REVIEW / SYNTHÈSE

mTORC1 and the regulation of skeletal muscle anabolism and mass

Olasunkanmi A.J. Adegoke, Abdikarim Abdullahi, and Pegah Tavajohi-Fini

Abstract: The mass and integrity of skeletal muscle is vital to whole-body substrate metabolism and health. Indeed, defects in muscle metabolism and functions underlie or exacerbate diseases like diabetes, rheumatoid arthritis, and cancer. Physical activity and nutrition are the 2 most important environmental factors that can affect muscle health. At the molecular level, the mammalian target of rapamycin complex 1 (mTORC1) is a critical signalling complex that regulates muscle mass. In response to nutrition and resistance exercise, increased muscle mass and activation of mTORC1 occur in parallel. In this review, we summarize recent findings on mTORC1 and its regulation in skeletal muscle in response to resistance exercise, alone or in combination with intake of protein or amino acids. Because increased activity of the complex is implicated in the development of muscle insulin resistance, obesity, and some cancers (e.g., ovarian, breast), drugs that target mTORC1 are being developed or are in clinical trials. However, various cancers are associated with extensive muscle wasting, due in part to tumour burden and malnutrition. This muscle wasting may also be a side effect of anticancer drugs. Because loss of muscle mass is associated not only with metabolic abnormalities but also dose limiting toxicity, we review the possible implications for skeletal muscle of long-term inhibition of mTORC1, especially in muscle wasting conditions.

Key words: mTORC1, protein synthesis, proteolysis, resistance exercise.

Résumé : La masse et l'intégrité du muscle squelettique sont essentielles à la santé et au métabolisme des substrats dans l'organisme en entier. De fait, des troubles du métabolisme et des fonctions des muscles sous-tendent ou aggravent des maladies comme le diabète, la polyarthrite rhumatoïde et le cancer. L'activité physique et l'alimentation sont les deux plus importants facteurs environnementaux ayant un impact sur la santé des muscles. Au niveau moléculaire, le complexe 1 de la cible de la rapamycine chez les mammifères (mTORC1) est un complexe de signalisation essentielle à la régulation de la masse musculaire. En réponse à l'alimentation et aux exercices contre résistance, on observe en parallèle l'augmentation de la masse musculaire et l'activation de mTORC1. Dans cette analyse documentaire, on présente les dernières observations au sujet du mTORC1 et de sa régulation dans le muscle squelettique en réponse aux exercices contre résistance isolément ou en combinaison avec l'apport d'acides aminés ou de protéines. En raison de l'augmentation de l'activité de ce complexe dans le développement de l'insulinorésistance musculaire, de l'obésité et de cancers (ex., ovaire, sein), des chercheurs travaillent à la conception de médicaments ciblant le mTORC1 ou les testent dans des essais cliniques. Cependant, divers cancers sont associés à l'amyotrophie généralisée, et ce, partiellement à cause de la charge tumorale et de la malnutrition. Cette amyotrophie est peut-être un effet secondaire des médicaments anticancéreux. Comme la perte de masse musculaire est non seulement associée à des troubles du métabolisme, mais présente aussi une toxicité dose-limitante, on analyse les implications probables du muscle squelettique dans l'inhibition à long terme du mTORC1, particulièrement en présence d'amyotrophie.

Mots-clés : mTORC1, synthèse protéique, protéolyse, exercice contre résistance.

[Traduit par la Rédaction]

Introduction

The mass and integrity of skeletal muscle is an important determinant of whole-body health. Defects in skeletal muscle growth and metabolism underlie or exacerbate diabetes (Ferrucci and Studenski 2009), congestive heart failure (Mafra et al. 2008), rheumatoid arthritis (Roubenoff 2009), chronic kidney diseases (Mafra et al. 2008), peripheral artery disease (Mafra et al. 2008), cancer, and HIV (Little and Phillips 2009). Skeletal muscle plays major roles in whole-body disposal of glucose (DeFronzo and Tripathy 2009), amino acids (Marliss and Gougeon 2002), and fatty acids (Franklin and

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Kanaley 2009), the accumulation of which can worsen the metabolic state (Marliss and Gougeon 2002). In diverse diseases, muscle mass is a predictor of disease prognosis, length of hospital stay, and treatment outcomes (Marliss and Gougeon 2002; Pilz et al. 2006).

Two important factors that regulate muscle mass and function are nutrition and physical activity. Compared with other macronutrients, proteins and branched-chain amino acids have pronounced effects in stimulating muscle protein anabolism (Kimball and Jefferson 2010; Little and Phillips 2009). The anabolic effects of resistance exercise on muscle protein synthesis and mass are also well documented (reviewed in Little and Phillips 2009). At the molecular level, the nutrient or energy sensor mammalian target of rapamycin complex 1 (mTORC1) appears to mediate the anabolic effect of amino acids and resistance exercise (Tanti and Jager 2009; Wackerhage and Ratkevicius 2008). In this review, we first summarize recent findings on mTORC1 and its regulation. We then discuss recent data on the role of the complex on skeletal muscle anabolism induced by resistance exercise, alone or in combination with the consumption of proteins or amino acids. Elevated mTORC1 activity, as reflected in increased ribosomal protein S6 kinase 1 (S6K1) phosphorylation and decreased abundance of eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1), is implicated in muscle insulin resistance, obesity (Tremblay et al. 2007a), and some cancers (e.g., ovarian, breast, and kidney) (Dowling et al. 2010a; Guertin and Sabatini 2009). As a result, inhibitors of the complex are in use or being proposed to treat those conditions. Since mTORC1 is required for muscle growth, we discuss the possible implication of long-term use of these inhibitors. Although mTORC1 is also implicated in regulating lipogenesis (Laplante and Sabatini 2009b), the focus of this review is on mTORC1 and mRNA translation and muscle mass.

