rüegg & Glass, 2011). Their normal upregulation was demonstrated to require the FOXO (also known as Forkhead) family of transcription factors (Sandri *et al*., 2004; Stitt *et al*., 2004). FOXO transcription factors are excluded from the nucleus when phosphorylated by Akt and translocate to the nucleus upon dephosphorylation (Brunet *et al*., 1999). The translocation and activity of FOXO transcription factors are required for upregulation of MuRF1 and MAFbx – in the case of FOXO3, the activation was demonstrated to be sufficient to induce atrophy (Mammucari *et al*., 2007; Zhao *et al*., 2007), a finding that was subsequently supported by the transgenic expression of FOXO1, which also resulted in an atrophic phenotype (McLoughlin *et al*., 2009; Southgate *et al*., 2007).

MST1, a kinase that is highly expressed in the skeletal muscle, is up-regulated in fast but not slow skeletal muscle upon denervation (Wei *et al*., 2013). Deletion of the MST1 kinase significantly blocked loss of skeletal muscle normally caused by denervation and decreased the expression of

MAFbx, along with LC3. These effects of MST1 are apparently due to its ability to phosphorylate FOXO3a l at Ser207, promoting its nuclear translocation in atrophic fast-dominant muscles (Wei *et al*., 2013).

Glucocorticoid-mediated activation of MuRF1 transcription

High concentrations of glucocorticoids can induce muscle atrophy, in part by upregulation of MuRF1 and MAFbx (Wray *et al*., 2003; Stitt *et al*., 2004). It was shown that glucocorticoids synergize with FOXO1 in inducing transcription of the *MuRF1* gene (David *et al*., 2008; Zhao *et al*., 2009). Indeed, sepsis induces MuRF1 activation in part by glucocorticoid activation of its ligand-dependent transcription factor, the glucocorticoid receptor (GR) (Smith *et al*., 2010). Target genes of the GR were identified in the skeletal muscle. One such gene, *KLF15*, was found to upregulate the expression of both MAFbx and MuRF1, resulting in myotube atrophy (Shimizu *et al*., 2011).

In addition to the regulation by Akt/FOXO signaling, MuRF1 and MAFbx transcription can be at least partially inhibited by the activation of TORC1 (Herningtyas *et al*., 2008), although this evidence is not sufficient to block cachexia, since supplementation by amino acids, which induce TORC1 activation, cannot block muscle atrophy seen in cachexia. However, it has been reported that mTOR activation inhibits GR (Shimizu *et al*., 2011), which gives one mechanism by which mTOR signaling blocks the upregulation of these E3s. While activation of TORC1 might not be sufficient to block MuRF1 and MAFbx upregulation, the inhibition of mTOR independently can induce activation of these E3 ligases. This was shown, for example, by the use of AMPK, which can block mTOR signaling, and which is sufficient to upregulate MuRF1 and MAFbx (Tong *et al*., 2009).

TORC2, in a positive feedback loop, phosphorylates Akt at serine 473, thereby permitting maximum Akt activation (Sarbassov & Sabatini, 2005; Sarbassov *et al*., 2005). Recently Bentzinger *et al.* (2013) demonstrated that deletion or knockdown of RAPTOR, resulting in an inhibition of TORC1 signaling, was sufficient to increase muscle atrophy (Bentzinger *et al*., 2013). Surprisingly, a sustained activation of TORC1 actually caused muscle atrophy, due to the suppressed phosphorylation of Akt via feedback inhibition by mTORC1 (Bentzinger *et al*., 2013). Indeed, this negative feedback signaling, resulting in a blockade of Akt, is due to a mechanism involving feedback phosphorylation and inhibition of the upstream mediator IRS by p70S6K downstream of mTOR (Tremblay & Marette, 2001), causing inhibition of PI3K and, therefore, Akt activation. In the recent study by Bentzinger *et al*., this feedback loop resulted in a paradoxical activation of MuRF1 and MAFbx, since in this case FOXO signaling was derepressed. This surprising finding seems to indicate that long-term stimulation of TORC1, caused for example by amino acid-mediated stimulation without coincident activation of Akt, which can be induced by exercise-mediated activation of IGF1, might paradoxically result in muscle atrophy – giving a mechanism whereby it may be counterproductive to eat protein without exercising. Indeed, this same group also showed that sustained activation of TORC1 could eventually result in actual myopathy (Castets *et al*., 2013). Another mechanism for this effect is a disregulation of autophagy, which is usually induced when mTOR signaling is blocked (Mordier *et al*., 2000), and which is required for the normal maintenance of skeletal muscle (Sandri, 2013).

Recently discovered E3 ligases that regulate muscle mass and differentiation

While acute atrophy results in upregulation of MuRF1 and MAFbx, which are sufficient to cause breakdown of the myosin-containing thick filament of the sarcomere and protein translation factors like eIF3f (Sanchez *et al*., 2013), respectively; other E3 ligases come into play during muscle atrophy. Such E3 ligases include the already-mentioned Fbx-containing protein Fbxo40, which degrades IRS1 upon IGF1 signaling (Shi *et al*., 2011), TRIM32, an E3 ligase that degrades actin (Kudryashova *et al*., 2005) and desmin (Cohen *et al*., 2012). Loss of desmin is responsible for a particular form of limb girdle muscular dystrophy (Frosk *et al*., 2002). In contrast to MuRF1, whose deletion seems to spare muscle and block atrophy, loss of TRIM32 results in pathologic, or dystrophic, skeletal muscle. The E3 ligase Trip12, a HECT domain E3 ubiquitin ligase, has been recently shown to bind and induce the polyubiquitination of a protein called Sox6, a transcription factor which plays a role in fiber-type switching (An *et al*., 2013). Knockdown of Trip12 in myotubes resulted in an increase in Sox6 protein levels and a concurrent decrease in slow fiber-specific Myh7 expression, along with a coincident increase in the fast fiber-specific marker, Myh4 (An *et al*., 2013).

