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REVIEW ARTICLE

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Regulation of mTOR by amino acids and resistance exercise in skeletal muscle

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Abstract Resistance exercise disturbs skeletal muscle homeostasis leading to activation of catabolic and anabolic processes within the muscle cell. A current challenge of exercise biology is to describe the molecular mechanisms of regulation by which contractile activity stimulates net protein breakdown during exercise and net protein synthesis during recovery. Muscle growth is optimized by combining exercise and appropriate nutritional strategies, such as amino acid (AA) and carbohydrate ingestion. The effects are integrated at the level of one central regulatory protein, mTOR (mammalian target of rapamycin). mTOR is a complex protein integrating signals of the energetic status of the cell and environmental stimuli to control protein synthesis, protein breakdown and therefore cell growth. mTOR is known to be activated by insulin, and the mechanisms involved are well documented. The ways by which exercise and AA lead to mTOR activation remain partially unclear. Exercise and AA use different signalling pathways upstream of mTOR. Exercise seems to recruit partially the same pathway as insulin, whereas AA could act more directly on mTOR. During resistance exercise, the activity of mTOR could be acutely blunted by AMP-activated protein kinase (AMPK), thus inhibiting protein synthesis and enhancing AA availability for energy metabolism. During recovery, the inhibition of mTOR by AMPK is suppressed, and its activation is maximized by the presence of AA. There appears to be a requirement for a minimal concentration of plasma insulin to stimulate muscle protein synthesis in response to resistance exercise and AA ingestion.

Keywords Protein synthesis · p70 ribosomal S6 kinase · eukaryotic initiation factor 4E-binding protein · protein kinase B/Akt · AMP-activated protein kinase

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Introduction

Resistance exercise leads to changes in protein turnover in skeletal muscle up to 48 h after the completion of a training session (Phillips et al. 1997). At rest in the fasted state, the net protein balance is negative, because protein breakdown largely exceeds protein synthesis. Following exercise in the fasted state, protein synthesis as well as protein breakdown are enhanced, although the latter is less so, resulting in a less negative net balance (Biolo et al. 1995; Phillips et al. 1997; Tipton and Wolfe 1998). Most athletes involved in resistance training are looking for increased muscle mass. Ingesting a mixture containing carbohydrate (CHO) and amino acids (AA), preferentially before (Tipton et al. 2001) or immediately after (Biolo et al. 1997) completion of the training session, counteracts the catabolic state by increasing AA transport and availability into the muscles (Biolo et al. 1995). In this situation, protein synthesis (Louis et al. 2003; Tipton and Wolfe 1998), but not protein breakdown (Biolo et al. 1995; Phillips et al. 1997), is enhanced, and the net protein balance becomes positive. The effect of AA is enhanced by an elevated insulin concentration subsequent to CHO supplementation (Rasmussen et al. 2000). Nutritional strategies centred around the ingestion of AA are essential to maximize the effect of resistance training and therefore to accumulate muscle mass (Wolfe 2000).

The mechanisms of activation of protein synthesis by AA and exercise are somewhat different. Both use distinct signalling pathways converging at the level of one central protein involved in cell growth called mTOR (mammalian target of rapamycin). mTOR is a 289 kDa serine/threonine kinase composed of 2,459 AA. This large protein has also been called FRAP [FK506-binding protein 12 (FKBP-12) and rapamycin-associated

protein], RAFT1 (rapamycin and FKBP-12 target-1), RAPT1 (rapamycin target-1) or SEP (sirolimus effector protein). mTOR stimulates protein synthesis mainly via three key regulatory proteins: $p70^{s6k}$ (p70 ribosomal S6 kinase), 4E-BP1 (eukaryotic initiation factor 4E-binding protein 1) (Brunn et al. 1997; Burnett et al. 1998) and eIF4G (eukaryotic initiation factor 4G) (Raught et al. 2001). The purpose of this review is to describe the roles and the mechanisms of regulation of the mTOR-mediated signalling by AA and exercise. The first part will describe the roles and the regulation mechanisms of mTOR and its downstream effectors. Next, the role of AA in mTOR activation will be discussed. Finally, the effect of exercise and AA on mTOR signalling will be analyzed. Optimal stimulation of muscle growth is highly dependent on the fine-tuned integration of these two pathways.