mTOR complexes

Much of our understanding of the composition and mechanisms of regulation of mTORC1 action (Fig. 1) comes from studies with non-muscle cells. Although recent findings still rely on these systems, the fact that earlier discoveries made in other cell types and tissues have been confirmed in muscle suggests that the recent discoveries too likely cut across many cell types.

mTOR is a conserved serine threonine kinase that nucleates at least 2 distinct complexes, mTORC1 and 2 (Laplante and Sabatini 2009a). The 2 complexes differ in subunit composition, mode of regulation, substrate specificity, and cellular functions, although there is some evidence of cross-talk between them (Dibble et al. 2009). mTORC1 exists as a homodimer and contains mTOR, the substrate adaptor regulatory associated protein of mTOR (Raptor), mammalian lethal with Sec 13 protein 8 (mLST8, also known as GβL), proline-rich AKT substrate 40kDA (PRAS40), and DEP-domain-containing mTOR-interacting protein (Deptor) (Fig. 1). On the other hand, mTORC2 contains, in addition to mTOR, the adaptor protein rapamycin insensitive companion of mTOR (Rictor), mammalian stress-activated protein kinase interacting protein (mSin1), protein observed with Rictor-1 (Proctor-1), mLST8, and Deptor. Unlike the other subunits

of the complexes, PRAS40 (Laplante and Sabatini 2009a) and Deptor (Peterson et al. 2009) are negative regulators of the 2 mTOR complexes.

A distinguishing feature of the complexes is that while mTORC1 is sensitive to the immunosuppressant drug rapamycin, mTORC2 in general is not (Dowling et al. 2010a). Furthermore, mice with muscle specific knockout of Raptor (i.e., mTORC1), but not of Rictor (mTORC2), have reduced muscle mass (Bentzinger et al. 2008). In addition, overexpression of Ras homologue enriched in brain (Rheb, an mTORC1 activator, see below) in mouse tibialis anterior muscle activates mTORC1, but not mTORC2, and increases cap-dependent mRNA translation and fibre cross sectional area (Goodman et al. 2010). These results indicate that mTORC1 plays a dominant role in regulating muscle metabolism.

Recent findings have blurred the demarcation between the 2 complexes. Although mTORC2 is described as rapamycin insensitive, prolonged incubation with the drug can suppress mTORC2 assembly and function (Sarbassov et al. 2006). A number of interacting partners of mTOR, including Tel2 interacting protein 1 (Tti1) (Kaizuka et al. 2010) and Ras-related C3 botulinum toxin substrate 1 (Rac 1) (Saci et al. 2011), positively regulate the functions of both mTORC1 and 2. Furthermore, contrary to initial reports, there is evidence that mTORC2, like mTORC1, is activated by amino acids (Tato et al. 2011), and that it can associate with ribosomes to enhance translation (Oh et al. 2010). Moreover, targeting of mTOR to intracellular membranes by insulin or amino acid stimulation activates not only mTORC1 but also mTORC2 (Saci et al. 2011). Thus, mTORC2 likely plays a role in many functions typically attributed to mTORC1.
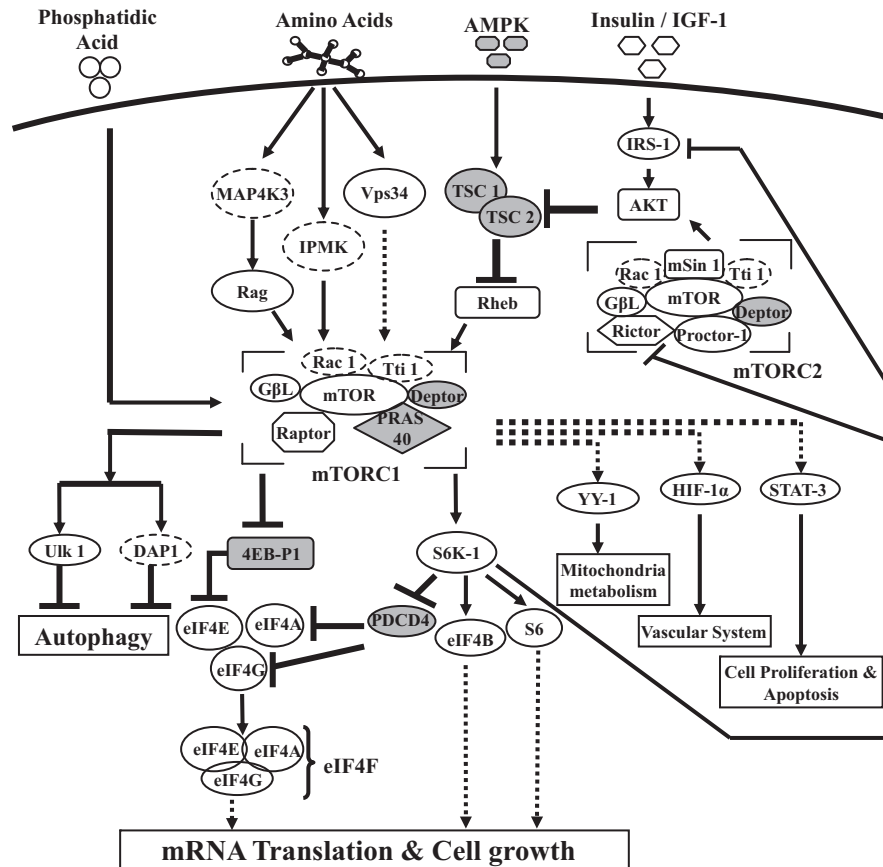
Regulation of mTORC1 signalling

Although the activity of mTORC1 can be regulated by DNA damage, energy (ATP), and oxygen levels (Sengupta et al. 2010; Wang and Proud 2011), it is the regulation of the complex by amino acids and by growth factors (insulin–IGF-1) that has been the most actively studied.