An interesting recent finding is that an E3 ligase called Mul1 controls ''mitophagy'' – the turnover of mitochondria (Lokireddy *et al*., 2012). Loss of mitochondria was noted early on in settings of muscle atrophy (Pellegrino & Franzini, 1963), and this loss has been thought to contribute to the phenotype, due to the decreased ability to generate ATP, among other sequellae. Overexpression of Mul1 was sufficient for the induction of mitophagy in skeletal muscle myotubes, and suppression both protected against mitophagy and partially rescued the muscle subjected to atrophy-inducing stimuli (Lokireddy *et al*., 2012).

Myostatin, activin, and other TGFβ family members

Myostatin, or growth and differentiation factor 8 (GDF-8), is a member of the transforming growth factor-β (TGF-β) superfamily that acts as a negative regulator of muscle growth (Lee & McPherron, 1999, 2001; McPherron & Lee, 1997, 2002; McPherron *et al*., 1997, 1999). Genetic mutations in *MSTN* and therapeutic inhibition against myostatin result in an increase in the overall skeletal muscle mass, a phenotype conserved across multiple species, including mice, cattle, and humans (Grobet *et al*., 1997; Lee, 2007; McPherron & Lee, 1997; McPherron *et al*., 1997). Once bound to its type I and type II receptors, Activin Receptor II A or B (ActRIIA or B) and Activin-Like Kinase-4 or 5 (ALK-4 or 5), respectively, intracellular signaling is initiated via phosphorylation and activation of the transcription factors Smad2 and 3, which translocate to the nucleus and

activate target genes (McCroskery *et al*., 2003; Rebbapragada *et al*., 2003). The existence of non-Smad-mediated pathways has been only recently reported and the identities of downstream targets of myostatin intracellular signals are unclear (Huang *et al*., 2007; Philip *et al*., 2005).

In skeletal muscle, myostatin negatively regulates Akt signaling (Sartori *et al*., 2009; Trendelenburg *et al*., 2009). Interestingly, IGF1 can rescue this effect on Akt; when myostatin and IGF1 are given together, Akt phosphorylation is indistinguishable versus myotubes stimulated with IGF1 alone (Trendelenburg *et al*., 2009), despite the fact that there is no obvious direct effect of IGF1 on myostatin-mediated Smad signaling (Sartori *et al*., 2009; Trendelenburg *et al*., 2009). One mechanism by which IGF1 may inhibit or at least restrict Smad2/3 activation is by TORC1 signaling. The roles of TORC1 and TORC2 in myostatin's inhibition of muscle growth were investigated, and an increase in myostatin-induced Smad2 phosphorylation was observed following inhibition of TORC1 (by siRNA knockdown of RAPTOR), potentiating myostatin's inhibitory effects on muscle (Trendelenburg *et al*., 2009). An additional component of a myostatin-Akt cross-talk model may, therefore, include a feedback loop, with TORC1 capable of negatively influencing myostatin signaling. Although myostatin and IGF1 have antagonistic effects on mTOR phosphorylation, it is not clear whether the regulation of mTOR represents a necessary central nexus of myostatin-induced effects or, rather, plays a more supportive role, downstream of Akt (Figure 1). Recent studies have shown that blocking mTOR activity does not fully prevent the increases in protein synthesis and hypertrophy phenotype associated with myostatin inhibition (Sartori *et al*., 2009; Welle *et al*., 2009).

Myostatin's negative regulation of skeletal muscle growth may be due, in part, to its interference with myoblast differentiation (Lin *et al*., 2002; McPherron *et al*., 1999; Rios *et al*., 2001). Trendelenburg *et al.* (2009) reported that treatment of primary human skeletal myoblasts and myotubes with physiologic concentrations of myostatin resulted in an inhibition of differentiation. siRNA-mediated knockdown of Smad2 and Smad3 was shown to be sufficient to inhibit myostatin signaling and rescue differentiation (Trendelenburg *et al*., 2009; Sartori *et al*., 2009). Previous research has suggested that other members of the TGF-β superfamily may cooperate with myostatin in regulating differentiation. Specifically, expression of the TGF-β inhibitor follistatin, coupled with myostatin inhibition, exhibits a synergistic effect on increasing muscle mass. TGF-β1, GDF-11, and Activin A, all members of the TGF-β superfamily, have been shown to block muscle differentiation with similar or even greater potencies than that of myostatin (Trendelenburg *et al*., 2009; Figure 1). It is currently unclear whether endogenous levels of these molecules are capable of modulating skeletal muscle and, as such, further studies are required to determine the specific roles and physiological importance of TGF-β molecules in skeletal muscle.

Whether or not myostatin directly induces atrophy signaling is somewhat less clear. One study showed that rather than inducing MuRF1 and MAFbx, myostatin signaling actually decreased transcription of these genes, along with other genes normally induced upon muscle differentiation (Trendelenburg *et al*., 2009). The conclusion from that study, therefore, was that myostatin induces muscle atrophy both by blocking Akt mediated protein synthesis and by downregulating genes required for muscle homeostasis normally induced upon differentiation, even in post-differentiated muscle fibers. Other studies, however, have shown that myostatin, albeit at quite high concentrations, can in fact induce upregulation of the E3 ligases (Lokireddy *et al*., 2012; Sartori *et al*., 2009).

Myostatin itself can be regulated by multiple mechanisms, including via the CCAAT/enhancer (David *et al*., 2010), hypoxia (Hayot *et al*., 2011), and microRNA27-a (David & Amanda, 2010). Furthermore, it has been shown that inflammatory signaling, downstream of cytokine activation, induces endogenous expression of the TGFβ family member Activin, demonstrating an important instance of cytokine/TGFβ signaling (Trendelenburg *et al*., 2012; Figure 1).