Roles of mTOR

Some authors present mTOR as an energetic sensor, although mTOR has a relatively high K_m for ATP (~1 mM) (Dennis et al. 2001). Therefore, a large drop in ATP is needed to induce a substantial effect on mTOR activity, as opposed to AMP-activated protein kinase (AMPK) which is a more sensitive energetic sensor to detect small variations in AMP concentration. A major role of mTOR is to integrate environmental stimuli (nutrient and growth factor levels, mitochondrial signals and exercise) in order to control cellular growth (Fig. 1) (for reviews, see Proud 2002; Raught et al. 2001). Protein synthesis is one of the most highly regulated processes. Cell growth is well organized and occurs at specific moments and in well-defined cellular compartments. To develop, the cell must integrate mitogenic and nutritional signals. One of the most powerful anabolic signals is insulin, which acts, among others, through mTOR-mediated signalling. Once bound to its receptor, insulin activates the intrinsic kinase activity of the receptor leading to its autophosphorylation (Kasuga et al. 1982) and phosphorylation of several substrates, including members of the insulin receptor substrate (IRS) family (White et al. 1985). Phosphorylation of IRS-1 recruits another signalling molecule, the phosphatidylinositol 3-kinase (PI3K) (Backer et al. 1993). One downstream target of PI3K is the serine/threonine protein kinase B (PKB) which activates mTOR directly and indirectly (see TSC1/TSC2 and Rheb) and its downstream effectors involved in the control of protein translation, $p70^{s6k}$, 4E-BP1 and eIF4G (Fig. 2).

mTOR could control other processes such as autophagy (Blommaart et al. 1995; Noda and Ohsumi 1998), cell proliferation (Coolican et al. 1997; Gao et al. 2004; Shah et al. 2001) and cytoskeletal organization (Berven and Crouch 2000; Berven et al. 2004; Qian et al. 2004) (Fig. 1). Although the main focus of this review is on protein translation, it should be noted that mTOR also regulates gene transcription (Mayer et al. 2004), as demonstrated by studies using rapamycin, an inhibitor of mTOR.

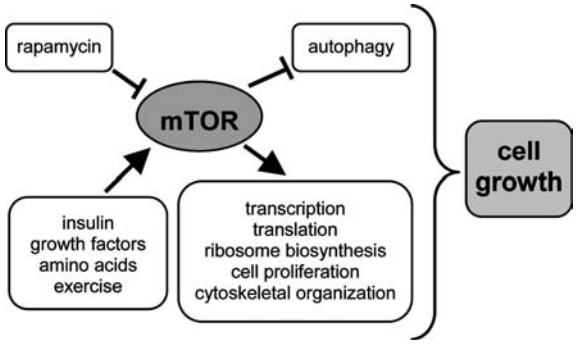

Fig. 1 Roles of *mTOR* (mammalian target of rapamycin). mTOR signalling is activated in the presence of insulin, growth factors, amino acids and during exercise, whereas it is repressed by rapamycin. When activated, mTOR inhibits autophagy, and stimulates transcription, translation of mRNAs coding for components of the translation machinery, ribosome biosynthesis, cell proliferation and cytoskeletal organization to favour cell growth

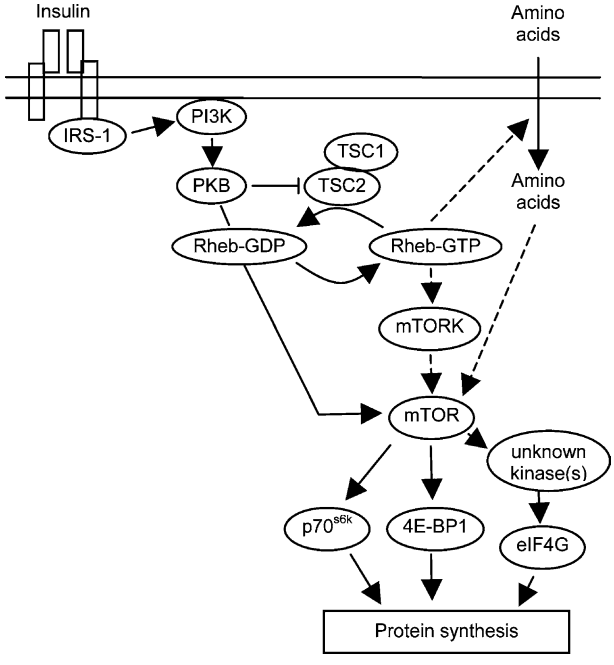

Fig. 2 Inhibition of mTOR by *TSC1*/*TSC2* (tuberous sclerosis complex 1/2). TSC2 is a GTPase-activating protein for the small G protein *Rheb* (Ras homologue enriched in brain). The GAP domain of TSC2 increases the hydrolysis of the GTP form of Rheb, inhibiting the signal to propagate through mTOR. Rheb, under active form (Rheb–GTP), could facilitate the transport of amino acids into the cell, which in turn could activate mTOR. *IRS-1* Insulin receptor substrate 1, *PI3K*, phosphatidylinositol 3-kinase, *PKB* protein kinase B, *mTORK* mammalian target of rapamycin kinase, $p70^{s6k}$ p70 ribosomal S6 kinase, *4E-BP1* eukaryotic initiation factor 4E-binding protein 1