Activation by insulin or IGF-1

The binding of insulin or IGF-1 to its receptors ultimately leads to the activation of AKT (also called protein kinase B, PKB) pathway. Activated AKT stimulates mTORC1 by phosphorylating the tuberous sclerosis complex (TSC1/2). This phosphorylation prevents TSC1/2 from inhibiting mTORC1 activator Rheb (Laplante and Sabatini 2009a). The second messenger cyclic AMP (cAMP) also activates mTORC1 by promoting interaction of Rheb with mTOR (Kim et al. 2010). When bound to GTP, Rheb activates mTORC1 by ill-defined mechanisms. AKT activates mTORC1 also by phosphorylating PRAS40, which prevents the inhibitory binding of the latter to mTOR (Sengupta et al. 2010). In addition to this canonical PI3K–AKT-dependent growth factor activation of mTORC1, Rac1 is able to activate both mTORC1 and mTORC2 independently of PI3K (Saci et al. 2011). Finally, the RAS or mitogen activated protein kinase (MAPK) has also been implicated in activating mTORC1. This occurs via extracellular signal regulated kinase (ERK1/2)-mediated phosphorylation of Raptor on serine 8, 696, and 863. Raptor

Fig. 1. The 2 mTOR complexes. Dashed arrows indicate pathways for which mechanisms of actions are not clear. Components in shapes with solid lines are those for which several lines of evidence exist or whose activities have been demonstrated in skeletal muscle or muscle cells; for those with dashed lines, significance in muscle has not been demonstrated. Components that inhibit mTORC1 or mRNA translation are shown with grey background. Other activators or substrates of mTORC1 are discussed in the text. 4E-BP1, eIF4E binding protein 1; DAP1, death-associated protein 1; Deptor, DEP-domain-containing mTOR-interacting protein; eIF4A, eukaryotic translation initiation factor 4A; eIF4B, eukaryotic translation initiation factor 4B; eIF4E, eukaryotic translation initiation factor 4E; eIF4F, eukaryotic translation initiation factor 4F; eIF4G, eukaryotic translation initiation factor 4G; GßL, G-protein β-subunit-like protein; HIF-1α, hypoxia inducible factor 1α; IPMK, inositol polyphosphate multikinase; IRS-1, insulin receptor substrate 1; MAP4K3, mitogen-activated protein 4-kinase 3; mSin1, mammalian stress-activated protein kinase interacting protein; PDCD4, programmed cell death protein 4; PRAS 40, proline-rich PKB substrate 40 kDa; Proctor-1, protein observed with Rictor-1; Rac 1, ras-related C3 botulinum toxin substrate 1; Rag, recombination activation gene; Raptor, regulatory associated protein of mTOR protein; Rheb, Ras homologue enriched in the brain; Rictor, rapamycin insensitive companion of mTOR; S6, ribosomal protein S6; S6K1, S6 kinase 1; STAT-3, signal transducer and activator transcription 3; TSC, tuberous sclerosis complex; Tti 1, Tel2 interacting protein 1; Ulk 1, unc-51-like kinase 1; Vps34, vacuolar protein sorting 34; YY-1, yin yang protein 1.

mutated at these sites is unable to signal to mTORC1 substrates 4E-BP1 and S6K1 (Carriere et al. 2011). Therefore, insulin can activate mTORC1 via multiple pathways.

Activation by amino acids

Signals from amino acids, especially leucine and arginine (Hara et al. 1998), to mTORC1 are relayed via 6 different mechanisms. Entry of amino acids triggers an increase in intracellular Ca^{2+}, leading to increased binding of calmodulin to a class III PI3K family member human vacuolar protein sorting 34 (hVps34). As a result, there is an increase in phosphatidylinositol 3-phosphate (PI3P) level, which then activates mTORC1 via unknown mechanisms (Gulati et al. 2008). Second, amino acids induce the binding of mTORC1 to RAG proteins, a family of small guanosine triphosphatases (GTPases) (reviewed in Sengupta et al. 2010). RAG proteins target mTORC1 to lysosomal membranes via a heterotrimeric

protein complex termed the Ragulator. Since mTORC1 activator Rheb is also located on this membrane, the complex is thus activated. Third, in studies utilizing non-muscle cells, amino acid and growth factor withdrawal increases intracellular pH from 7.0 to 7.7 and causes mTORC1 to be associated with predominantly perinuclear lysosomes. The addition of nutrients lowers the pH and restores mTORC1 to peripheral (including plasma) membranes. Significantly, changing intracellular pH in the absence of nutritional manipulations reversibly modifies mTORC1 association with peripheral membranes and mTORC1 activity (Korolchuk et al. 2011), suggesting that growth factor and amino acids may regulate mTORC1 by modifying intracellular pH. Fourth, amino acids induce the phosphorylation and activation of MAP4K3, and this is necessary for amino acid signalling to mTORC1 in human embryonic kidney cells (Yan et al. 2010). Fifth, in mouse embryonic fibroblasts, the lipid kinase inositol poly-

phosphate multikinase (IPMK) can mediate amino acid signalling to mTORC1 by stabilizing the interaction between Raptor and mTOR (Kim et al. 2011). Cells depleted of IPMK have impaired amino acid-induced signalling to mTORC1. Finally, Ra1A, a member of the Ras superfamily, can mediate amino acid signalling to mTORC1 (Maehama et al. 2008). Hela cells depleted of Ra1A are defective in phosphorylating S6K1 and 4E-BP1 in response to amino acids. Thus, the effects of amino acids can be mediated by multiple mechanisms.