G-Protein induced activation of hypertrophy signaling

Independent of IGF1-mediated mTOR activation, signaling through heterotrimeric guanine nucleotide-binding proteins (G protein)-coupled receptors (GPCRs) (Pierce *et al*., 2002) has emerged as a novel mechanism in the regulation of skeletal muscle hypertrophy (Jean-Baptiste *et al*., 2005). Upon ligand binding, GPCRs undergo a conformational shift, permitting their activation and signaling via recruitment of intracellular heterotrimeric G proteins. Activation of four G protein-coupled receptors, CRFR2, β2-AR, the LPA receptor, and Fzd7, have shown to induce skeletal muscle hypertrophy (Lynch & Ryall, 2008; Rebecca & Randi, 2012). More recently, Minetti *et al.* (2011) demonstrated that a G protein, specifically Gαi2, was essential for the induction of muscle hypertrophy mediated by LPA receptor signaling

G proteins, expressed in multiple tissue types including skeletal muscle, consist of a GTP-binding alpha subunit (Gα) and a heterodimer of beta and gamma subunits (Gβγ) – once activated, Gα subunits bind GTP, thereby releasing bound Gβγ subunits and allowing Gα to mediate downstream signaling. Among the four classes of Gα subunits, $G\alpha_i$ proteins ($G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$) are widely distributed and highly homologous, capable of regulating key signaling mediators such as phospholipase C and protein kinase C (PKC) (Wettschureck & Offermanns, 2005). Previous research has suggested a possible link between G protein and Akt signaling events, with GPCR β2-AR-mediated skeletal muscle hypertrophy, at least, accompanied by the activation of Akt in a manner dependent of mTOR (Kline *et al*., 2007; Koopman *et al*., 2010).

Constitutively active Gαi2 by itself was sufficient to promote hypertrophy in cultured myotubes as well as in mouse models (Minetti *et al*., 2011). However, while rapamycin and PKC inhibitors blocked the resulting hypertrophic phenotype, PI3K inhibitors did not have an effect. Consistent with these data, Gαi2 activity drove phosphorylation of targets downstream of mTOR, specifically p70S6K and GSK3β, but not that of Akt, suggesting a linear pathway between the G protein and the mTOR via PKC. Surprisingly, the results offer a novel mechanism for Gαi-mediated hypertrophy signaling in skeletal muscle that is dispensable of PI3K and Akt.

In addition to its role in hypertrophy, GPCR signaling may also directly influence the atrophy program in skeletal muscle. In multiple rodent models of atrophy, including unloading and aging, ligands for the GPCRs promoted atrophy resistance (Carter & Lynch, 1994; Kline *et al*., 2007). Similarly, β2-AR agonists exhibit an inhibitory effect on muscle atrophy in cancer cachexia models (Carbó *et al*., 1997; Costelli *et al*., 1995). Minetti *et al*. (2011) demonstrated that activation of Gαi2 can block the up-regulation in the expression *MuRF1* and *MAFbx* associated with proinflammatory cytokine TNFα-induced atrophy. In contrast to the Gαi2/PKC signaling for hypertrophy, this appears to be PKC-independent, mediated instead by HDAC4 localization/activity – Gαi2 drove HDAC4 cytoplasmic localization, thereby preventing its nuclear functions (Figure 1).

PGC-1α, mitochondria, and sarcopenia

Mitochondrial oxidative metabolism and energy transduction pathways are critical for skeletal muscle function, and it has been recognized for quite some time that another major effect of long-term muscle atrophy is a decrease in mitochondria (Pellegrino & Franzini, 1963). The transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α) is sufficient to induce mitochondriogenesis (Wu *et al*., 1999), along with other effects on muscle, including fiber-type switching (Puigserver & Spiegelman, 2003). There are two reports that over-expression of PGC1α can also result in the sparing of skeletal muscle under atrophy conditions, perhaps by negative regulation of FOXO signaling (Brault *et al*., 2010; Sandri *et al*., 2006). Paradoxically, a signaling pathway that increases mitochondriogenesis via PGC1α is the activation of AMPK, which at the same time actually decreases protein synthesis, by blocking mTOR (Bolster *et al*., 2002; Mounier *et al*., 2009). Therefore, it would be important to determine whether it is possible to positively modulate the PGC1α pathway without interfering with protein synthesis, since there is a net loss of protein in atrophy, and therefore, an additional inhibition in synthesis pathways may not be desirable.

In addition to the transcriptional co-activator PGC1α, the expression of genes important for mitochondrial biogenesis is also in part controlled by the estrogen-related receptor (ERR) subfamily of nuclear receptors (Soriano *et al*., 2006). PGC-1α and ERRs, working together, induce the expression of a muscle-specific protein, Perm1, which regulates the expression of genes with roles in glucose and lipid metabolism, energy transfer, and contractile function (Cho *et al*., 2013).

The age-related loss of skeletal muscle is called ''sarcopenia'' (Glass & Roubenoff, 2010; Hughes & Roubenoff, 2000). This loss of muscle mass and function results in frailty of the elderly, a considerable degree of morbidity, such as an enhanced risk of falls, and the loss of the ability to maintain an independent lifestyle. In an unbiased survey of gene changes which occur upon sarcopenia in rats, the most downregulated pathway was that associated with PGC1α and mitochondriogenesis (Ibebunjo *et al*., 2013). While this simply establishes an association, when transgenic mice overexpressing PGC1α in skeletal muscle were followed in an aging colony, they were found to be resistant to the onset of sarcopenia (Wenz *et al*., 2009); even more provocative was the supplemental figure in this study, demonstrating that the transgenics had a significant increase in life-span. This last piece of data is especially surprising, since PGC1α was only overexpressed in the muscle; the implication is that the muscle may be a source of secreted ''myokines'' which have a protective effect on the rest of the organism.