Regulation of mTOR and its downstream targets

In the N-terminal region, mTOR possesses 16 HEAT domains which allow the binding of various regulatory proteins. The activity of mTOR is also influenced by changes in its phosphorylation state. Up to now, three phosphorylation sites have been discovered: Ser 2,481, an autophosphorylation site, Ser 2,448 and Thr 2,446, the roles of which are described in later sections. Once phosphorylated, mTOR can in turn phosphorylate downstream targets. The major proteins regulated by mTOR and playing a role in translation are 4E-BP1, eIF4G, and $p70^{s6k}$ (Raught et al. 2001). The mechanism by which mTOR activates its effectors is not fully elucidated. mTOR could stimulate 4E-BP1, eIF4G, and $p70^{s6k}$ either directly by phosphorylation (Raught et al. 2001), indirectly by activating another protein kinase (Raught et al. 2001), or by inhibiting a phosphatase (e.g. protein phosphatase 2A, PP2A) (Li et al. 2004).

4E-BP1 is an inhibitor of the initiation translation factor eIF4E (eukaryotic initiation factor 4E). When 4E-BP1 is phosphorylated, eIF4E is released and can form the eIF4F complex together with eIF4G, which is also under the control of mTOR (Raught et al. 2001), and eIF4A. The assembly of this complex is necessary for initiation to continue. 4E-BP1 possesses many phosphorylation sites. The phosphorylation of Ser 65 and Thr 70 (and to a lesser extent Thr 37 and 46) is blocked in the presence of rapamycin suggesting an essential role of mTOR in the regulation of 4E-BP1 (Proud 2002).

$p70^{s6k}$ is a member of the S6K family. The S6Ks are a product of two genes, S6K1 and S6K2, $p70^{s6k}$ being encoded by the first one. Up to now 12 sites of phosphorylation have been described for $p70^{s6k}$ (Shah et al. 2000). The Thr 389 is a direct target of mTOR in vitro (Dennis et al. 1999). The phosphorylation of Thr 389 by mTOR allows subsequent phosphorylation of Thr 229 by the phosphoinositide-dependant kinase 1 (PDK1). It is generally assumed that, when activated, the S6K proteins phosphorylate the ribosomal S6 protein. The latter controls the translation of a class of mRNAs called the 5′TOP (tract of pyrimidine) mRNAs, although, for some authors, this process does not require S6K or S6 phosphorylation (Stolovich et al. 2002). The TOP mRNAs encode for ribosomal proteins and proteins implicated in translation, such as eEF2 (eukaryotic elongation factor 2) (Proud 2002). eEF2 mediates the translocation during the elongation phase. It binds the guanidic nucleotides and is active when bound to GTP. Furthermore, the activity of eEF2 can be inhibited by phosphorylation on Thr 56 via its kinase eEF2K. $p70^{s6k}$ can inhibit eEF2K by phosphorylation, thus increasing the activity of eEF2 (Browne et al. 2004). In summary, $p70^{s6k}$ is involved in the expression as well as in the activity of eEF2. Another potent target of the S6K family is the phosphoprotein eIF4B which stimulates the activity of eIF4A (see previous paragraph) (Raught et al. 2001).

Rapamycin-FKBP12

Rapamycin regulates the protein kinase activity of mTOR. Once bound to its intracellular receptor FKBP12 (also called FK506-binding protein), rapamycin specifically interacts with mTOR and inhibits the signalling downstream of mTOR in vitro. However, the concentrations of rapamycin required to inhibit the activity of mTOR in vitro are much higher than in vivo (500 nM vs 25 nM). It is likely that rapamycin does not directly modulate the activity of mTOR, but when bound to it, rapamycin could activate a phosphatase which could act on the effectors downstream of mTOR (Raught et al. 2001).

Raptor, rictor and GβL

The activity of mTOR is also controlled by several binding partners such as the regulatory associated protein to mTOR (raptor), the rapamycin-insensitive companion of mTOR (rictor) and GβL (also called mLST8). Raptor and rictor independently associate with mTOR and GβL and define two distinct mTOR complexes (Sarbassov et al. 2004). Raptor seems to serve as an mTOR scaffolding protein whose association with mTOR is required for an efficient phosphorylation of 4E-BP1 and $p70^{s6k}$ (Nojima et al. 2003). Binding of raptor to mTOR could influence the sensitivity of mTOR to rapamycin and AA (Kim et al. 2002). However, further investigations are needed to clarify the interaction between mTOR and raptor, and how raptor alters the kinase activity of mTOR.