There is some evidence that the 6 proposed mechanisms are linked. The hVps34, Rag–Ragulator, and Ra1A pathways of mTORC1 activation by amino acids are Rheb-dependent. Also, hpVs34 and Rag–Ragulator pathways involve the localization of mTOR to an endomembrane compartment. Finally, MAP4K3 signalling appears dependent on the RAG proteins as depletion of the later inhibits MAP4K3 signalling to mTORC1 (Yan et al. 2010). Since many of these findings were made in non-muscle cells or tissues, the significance of these mechanisms in skeletal muscle metabolism remains unclear. In studies with piglets, higher skeletal muscle protein synthesis is associated with a greater abundance of RagB and an increased binding of RagB with Raptor; however, neither insulin nor infusion of amino acids has an effect on RagB nor on its interaction with Raptor (Suryawan and Davis 2010), suggesting that the changes seen in this RAG protein may not mediate the effect of amino acid and insulin on muscle protein synthesis. In another study, knock-down of Deptor in myotubes increases the phosphorylation of 4E-BP1 and S6K1 as well as myotubes protein synthesis (Kazi et al. 2011).

Consequences of mTORC1 activation

Whether induced by amino acids or growth factors, activated mTORC1 promotes diverse cellular outcomes, including stimulation of mRNA translation, ribosome biogenesis, and inhibition of apoptosis (Laplante and Sabatini 2009a). This leads to increased cell size and cell number. Those outcomes result from the action of mTORC1 on its substrates, especially 4E-BP1 and S6K1 (Laplante and Sabatini 2009a; Ma and Blenis 2009). Other substrates of mTORC1 are involved in regulating the transcription of genes required for the production of ribosomes. mTORC1 is required for inactivating MAF1, a protein that represses RNA polymerase III in response to serum starvation. RNA polymerase III is needed for the transcription of genes involved in ribosome production. mTORC1 represses MAF1 by phosphorylating it on multiple serine residues, especially serine 75, within the nucleus (Kantidakis et al. 2010; Michels et al. 2010; Shor et al. 2010). Phosphorylation of MAF1 (Shor et al. 2010) and recruitment of mTORC1 to gene promoter regions (Shor et al. 2010; Tsang et al. 2010) are sensitive to both growth factor and amino acid stimulation. Finally, mTORC1 has also been implicated in regulating micro RNA (miRNA). This class of small RNAs regulates gene expression by binding to the 3′ end of candidate mRNA, thereby inhibiting translation and (or) destabilizing the target mRNA (Braun and Gautel 2011). Among them, miR-1 is a muscle-specific miRNA implicated in regulating myogenesis. mTORC1 regulates the transcrip-

tion of this miRNA in myotubes, as treatment with rapamycin reduces its abundance (Sun et al. 2010).

mTORC1 regulates autophagy in human embryonic kidney and Hela cells by phosphorylating, thereby inactivating, the autophagy inhibitor death-associated protein 1 (DAP1) (Koren et al. 2010). Although this appears inconsistent with the anti-autophagy functions of mTORC1, the activation of DAP1 in the absence of mTORC1 is consistent with the cell trying to limit autophagy in times of limited energy or nutrient abundance. Another autophagy related substrate of mTORC1 is Unc-51-like kinase 1 (Ulk1). When nutrients abound, mTORC1 phosphorylates Ulk1 at multiple serine residues, especially serine 638 and 758. However, within minutes of nutrient withdrawal, Ulk1 is dephosphorylated, allowing it to activate autophagy in mouse fibroblasts (Shang et al. 2011). While inhibition of mTORC1 during starvation permits autophagy in kidney cells, the functions of the complex are necessary to restore lysosomal homeostasis (i.e., regeneration of free lysosomes to a pre-autophagy level), even when starvation continues (Yu et al. 2010). The reactivation of mTORC1 following sustained (>4 h) starvation is autophagy-dependent, and results from the breakdown products of autophagy reactivating the complex. In summary, while inactivation of mTORC1 promotes autophagy, products of this cellular degradative pathway help to reactivate mTORC1, thereby limiting autophagy and restoring homeostasis.

Finally, mTORC1 controls the mRNA expression of yin-yang 1 (YY1), a transcriptional factor that regulates mitochondrial gene expression and oxidative functions (Cunningham et al. 2007). Other putative substrates of this complex are hypoxia inducible factor 1 alpha (HIF-1α), serum- and glucocorticoid-induced protein kinase 1 (SGK1), and signal transducer and activator of transcription 3 (STAT3) (Laplante and Sabatini 2009a). Except for YY1, whose regulation by mTORC1 was first described in muscle cells (Cunningham et al. 2007), and HIF-1α, which has been shown to inhibit IGF-1-induced myogenesis (Ren et al. 2010), the regulation of these other substrates of mTORC1 in skeletal muscle is yet to be described.