The anabolic sex hormone testosterone is also capable of inducing PGC1α, in addition to IGF-1 (Ibebunjo *et al*., 2011); testosterone and the consequent activation of the Androgen Receptor provide one of the few known mechanisms that can simultaneously induce mitochondriogenesis and anabolism via the IGF1/Akt pathway (Ibebunjo *et al*., 2011).

Conclusion

It has only been in the last 15 years or so that the signaling pathways controlling skeletal muscle mass and function have begun to be elucidated. Therefore, it should not be surprising that new mechanisms and refinements to these pathways continue to be discovered, and indeed very recently quite considerable progress has been made in this area. Still, the fact remains that, in contrast to other well-studied diseases, there is an almost entire lack of approved medications for skeletal muscle disease – despite the great need accentuated by an aging population, where muscle frailty and weakness are an almost universal sign of ''normal aging''. Further, the finding that in settings such as cancer, the simple treatment of cachexia – distinct from the tumor itself – can increase survival which in turn greatly increases the interest in preserving skeletal mass and function in settings of disease. The enhanced understanding of the mechanisms controlling skeletal muscle maintenance gives increased hope that such treatments will be developed in the near future.

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Declaration of interest

Both authors are employees of Novartis Institutes for Biomedical Research.

References

Alessi DR, Caudwell FB, *et al*. (1996). Molecular basis for the substrate specificity of protein kinase B; comparison with MAPKAP kinase-1 and p70 S6 kinase. FEBS Lett 399:333–8.

An C-I, Ganio E, Hagiwara N. (2013). Trip12, a HECT domain E3 ubiquitin ligase, targets Sox6 for proteasomal degradation and affects fiber type-specific gene expression in muscle cells. Skeletal Muscle 3: 11.

Bentzinger C, Lin S, Romanino K, *et al*. (2013). Differential response of skeletal muscles to mTORC1 signaling during atrophy and hypertrophy. Skeletal Muscle 3:6.

Berset C, Trachsel H, Altmann M. (1998). The TOR (target of rapamycin) signal transduction pathway regulates the stability of translation initiation factor eIF4G in the yeast *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 95:4264–9.

Blaauw B, Canato M, Agatea L, *et al*. (2009). Inducible activation of Akt increases skeletal muscle mass and force without satellite cell activation. FASEB J 23:3896–905.

Bodine SC, Latres E, Baumhueter S, *et al*. (2001a). Identification of ubiquitin ligases required for skeletal muscle atrophy. Science 294: 1704–8.

Bodine SC, Stitt TN, Gonzalez M, *et al*. (2001b). Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nat Cell Biol 3:1009–13.

Bohni R, Riesgo-Escovar J, Oldham S, *et al*. (1999). Autonomous control of cell and organ size by CHICO, a Drosophila homolog of vertebrate IRS1-4. Cell 97:865–75.

Bolster DR, Crozier SJ, Kimball SR, Jefferson LS. (2002). AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. J Biol Chem 277:23977–80.

Borden KL, Freemont PS. (1996). The RING finger domain: a recent example of a sequence-structure family. Curr Opin Struct Biol 6: 396–401.

Brault JJ, Jespersen JG, Goldberg AL. (2010). Peroxisome proliferator-activated receptor gamma coactivator 1alpha or 1beta overexpression inhibits muscle protein degradation, induction of ubiquitin ligases, and disuse atrophy. J Biol Chem 285:19460–71.

Brunet A, Bonni A, Zigmond MJ, *et al*. (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96:857–68.

Brunn GJ, Hudson CC, Sekulic A, *et al*. (1997). Phosphorylation of the translational repressor PHAS-I by the mammalian target of rapamycin. Science 277:99–101.

Cai D, Frantz JD, Tawa Jr NE, *et al*. (2004). IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. Cell 119: 285–98.

Carbó N, López-Soriano J, Tarragó T, *et al*. (1997). Comparative effects of β2-adrenergic agonists on muscle waste associated with tumour growth. Cancer Lett 115:113–18.

Carter WJ, Lynch ME. (1994). Comparison of the effects of salbutamol and clenbuterol on skeletal muscle mass and carcass composition in senescent rats. Metabolism 43:1119–25.

Castets P, Lin S, Rion N, *et al*. (2013). Sustained activation of mTORC1 in skeletal muscle inhibits constitutive and starvation-induced autophagy and causes a severe, late-onset myopathy. Cell Metabol 17:731–44.

Centner T, Yano J, Kimura E, *et al*. (2001). Identification of muscle specific ring finger proteins as potential regulators of the titin kinase domain. J Mol Biol 306:717–26.

Cho Y, Hazen BC, Russell AP, Kralli A. (2013). Peroxisome proliferator-activated receptor γ coactivator 1 (PGC-1)- and estrogen-related receptor (ERR)-induced regulator in muscle 1 (PERM1) is a tissue-specific regulator of oxidative capacity in skeletal muscle cells. J Biol Chem 288:25207–18.

Clarke BA, Drujan D, Willis MS, *et al*. (2007). The E3 Ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle. Cell Metab 6:376–85.

Cohen S, Brault JJ, Gygi SP, *et al*. (2009). During muscle atrophy, thick, but not thin, filament components are degraded by MuRF1-dependent ubiquitylation. J Cell Biol 185:1083–95.

Cohen S, Zhai B, Gygi SP, Goldberg AL. (2012). Ubiquitylation by Trim32 causes coupled loss of desmin, Z-bands, and thin filaments in muscle atrophy. J Cell Biol 198:575–89.

Coleman ME, DeMayo F, Yin KC, *et al*. (1995). Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice. J Biol Chem 270:12109–16.

Costelli P, Garcia-Martinez C, Llovera M, *et al*. (1995). Muscle protein waste in tumor-bearing rats is effectively antagonized by a beta 2-adrenergic agonist (clenbuterol). Role of the ATP-ubiquitin-dependent proteolytic pathway. J Clin Invest 95:2367–72.

Crabtree GR, Olson EN. (2002). NFAT signaling: choreographing the social lives of cells. Cell 109:S67–79.