The best characterized functions of mTORC1 in skeletal muscle, however, are those mediated by the actions of the complex on 4E-BP1 and S6K1 (Ma and Blenis 2009). By phosphorylating 4E-BP1, mTORC1 relieves inhibition of eIF4E by 4E-BP1, thus favoring formation of eIF4F complex and mRNA translation. Hypertrophy induced by mTORC1 appears dependent on S6K1, while 4E-BPs mediate the effect on cell proliferation (Dowling et al. 2010b; Risson et al. 2009). The exact mechanisms by which phosphorylation of S6K1 leads to increased translation are not clear but may include phosphorylation of eIF4B, an event that promotes the helicase activity of eIF4A (Holz et al. 2005); phosphorylation of ribosomal protein S6, an event that is thought to promote translation of a specific class of mRNAs (Jefferies et al. 1997); and phosphorylation of the mRNA translation inhibitor programmed cell death 4 (PDCD4) (reviewed in Laplante and Sabatini 2009a). Much of what is known about PDCD4 is from non-muscle cells; however, PDCD4 protein accumulates in rat skeletal muscle during starvation. Its level decreases as animals are re-fed (Zargar et al. 2011). Collectively, these studies implicate effectors of mTORC1 in

the activation of muscle protein synthesis, although details of their mechanisms still need to be elucidated.

mTORC1 and skeletal muscle intracellular proteolysis

A change in muscle protein mass is the net result of the regulation of 2 opposing ongoing processes, protein synthesis and proteolysis. Intracellular proteolysis in skeletal muscle proceeds by (*i*) ubiquitin–ATP–proteasome-dependent pathway (ubiquitin system), (*ii*) autophagy–lysosomal pathway, (*iii*) Ca^{2+}-dependent proteases, and (*iv*) apoptosis. Of these, the ubiquitin system is the most studied. In this pathway, protein substrates are first conjugated to ubiquitin, a process that requires the sequential activities of ubiquitin activating enzymes (E1), ubiquitin conjugating enzymes (E2), and ubiquitin protein ligases (E3) (Ciechanover 2005; Hershko 2005). The polyubiquitinated substrate is then recognized and degraded by the 26S proteasome complex. Increases in expression (mRNA, protein) of E2, E3, subunits of the proteasome, and in the amount of ubiquitinated proteins correlate with increased proteolysis, and vice versa (Jagoe and Goldberg 2001).

The importance of the ubiquitin system in regulating skeletal muscle proteolysis is underlined by the presence of muscle specific ubiquitin protein ligases Muscle Atrophy F-box (MAFbx, also called Atrogin-1) and Muscle RING Finger 1 (MuRF1) (reviewed in Glass 2005). In muscle cells and in atrophying skeletal muscle, MuRF1 ubiquitinates myofibrillar proteins and targets them for degradation (Cohen et al. 2009). Furthermore, muscle proteolysis in diverse catabolic conditions are attenuated when the ubiquitin system is blocked (Jagoe and Goldberg 2001).

Increased activities of cathepsins (lysosomal enzymes) and autophagy (reviewed in Mammucari et al. 2008), and of m and k calpains (Ca^{2+} dependent system) (Ventadour and Attaix 2006) are also observed in catabolic states. In addition, increased apoptosis may contribute to reduced muscle mass (Ferreira et al. 2008).

An important question is whether mTORC1 regulates muscle proteolysis. Much of the evidence implicating the complex relates to the ability of activated mTORC1 to suppress autophagy (Jung et al. 2010). Although lysosomal proteolysis–autophagy is important in mediating increased muscle proteolysis seen in starvation, denervation, and glucocorticoid administration in rodents (Ogata et al. 2010; Schakman et al. 2009), activation of autophagy under those conditions is only minimally rapamycin-sensitive (Mammucari et al. 2008). Few studies have examined the role of mTORC1 in regulating the ubiquitin system. In a study with C2C12 myotubes, leucine, isoleucine, and arginine suppress the expression of Atrogin-1 and MuRF1. The suppression of Atrogin-1, but not of MuRF1, is attenuated in the presence of rapamycin (Herningtyas et al. 2008). Mice heterozygous for mTOR have reduced lean mass along with decreased muscle protein synthesis and increased mRNA expression of Atrogin-1 and MuRF1 (Lang et al. 2010). Clearly, more data are needed to ascertain whether mTORC1 is required for the regulation of skeletal muscle proteolysis.

mTORC1 and the anabolic response to resistance exercise

This will be discussed under 3 questions: (*i*) Are mTORC1 activators and (or) downstream effectors modulated during resistance exercise-induced skeletal muscle anabolism? (*ii*) What happens to exercise-induced anabolism when these regulators or effectors are inhibited, knocked-down, or knocked-out? (*iii*) Does inhibition of mTORC1 or knock-down or knockout of mTOR abolish resistance-exercise induced skeletal muscle anabolism? In the discussion that follows, although studies with human subjects are reviewed, much of our understanding of the roles of mTORC1 in exercise-induced muscle hypertrophy comes from rodent and cell culture studies. Except through the use of mTORC1 inhibitors, examining the requirement of mTORC1 for a specific phenotype often requires genetic approaches, studies that are not feasible in humans. While rodent and cell culture models cannot mimic what goes on in humans, they nevertheless can generate hypothesis that can be tested in human studies.

Resistance exercise and upstream activators of mTORC1

Functional overload-induced hypertrophy in rodent muscles occurs in parallel with increased phosphorylation of serine 2448 of mTOR (Reynolds et al. 2002) and serine 473 of AKT (Spangenburg et al. 2008). Resistance exercise increases human muscle protein synthesis at 1–2 h postexercise in parallel with elevated AKT phosphorylation (Dreyer et al. 2008). However, electrical stimulation in rat EDL induces phosphorylation of the mTORC1 substrate p70S6K1 and this regulation is insensitive to Wortmannin, the inhibitor of PI3K-AKT pathway (O'Neil et al. 2009). Thus, while resistance activity increases AKT activation, it is not clear whether this is needed for mTORC1 activation.

Phospholipase D (PLD) and its product, phosphatidic acid (PA), are upstream positive regulators of insulin-activated signalling to mTORC1 (Fang et al. 2001). Mechanically stimulated rodent muscles activate PLD and accumulate PA at various points after initiation of stimulation (Hornberger et al. 2006; O'Neil et al. 2009). Inhibition of PLD with 1-butanol or neomycin blocks PA accumulation and abrogates mechanically induced mTORC1 signalling (Hornberger et al. 2006; O'Neil et al. 2009). Because protein synthesis was not determined in the cited studies, it remains to be seen whether inhibition of PA production during resistance activity also blocks exercise-induced stimulation of protein synthesis and muscle hypertrophy.

Another exercise-activated upstream (negative) regulator of mTORC1 is AMP-activated protein kinase (AMPK). In a human study, AMPKα2 activity was increased while protein synthesis and mTOR phosphorylation were suppressed in vastus lateral muscle immediately after resistance exercise. However, 1 h postexercise, at which time protein synthesis and mTOR phosphorylation were elevated, AMPK activity remained elevated (Dreyer et al. 2006). Another study with resistance exercise in recreationally active men did not show regulation of this kinase (Hulmi et al. 2009). Nevertheless, the involvement of AMPK in exercise-induced muscle anabolism, and that such an effect is at least in part related to mTORC1, can be inferred from recent studies that show that

myotubes deficient in AMPK (Lantier et al. 2010) or muscle from mice lacking AMPK (Mounier et al. 2009) are bigger in size. The increased size of myotubes deficient in AMPK is attenuated in the presence of rapamycin. Further activation of AKT, and therefore of mTORC1, confers no further anabolism than that imposed by AMPK knockout or knock-down (Lantier et al. 2010). In the mouse study, in addition to the bigger size of muscles from mice lacking AMPK, resistance activity-induced muscle anabolism was about 20% greater in muscles from AMPK knock-out animals, and this occured in parallel with greater activation of mTORC1 (Mounier et al. 2009).

Hypoxia inducible protein REDD1 or RTP801 is another negative regulator of mTORC1 (Brugarolas et al. 2004). Although low-intensity resistance exercise increases human muscle REDD1 (Drummond et al. 2008a), the significance of this protein in mediating muscle hypertrophy in response to resistance exercise remains to be demonstrated.

Resistance exercise and downstream effectors of mTORC1

During resistance exercise in humans, there is increased phosphorylation of S6K1, 4E-BP1, and mTOR, sometimes along with enhancement of muscle protein synthesis and fiber size (Tannerstedt et al. 2009; Witard et al. 2009). Phosphorylation of these proteins peak at 30–60 min of recovery from leg extension at 80% 1 repetition maximum (Camera et al. 2010), which is likely too soon for changes in protein synthesis to be observed. In rat studies, although the observed effects are greater in muscles composed of type II fibres (Parkington et al. 2003), muscles that consist of predominantly oxidative fibres also show a response (Agata et al. 2009; Miyazaki et al. 2008).

In a study comparing young and old (28 vs. 64 years) human subjects, acute and chronic resistance exercises increased muscle protein synthesis in parallel with increased phosphorylation of AKT, mTOR, S6 (but not S6K1), and 4E-BP1 (Mayhew et al. 2009). Resistance exercise stimulated the synthesis of both myofibrillar and sarcoplasmic proteins by ~68% in untrained individuals but stimulated the synthesis of only myofibrillar proteins by 36% after training. In contrast, endurance exercise stimulated only the synthesis of mitochondrial proteins irrespective of training status. Phosphorylation of components of the mTORC1 signalling pathway was also observed, but type of training had no effect on this parameter (Wilkinson et al. 2008).

Resistance exercise-induced increases in human muscle protein synthesis and regulation of mTORC1 and its substrates is enhanced with consumption of whey proteins (Hulmi et al. 2009), or a solution that contains leucine-enriched essential amino acids and carbohydrate (Dreyer et al. 2008), or a diet that contains casein protein and carbohydrate (Glover et al. 2008). In another study, rates of protein synthesis in muscle at different times after resistance exercise and amino acid ingestion were compared in young and old human subjects (Drummond et al. 2008b). Exercise had no effect 1 h postexercise. However, exercise combined with amino acid ingestion stimulated protein synthesis only in the young individuals 3 h after exercise; by 6 h, stimulation of protein synthesis was similar in both groups. In that study, treatment-induced increases in phosphorylation of

mTOR, S6K1, and 4E-BP1 did not correlate with changes in protein synthesis. Thus, whereas changes in muscle protein synthesis and signalling proteins often occur in concert, it is unclear if there is a causative link.

Is mTORC1 required for resistance exercise-induced skeletal muscle anabolism?

Studies that examined this question used 2 approaches: (i) use of agents that inhibit mTOR or mTORC1, and (ii) employing whole animal or tissue-specific knockout of mTOR or mTORC1 components. For the inhibition studies, the most popular drug is rapamycin, an allosteric inhibitor of mTOR. Use of rapamycin has shown that activation of mTORC1 is needed for resistance-exercise induced stimulation of rat muscle protein synthesis (Fluckey et al. 2006; Kubica et al. 2005). In line with these findings, 2-week repetitive stretching suppresses atrophy in rat denervated soleus muscle and increases S6K1 phosphorylation (Agata et al. 2009); both effects are blocked by rapamycin. Furthermore, passive cyclic stretching in cell culture induces hypertrophy, which is attenuated when the myotubes are pre-treated with Wortmannin or rapamycin (Sasai et al. 2010). Interestingly, greater suppression is observed with the mTORC1 inhibitor (rapamycin) compared with the inhibitor of PI3K. Although protein synthesis was not measured in those 2 studies, the data implicate mTORC1 as a critical signalling node in the contraction-induced hypertrophy observed.