Cross DA, Alessi DR, Cohen P, *et al*. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378:785–9.

Csibi A, Leibovitch MP, Cornille K, *et al*. (2009). MAFbx/Atrogin-1 controls the activity of the initiation factor eIF3-f in skeletal muscle atrophy by targeting multiple C-terminal lysines. J Biol Chem 284:4413–21.

David LA, Allison SC, Andrea MH, *et al*. (2010). CCAAT/enhancer binding protein-δ expression is increased in fast skeletal muscle by food deprivation and regulates myostatin transcription in vitro. Am J Physiol: Regulat Integr Comparat Physiol 299:R1592–601.

David LA, Amanda SL. (2010). Posttranscriptional mechanisms involving microRNA-27a and b contribute to fast-specific and glucocorticoid-mediated myostatin expression in skeletal muscle. Am J Physiol Cell Physiol 300:C124–37.

David SW, Leslie MB, Jens van den B, *et al*. (2008). The glucocorticoid receptor and FOXO1 synergistically activate the skeletal muscle atrophy-associated MuRF1 gene. Am J Physiol: Endocrinol Metabol 295:E785–97.

Friday BB, Horsley V, Pavlath GK. (2000). Calcineurin activity is required for the initiation of skeletal muscle differentiation [In Process Citation]. J Cell Biol 149:657–66.

Frosk P, Weiler T, Nylen E, *et al*. (2002). Limb-girdle muscular dystrophy type 2H associated with mutation in TRIM32, a putative E3-ubiquitin ligase gene. Am J Human Genet 70:663–72.

Gingras AC, Kennedy SG, O'Leary MA, *et al*. (1998). 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt(PKB) signaling pathway. Genes Dev 12:502–13.

Gingras A-C, Raught B, Sonenberg N. (2001). Regulation of translation initiation by FRAP/mTOR. Genes Dev 15:807–26.

Glass D, Roubenoff R. (2010). Recent advances in the biology and therapy of muscle wasting. Ann New York Acad Sci 1211: 25–36.

Gomes MD, Lecker SH, Jagoe RT, *et al*. (2001). Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. Proc Natl Acad Sci USA 98:14440–5.

Gregorio CC, Perry CN, McElhinny AS. (2005). Functional properties of the titin/connectin-associated proteins, the muscle-specific RING finger proteins (MURFs), in striated muscle. J Muscle Res Cell Motil 26:389–400.

Grobet L, Martin LJ, Poncelet D, *et al*. (1997). A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nat Genet 17:71–4.

Guertin DA, Stevens DM, Thoreen CC, *et al*. (2006). Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. Dev Cell 11:859–71.

Hara K, Yonezawa K, Kozlowski MT, *et al*. (1997). Regulation of eIF-4E BP1 phosphorylation by mTOR. J Biol Chem 272:26457–63.

Hardt SE, Tomita H, Katus HA, Sadoshima J. (2004). Phosphorylation of eukaryotic translation initiation factor 2Bε by glycogen synthase kinase-3β regulates β-adrenergic cardiac myocyte hypertrophy. Circul Res 94:926–35.

Haruta T, Uno T, Kawahara J, *et al*. (2000). A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. Mol Endocrinol 14:783–94.

Hayot M, Rodriguez J, Vernus B, *et al*. (2011). Myostatin up-regulation is associated with the skeletal muscle response to hypoxic stimuli. Mol Cell Endocrinol 332:38–47.

Herningtyas EH, Okimura Y, Handayaningsih AE, *et al*. (2008). Branched-chain amino acids and arginine suppress MaFbx/atrogin-1 mRNA expression via mTOR pathway in C2C12 cell line. Biochim Biophys Acta (BBA): General Sub 1780:1115–20.

Horsley V, Friday BB, Matteson S, *et al*. (2001). Regulation of the growth of multinucleated muscle cells by an NFATC2-dependent pathway. J Cell Biol 153:329–38.

Horsley V, Pavlath GK. (2002). Nfat: ubiquitous regulator of cell differentiation and adaptation. J Cell Biol 156:771–4.

Hu S-I, Katz M, Chin S, *et al*. (2012). MNK2 inhibits eIF4G activation through a pathway involving serine–arginine-rich protein kinase in skeletal muscle. Sci Signal 5:ra14.

Huang Z, Chen D, Zhang K, *et al*. (2007). Regulation of myostatin signaling by c-Jun N-terminal kinase in C2C12 cells. Cell Signal 19: 2286–95.

Hughes V, Roubenoff R. (2000). Sarcopenia: current concepts. J Gerontol Med Sci 55A:M716–24.

Ibebunjo C, Chick JM, Kendall T, *et al*. (2013). Genomic and proteomic profiling reveals reduced mitochondrial function and disruption of the