Muscle or muscle cell size is smaller in the absence of S6K1 (Ohanna et al. 2005) and in muscle-specific Raptor knockout (Bentzinger et al. 2008), which is consistent with data from the rapamycin studies. In addition, muscle-specific knockout of mTOR in mice leads to muscle atrophy, especially in the fast-twitch muscles, in which muscle weights are about 20% smaller compared with the control. The decrease in muscle weights fully accounts for the reduction in body weight in knockout animals. The reduction in muscle weight is also associated with reduced fibre cross-sectional area (Risson et al. 2009). Interestingly, the severity of muscle specific mTOR knockout is greater than that observed in Raptor knockout or in Raptor and Rictor double knockout animals, suggesting additional contributions by mTOR to muscle metabolism beyond those mediated by mTORC1 and 2. Regarding contractile functions, force production is reduced in muscles lacking mTOR, an observation not made in muscle-specific Raptor knockout. Clearly then, mTOR or mTORC1 is important in regulating muscle mass and function. However, except for the examination of response to electrical stimulation, we are unaware of any study that examines changes in muscle mass and metabolism in response to resistance exercise in animals lacking mTOR or other mTORC1 components or substrates.

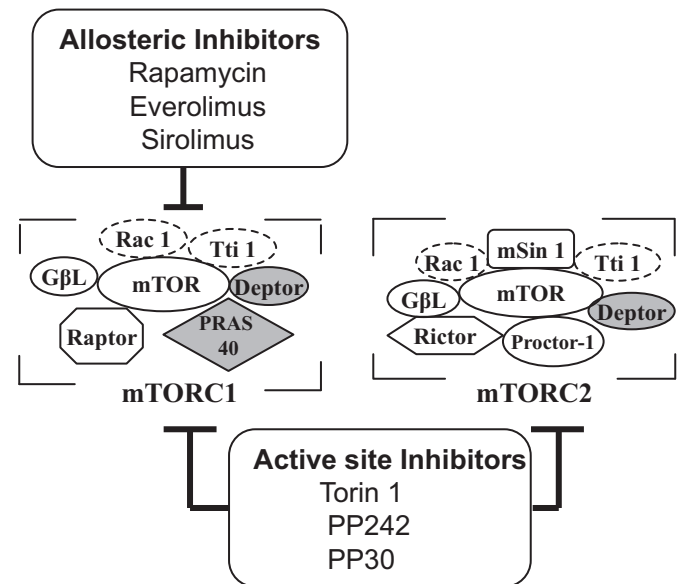## mTORC1 and the regulation of intracellular proteolysis in response to resistance exercise

Consistent with earlier reports (Glass 2005), during 4–8 days of rat leg immobilization, increased activities of the ubiquitin system and apoptosis were recorded, which were reversed as the animals were allowed to move around from days 9 to 40 (Vazeille et al. 2008). The time course of

changes in the component of the ubiquitin system, rather than of apoptosis, mirrored the changes in muscle mass during both immobilization and recovery phases. In another study, during muscle recovery following 14-day hind limb unloading, soleus muscle weight and fibre size were restored by day 5 of recovery. In that study, mRNA levels of m-calpain, atrogin-1 or MAFbx, and MuRF1, which were elevated 50%–70% during unweighting, returned to baseline by 1 day of recovery (Andrianjafiniony et al. 2010). Results from human studies are divided, perhaps because of the different endpoints measured. Some labs did not see substantial changes in markers of proteolysis during leg immobilization (Glover et al. 2010; Phillips et al. 2009). In contrast, but consistent with rodent studies (Bodine et al. 2001; Glass 2005; Reid 2005), another lab showed ~400% increase in 3-methyl histidine release (a measure of myofibrillar proteolysis) and in Atrogin-1 and MuRF1 mRNA in vastus lateral muscle following 72 h of lower limb suspension (Gustafsson et al. 2010; Tesch et al. 2008). Because of lack of agreement amongst data from different laboratories, more studies are needed to ascertain the contribution of altered proteolysis to human protein anabolism during muscle re-loading following a period of unloading. In addition, studies that employ inhibitors of mTORC1 will be needed to ascertain the significance of this pathway in regulating proteolysis in response to unloading and resistance exercise.

mTORC1 inhibitors and skeletal muscle metabolism

In spite of its significance in regulating muscle growth, overactivation of mTORC1 is implicated in human and rodent models of obesity, in muscle insulin resistance (Newgard et al. 2009; Tremblay et al. 2007b), and in cancer (Guertin and Sabatini 2009). The link to insulin resistance is because activated mTORC1–S6K1 phosphorylates insulin receptor substrate 1 (IRS-1) on multiple serine residues, an event that destabilizes IRS-1 and negatively impinges on its signalling to PI3K. In addition, the growth factor receptor-bound protein 10 (Grb-10) is phosphorylated by activated mTORC1, and phosphorylated Grb-10 mediates inhibition of PI3K and ERK-MAPK pathways (Hsu et al. 2011; Yu et al. 2011). Human cancers, including ovarian, breast, and kidney, are associated with aberrant activation of PI3K–mTORC1 pathway (Guertin and Sabatini 2009). Several inhibitors of mTORC1 are either in clinical trials or are approved for treatment of mainly cancers. These inhibitors include rapamycin and its derivatives (Fig. 2): sirolimus, everolimus, and AP-23573 (Dancey 2005). While some beneficial anti-tumour effects have been described, the efficacy of rapamycin and rapalogs has been less than anticipated (Guertin and Sabatini 2009). This suboptimal performance is attributed to the loss of negative feedback control that activation of mTORC1 confers on the IRS-1–PI3K–AKT pathway. Upon rapamycin treatment, mTORC1 is inhibited and IRS-1 signalling is restored, leading to the activation of IRS-1–PI3K–AKT signalling, a pathway that is implicated in abnormal cell proliferation. To circumvent this, a number of active site inhibitors of mTOR have been developed (Fig. 2). This class targets the kinase activity of mTOR and therefore will inhibit both mTORC1 and 2. These second generation inhibitors, including Torin 1