neuromuscular junction driving rat sarcopenia. Mol Cell Biol 33:194–212.
Ibebunjo C, Eash JK, Li C, *et al*. (2011). Voluntary running, skeletal muscle gene expression, and signaling inversely regulated by orchidectomy and testosterone replacement. Am J Physiol: Endocrinol Metabol 300:E327–40.
Izumiya Y, Hopkins T, Morris C, *et al*. (2008). Fast/glycolytic muscle fiber growth reduces fat mass and improves metabolic parameters in obese mice. Cell Metabol 7:159–72.
Jean-Baptiste G, Yang Z, Khoury C, *et al*. (2005). Peptide and non-peptide G-protein coupled receptors (GPCRs) in skeletal muscle. Peptides 26:1528–36.
Joazeiro CAP, Wing SS, Huang H-k, *et al*. (1999). The tyrosine kinase negative regulator c-Cbl as a RING-type, E2-dependent ubiquitin-protein ligase. Science 286:309–12.
Kamura T, Koepp DM, Conrad MN, *et al*. (1999). Rbx1, a component of the VHL tumor suppressor complex and SCF ubiquitin ligase [see comments]. Science 284:657–61.
Kang SA, Pacold ME, Cervantes CL, *et al*. (2013). mTORC1 phosphorylation sites encode their sensitivity to starvation and rapamycin. Science 341:1236566. doi: 10.1126/science.1236566.
Kim do H, Sarbassov dos D, Ali SM, *et al*. (2002). mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell 110:163–75.
Kline WO, Panaro FJ, Yang H, Bodine SC. (2007). Rapamycin inhibits the growth and muscle-sparing effects of clenbuterol. J Appl Physiol 102:740–7.
Koopman R, Gehrig SM, Leger B, *et al.* (2010). Cellular mechanisms underlying temporal changes in skeletal muscle protein synthesis and breakdown during chronic {beta}-adrenoceptor stimulation in mice. J Physiol 588:4811–23.
Kudryashova E, Kudryashov D, Kramerova I, Spencer MJ. (2005). Trim32 is a ubiquitin ligase mutated in limb girdle muscular dystrophy type 2H that binds to skeletal muscle myosin and ubiquitinates actin. J Mol Biol 354:413–24.
Lagirand-Cantaloube J, Cornille K, Csibi A, *et al*. (2009). Inhibition of atrogin-1/MAFbx mediated MyoD proteolysis prevents skeletal muscle atrophy *in vivo*. PLoS ONE 4:e4973.
Lagirand-Cantaloube J, Offner N, Csibi A, *et al*. (2008). The initiation factor eIF3-f is a major target for atrogin1/MAFbx function in skeletal muscle atrophy. EMBO J 27:1266–76.
Lamming DW, Ye L, Katajisto P, *et al*. (2012). Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science 30;335:1638–43.
Lai K-MV, Gonzalez M, Poueymirou WT, *et al*. (2004). Conditional activation of akt in adult skeletal muscle induces rapid hypertrophy. Mol Cell Biol 24:9295–304.
Langen RCJ, Haegens A, Vernooy JHJ, *et al*. (2012). NF-κB activation is required for the transition of pulmonary inflammation to muscle atrophy. Am J Respirat Cell Mol Biol 47:288–97.
Lawrence Jr JC, Fadden P, Haystead TA, Lin TA. (1997). PHAS proteins as mediators of the actions of insulin, growth factors and cAMP on protein synthesis and cell proliferation. Adv Enzyme Regul 37: 239–67.
Lee AV, Gooch JL, Oesterreich S, *et al*. (2000). Insulin-like growth factor I-induced degradation of insulin receptor substrate 1 is mediated by the 26S proteasome and blocked by phosphatidylinositol 3'-kinase inhibition. Mol Cell Biol 20:1489–96.
Lee S-J. (2007). Quadrupling muscle mass in mice by targeting TGFbeta signaling pathways. PLoS ONE 2:e789.
Lee SJ, McPherron AC. (1999). Myostatin and the control of skeletal muscle mass. Curr Opin Genet Dev 9:604–7.
Lee SJ, McPherron AC. (2001). Regulation of myostatin activity and muscle growth. Proc Natl Acad Sci USA 98:9306–11.
Li H-H. (2007). Atrogin-1 inhibits Akt-dependent cardiac hypertrophy in mice via ubiquitin-dependent coactivation of Forkhead proteins. J Clin Invest 117:3211–23.
Li HH, Kedar V, Zhang C, *et al*. (2004). Atrogin-1/muscle atrophy F-box inhibits calcineurin-dependent cardiac hypertrophy by participating in an SCF ubiquitin ligase complex. J Clin Invest 114:1058–71.
Lin J, Arnold HB, Della-Fera MA, *et al*. (2002). Myostatin knockout in mice increases myogenesis and decreases adipogenesis. Biochem Biophys Res Commun 291:701–6.
Lokireddy S, Wijesoma IW, Teng S, *et al*. (2012). The ubiquitin ligase Mul1 induces mitophagy in skeletal muscle in response to muscle-wasting stimuli. Cell Metabolism 16:613–24.
Lynch GS, Ryall JG. (2008). Role of beta-adrenoceptor signaling in skeletal muscle: implications for muscle wasting and disease. Physiol Rev 88:729–67.
Mammucari C, Milan G, Romanello V, *et al*. (2007). FoxO3 controls autophagy in skeletal muscle in vivo. Cell Metab 6:458–71.
McCroskery S, Thomas M, Maxwell L, *et al*. (2003). Myostatin negatively regulates satellite cell activation and self-renewal. J Cell Biol 162:1135–47.
McElhinny AS, Kakinuma K, Sorimachi H, *et al*. (2002). Muscle-specific RING finger-1 interacts with titin to regulate sarcomeric M-line and thick filament structure and may have nuclear functions via its interaction with glucocorticoid modulatory element binding protein-1. J Cell Biol 157:125–36.
McLoughlin TJ, Smith SM, DeLong AD, *et al*. (2009). FoxO1 induces apoptosis in skeletal myotubes in a DNA-binding-dependent manner. Am J Physiol Cell Physiol 297:C548–55.
McPherron AC, Lawler AM, Lee SJ. (1997). Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 387:83–90.
McPherron AC, Lawler AM, Lee SJ. (1999). Regulation of anterior/posterior patterning of the axial skeleton by growth/differentiation factor 11. Nat Genet 22:260–4.
McPherron AC, Lee SJ. (1997). Double muscling in cattle due to mutations in the myostatin gene. Proc Natl Acad Sci USA 94: 12457–61.
McPherron AC, Lee SJ. (2002). Suppression of body fat accumulation in myostatin-deficient mice. J Clin Invest 109:595–601.
Meroni G, Diez-Roux G. (2005). TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. BioEssays 27:1147–57.
Minetti GC, Feige JN, Rosenstiel A, *et al*. (2011). Gα_{i2} signaling promotes skeletal muscle hypertrophy, myoblast differentiation, and muscle regeneration. Sci Signal 4:ra80.
Mitch WE, Goldberg AL. (1996). Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. N Engl J Med 335: 1897–905.
Mittal A, Bhatnagar S, Kumar A, *et al*. (2010). The TWEAK, Fn14 system is a critical regulator of denervation-induced skeletal muscle atrophy in mice. J Cell Biol 188:833–49.
Moore-Carrasco R, Busquets S, Almendro V, *et al*. (2007). The AP-1/NF-kappaB double inhibitor SP100030 can revert muscle wasting during experimental cancer cachexia. Int J Oncol 30:1239–45.
Mordier S, Deval C, Bechet D, *et al*. (2000). Leucine limitation induces autophagy and activation of lysosome-dependent proteolysis in C2C12 myotubes through a mammalian target of rapamycin-independent signaling pathway. J Biol Chem 275:29900–6.
Morisco C, Zebrowski D, Condorelli G, *et al*. (2000). The Akt-glycogen synthase kinase 3beta pathway regulates transcription of atrial natriuretic factor induced by beta-adrenergic receptor stimulation in cardiac myocytes. J Biol Chem 275:14466–75.
Moriscot AS, Baptista IL, Bogomolovas J, *et al*. (2010). MuRF1 is a muscle fiber-type II associated factor and together with MuRF2 regulates type-II fiber trophicity and maintenance. J Struct Biol 170: 344–53.
Mounier R, Lantier L, Leclerc J, *et al*. (2009). Important role for AMPKalpha1 in limiting skeletal muscle cell hypertrophy. FASEB J 23:2264–73.
Mrosek M, Meier S, Ucurum-Fotiadis Zh, *et al*. (2008). Structural analysis of B-Box 2 from MuRF1: identification of a novel self-association pattern in a RING-like fold†‡. Biochemistry 47:10722–30.
Musaro A, McCullagh K, Paul A, *et al*. (2001). Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. Nat Genet 27:195–200.
Nakao R, Hirasaka K, Goto J, *et al*. (2009). Ubiquitin ligase Cbl-b is a negative regulator for insulin-like growth factor 1 signaling during muscle atrophy caused by unloading. Mol Cell Biol 29:4798–811.
Pellegrino C, Franzini C. (1963). An electron microscope study of denervation atrophy in red and white skeletal muscle fibers. J Cell Biol 17:327–49.
Philip B, Lu Z, Gao Y. (2005). Regulation of GDF-8 signaling by the p38 MAPK. Cell Signal 17:365–75.
Pierce KL, Premont RT, Lefkowitz RJ. (2002). Seven-transmembrane receptors. Nat Rev Mol Cell Biol 3:639–50.

Pizon V, Iakovenko A, van der Ven PFM, *et al*. (2002). Transient association of titin and myosin with microtubules in nascent myofibrils directed by the MURF2 RING-finger protein. J Cell Sci 115:4469–82.

Prevot D, Darlix JL, Ohlmann T. (2003a). Conducting the initiation of protein synthesis: the role of eIF4G. Biol Cell 95:141–56.

Prevot D, Decimo D, Herbreteau CH, *et al*. (2003b). Characterization of a novel RNA-binding region of eIF4GI critical for ribosomal scanning. EMBO J 22:1909–21.

Puigserver P, Spiegelman BM. (2003). Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α): transcriptional coactivator and metabolic regulator. Endocr Rev 24:78–90.

Pyronnet S, Imataka H, Gingras A-C, *et al*. (1999). Human eukaryotic translation initiation factor 4G (eIF4G) recruits Mnk1 to phosphorylate eIF4E. EMBO J 18:270–9.

Ramírez C, Russo TL, Sandoval MC, *et al*. (2011). Joint inflammation alters gene and protein expression and leads to atrophy in the tibialis anterior muscle in rats. Am J Phys Med Rehabil 90:930–9.

Rebbapragada A, Benchabane H, Wrana JL, *et al*. (2003). Myostatin signals through a transforming growth factor {beta}-like signaling pathway to block adipogenesis. Mol Cell Biol 23:7230–42.

Rebecca B, Randi S. (2012). cAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. Am J Physiol: Endocrinol Metabol 303:E1–7.

Rios R, Carneiro I, Arce VM, Devesa J. (2001). Myostatin regulates cell survival during C2C12 myogenesis. Biochem Biophys Res Commun 280:561–6.

Rommel C, Bodine SC, Clarke BA, *et al*. (2001). Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. Nat Cell Biol 3:1009–13.

Rüegg MA, Glass DJ. (2011). Molecular mechanisms and treatment options for muscle wasting diseases. Ann Rev Pharmacol Toxicol 51: 373–95.

Rui L, Yuan M, Frantz D, *et al*. (2002). SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. J Biol Chem 277:42394–8.

Sanchez AMJ, Csibi A, Raibon A, *et al*. (2013). eIF3f: a central regulator of the antagonism atrophy/hypertrophy in skeletal muscle. Int J Biochem Cell Biol 45:2158–62.

Sandri M. (2013). Protein breakdown in muscle wasting: role of autophagy-lysosome and ubiquitin-proteasome. Int J Biochem Cell Biol 45:2121–9.

Sandri M, Lin J, Handschin C, *et al*. (2006). PGC-1{alpha} protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. Proc Natl Acad Sci USA 103: 16260–5.

Sandri M, Sandri C, Gilbert A, *et al*. (2004). Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. Cell 117:399–412.

Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. (2005). Phosphorylation and regulation of Akt/PKB by the Rictor–mTOR complex. Science 307:1098–101.

Sarbassov DD, Sabatini DM. (2005). Redox regulation of the nutrient-sensitive raptor–mTOR pathway and complex. J Biol Chem 280: 39505–9.

Sartori R, Milan G, Patron M, *et al*. (2009). Smad2 and 3 transcription factors control muscle mass in adulthood. Am J Physiol Cell Physiol 296:C1248–57.