Fig. 2. Inhibitors of mTOR. The inhibitors fall into 2 classes: allosteric and active site inhibitors. Note that the latter class inhibits the 2 mTOR complexes. Please see text for a complete list of the inhibitors. See Fig. 1 for definitions.

(Thoreen et al. 2009), PP242 and PP30 (Feldman et al. 2009), Ku-0063794 (García-Martínez et al. 2009), AZD8055 (Chresta et al. 2010), and WYE-354 (Yu et al. 2009), inhibit the phosphorylation of substrates of mTORC1 (S6K1 and 4E-BP1) and of mTORC2 (AKT and SGK). They also suppress global protein synthesis in tumour cells with better potency than rapamycin, and WYE-354 inhibits tumour growth in rodents while AZD8055 inhibits growth and (or) promotes regression in xenografts.

For the reasons already mentioned, inhibition of mTORC1 will likely lead to an impairment of the regulation of skeletal muscle mass and metabolism. In this regard, mice overexpressing human TSC1 (a negative regulator of mTORC1) are smaller in size, have TA and EDL muscles that are ~23% smaller, and an 18% reduction in muscle fibre compared with wild-type animals (Wan et al. 2006). This suggests that prolonged mTORC1 inhibition may negatively affect skeletal muscle mass, especially given the fact that muscle protein metabolism is impaired in many cancers.

Extensive skeletal muscle wasting is a feature of cachexia, a condition often seen in cancer patients (Dodson et al. 2011; Prado et al. 2011). Cachexia can be attributed to malnutrition and hypermetabolism (Prado et al. 2011), and elevated circulating levels of catabolic inflammatory factors (Dodson et al. 2011; Eley et al. 2008). In addition, muscle wasting is an undesirable side effect of chemotherapy. For example, renal cancer patients treated with sorafenib, the antiangiogenic multikinase inhibitor that targets PI3K–AKT pathway, lose 5% to 8% of their skeletal muscle mass, compared with patients in the placebo group (Antoun et al. 2010b). Another study by the same group shows that dose-limiting toxicity (DLT) highly correlates with loss of lean mass in patients with metastic renal cancer patients treated with sorafenib, with DLT rate being 28% higher in sarcopenic vs. non-sarcopenic patients (Antoun et al. 2010a). In addition, lean body mass is a good predictor of adverse drug toxicity in cancer patients

treated with diverse chemotherapies (Prado et al. 2007, 2009). Thus, chemotherapy increases loss of lean mass and the resulting sarcopenia increases the risk of drug toxicity in diverse cancer groups treated with different drugs.

Given the established significance of mTORC1 in regulating muscle mass and the frequency with which impairments are observed in muscle protein content and mass in cancer patients, whatever successes that are achieved in reducing tumour burden by the use of mTOR inhibitors will possibly be associated with loss of muscle mass. No patient studies have examined the long-term effect of mTOR inhibitors on muscle metabolism. Given the data from rodent mTORC1 knockout studies, muscle wasting is likely a consequence of long-term use of rapamycin and rapalogs. In this respect, doses of everolimus that inhibit tumour growth in mice models of human tumours cause weight loss (O'Reilly et al. 2011).

Summary

Recent findings on the composition and regulation of mTORC1 have broadened our understanding of the link between the activation of this complex and specific cellular outcomes. However, many of these studies were conducted in non-muscle cells. Recent studies on the effect of resistance exercise suggest a link between the activation of mTORC1 and exercise-induced regulation of muscle protein synthesis. The significance of intracellular proteolysis during muscle loading and unloading is less clear: the role of mTORC1 in this process has received little attention. The few studies that are available suggest that long-term inhibition of mTORC1 may lead to undesirable side effects in skeletal muscle.

Concluding comments

Since some functions of mTORC1 are rapamycin-insensitive (Thoreen et al. 2009), studies using drugs that can inhibit the kinase activity of mTOR are needed to answer questions relating to the essentiality of mTORC1 in regulating muscle anabolic response to resistance exercise. In addition, detailed studies are needed to examine the implication of long-term inhibition of mTOR (in the context of mTORC1 or 2) on muscle mass, as this will have implication for treatment outcome and overall patient health. An alternative might be to target inhibition of mTORC1 to tissues and cells other than skeletal muscle. It will also be useful to examine whether resistance exercise ameliorates muscle wasting associated with chemotherapy.

Data from studies employing genetic approaches suggest that inhibiting mTORC2 may block tumour development without the side effects associated with mTORC1 inhibition. For example, deletion of Rictor has no effect on normal prostate cells, but Rictor is required for transformation induced by PTEN loss (Guertin and Sabatini 2009). If the observations made in prostate cancer hold true in other cancers, and since deletion of Rictor, unlike that of Raptor, has no effect on muscle metabolism (Guertin and Sabatini 2009), inhibiting mTORC2 may represent an attractive intervention that will limit or prevent tumour growth while having no negative effect on muscle metabolism.

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