Saurin AJ, Borden KL, Boddy MN, Freemont PS. (1996). Does this have a familiar RING? Trends Biochem Sci 21:208–14.

Schalm SS, Fingar DC, Sabatini DM, Blenis J. (2003). TOS motif-mediated raptor binding regulates 4E-BP1 multisite phosphorylation and function. Curr Biol CB 13:797–806.

Scheper GC, Morrice NA, Kleijn M, Proud CG. (2001). The mitogen-activated protein kinase signal-integrating kinase Mnk2 is a eukaryotic initiation factor 4E kinase with high levels of basal activity in mammalian cells. Mol Cell Biol 21:743–54.

Shi J, Luo L, Eash J, *et al*. (2011). The SCF–Fbxo40 complex induces IRS1 ubiquitination in skeletal muscle, limiting IGF1 signaling. Dev Cell 21:835–47.

Shimizu N, Yoshikawa N, Ito N, *et al*. (2011). Crosstalk between glucocorticoid receptor and nutritional sensor mTOR in skeletal muscle. Cell Metabol 13:170–82.

Smith IJ, Alamdari N, O'Neal P, *et al*. (2010). Sepsis increases the expression and activity of the transcription factor Forkhead Box O 1 (FOXO1) in skeletal muscle by a glucocorticoid-dependent mechanism. Int J Biochem Cell Biol 42:701–11.

Song R, Peng W, Zhang Y, *et al*. (2013). Central role of E3 ubiquitin ligase MG53 in insulin resistance and metabolic disorders. Nature 494:375–9.

Soriano FX, Liesa M, Bach D, *et al*. (2006). Evidence for a mitochondrial regulatory pathway defined by peroxisome proliferator-activated receptor-γ coactivator-1α, estrogen-related receptor-α, and mitofusin 2. Diabetes 55:1783–91.

Southgate RJ, Neill B, Prelovsek O, *et al*. (2007). FOXO1 regulates the expression of 4E-BP1 and inhibits mTOR signaling in mammalian skeletal muscle. J Biol Chem 282:21176–86.

Stitt T, Drujan D, Clarke B, *et al*. (2004). The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. Mol Cell 14:395–403.

Tintignac LA, Lagirand J, Batonnet S, *et al*. (2005). Degradation of MyoD mediated by the SCF (MAFbx) ubiquitin ligase. J Biol Chem 280:2847–56.

Tong JF, Yan X, Zhu MJ, Du M. (2009). AMP-activated protein kinase enhances the expression of muscle-specific ubiquitin ligases despite its activation of IGF-1/Akt signaling in C2C12 myotubes. J Cell Biochem 108:458–68.

Tremblay F, Marette A. (2001). Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway: a negative feedback mechanism leading to insulin resistance in skeletal muscle cells. J Biol Chem 276: 38052–60.

Trendelenburg A-U, Meyer A, Jacobi C, *et al*. (2012). TAK-1/p38/NFkappaB signaling inhibits myoblast differentiation by increasing levels of Activin A. Skeletal Muscle 2:3.

Trendelenburg AU, Meyer A, Rohner D, *et al*. (2009). Myostatin reduces Akt/TORC1/p70S6K signaling, inhibiting myoblast differentiation and myotube size. Am J Physiol Cell Physiol 296:C1258–70.

Tzatsos A, Kandror KV. (2006). Nutrients suppress phosphatidylinositol 3-kinase/Akt signaling via raptor-dependent mTOR-mediated insulin receptor substrate 1 phosphorylation. Mol Cell Biol 26:63–76.

Wei B, Dui W, Liu D, *et al*. (2013). MST1, a key player, in enhancing fast skeletal muscle atrophy. BMC Biol 11:12.

Welle S, Burgess K, Mehta S. (2009). Stimulation of skeletal muscle myofibrillar protein synthesis, p70 S6 kinase phosphorylation, and ribosomal protein S6 phosphorylation by inhibition of myostatin in mature mice. Am J Physiol Endocrinol Metab 296:E567–72.

Wenz T, Rossi SG, Rotundo RL, *et al*. (2009). Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging. Proc Natl Acad Sci USA 106:20405–10.

Wettschureck N, Offermanns S. (2005). Mammalian G proteins and their cell type specific functions. Physiol Rev 85:1159–204.

Winston JT, Koepp DM, Zhu C, *et al*. (1999). A family of mammalian F-box proteins [In Process Citation]. Curr Biol 9:1180–2.

Witt CC, Witt SH, Lerche S, *et al*. (2008). Cooperative control of striated muscle mass and metabolism by MuRF1 and MuRF2. EMBO J 27: 350–60.

Wray CJ, Mammen JM, Hershko DD, Hasselgren PO. (2003). Sepsis upregulates the gene expression of multiple ubiquitin ligases in skeletal muscle. Int J Biochem Cell Biol 35:698–705.

Wu Z, Puigserver P, Andersson U, *et al*. (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98:115–24.

Xu X, Sarikas A, Dias-Santagata DC, *et al*. (2008). The CUL7 E3 ubiquitin ligase targets insulin receptor substrate 1 for ubiquitin-dependent degradation. Mol Cell 30:403–14.

Yi J-S, Park JS, Ham Y-M, *et al*. (2013). MG53-induced IRS-1 ubiquitination negatively regulates skeletal myogenesis and insulin signalling. Nat Commun 4:Art no. 2354.

Zhande R, Mitchell JJ, Wu J, Sun XJ. (2002). Molecular mechanism of insulin-induced degradation of insulin receptor substrate 1. Mol Cell Biol 22:1016–26.

Zhao J, Brault JJ, Schild A, *et al*. (2007). FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. Cell Metabol 6:472–83.

Zhao W, Qin W, Pan J, *et al*. (2009). Dependence of dexamethasone-induced Akt/FOXO1 signaling, upregulation of MAFbx, and protein catabolism upon the glucocorticoid receptor. Biochem Biophys Res Commun 378:668–72.