Raptor not only binds to mTOR but also to $p70^{s6k}$ and 4E-BP1 through their respective TOS (conserved TOR signalling) motifs. The TOS motif is a short conserved sequence required for AA- and mTOR-dependent activation of the downstream effectors of mTOR. A mutation in the TOS motif of $p70^{s6k}$ and 4E-BP1 that prevents both AA- and mTOR-regulation also abolishes their binding to raptor.

Unlike raptor, the recently discovered rictor is insensitive to rapamycin and does not regulate $p70^{s6k}$. Rather, the rictor–mTOR complex modulates the organization of the actin cytoskeleton and cell morphology. PKCα (protein kinase Cα) is a mediator of this function, but it is likely that other effectors are involved in controlling the actin cytoskeleton (Sarbassov et al. 2004).

TSC1/TSC2 and Rheb

mTOR can be directly activated by PKB phosphorylating Ser 2,448 (Nave et al. 1999). Recently, an additional control mechanism of mTOR via PKB has been suggested, implicating the proteins TSC1 and TSC2 (tuberous sclerosis complex 1 and 2). TSC1 and TSC2, also called hamartin and tuberin respectively, form a complex and repress mTOR. This inhibition on mTOR can be reversed when TSC2 is phosphorylated by PKB,

which induces the dissociation of the complex (Fig. 2). PKB phosphorylates TSC2 on Ser 939, Ser 1,086, Ser 1,088 and Thr 1,422. According to some authors (Liu et al. 2002; Nellist et al. 2003), the phosphorylation of these sites by PKB is responsible for the binding of the 14-3-3 protein to TSC2. The 14-3-3 protein family has many roles in cell metabolism (Siles-Lucas and Gottstein 2003), amongst which the modulation of the TSC2 activity. According to others (Li et al. 2002; Shumway et al. 2003), it is the phosphorylation of TSC2 on Ser 1,210 by the p38 mitogen-activated protein-kinase-activated protein kinase 2 (MAPKAPK2) that would be essential for the binding of the 14-3-3 protein.

TSC2 is a GTPase-activating protein (GAP) for the small G protein Rheb (Ras homologue enriched in brain). When Rheb is bound to GTP, mTOR is activated (Fig. 2). The GAP domain of TSC2 increases the hydrolysis of the GTP form of Rheb preventing the signal propagating through mTOR. Up to now no guanine-nucleotide exchange factor (GEF) to counter the GAP activity of TSC2 has been discovered. It has been proposed that Rheb might not require a GEF for its activation because Rheb exists in a highly GTP-bound state and has low intrinsic GTPase activity (Im et al. 2002). Unlike TSC2, TSC1 has no GAP activity. Its role is rather to stabilize TSC2 and thereby to prevent TSC2 from ubiquitinization and degradation (Li et al. 2004).

Several mechanisms have been proposed to explain how Rheb could activate mTOR (Fig. 2). First, Rheb could be a component of the complex formed by mTOR and other proteins, thus regulating mTOR directly or via these binding proteins. As up to now no direct association between Rheb and mTOR has been demonstrated, it is likely that Rheb activates a binding protein of mTOR rather than mTOR directly. Secondly, mTOR could play a role in the detection of the intracellular AA level. It is then possible that Rheb activates mTOR indirectly by facilitating the transport of AA into the cell (Li et al. 2004). Thirdly, Rheb could activate an unknown kinase that phosphorylates mTOR. In HEK293 cells, overexpression of Rheb has been shown to increase the phosphorylation of mTOR on Ser 2,448 (Inoki et al. 2003a). This activation of mTOR by overexpressing Rheb can even occur in the absence of growth factors or AA (Inoki et al. 2003a; Tee et al. 2003). Conversely, overexpression of TSC1 and TSC2 blocks the ability of AA (Gao et al. 2002; Inoki et al. 2002; Tee et al. 2002) and Rheb (Inoki et al. 2003a; Tee et al. 2003) to activate mTOR. In summary, several hypotheses have been proposed to explain the mechanism of activation of mTOR by Rheb, but the effector(s) of Rheb that regulate(s) mTOR directly or indirectly remain(s) unknown.

AMP-activated protein kinase

AMPK is a heterotrimeric complex including one catalytic subunit, α, and two non-catalytic subunits, β and γ. AMPK is activated when AMP increases and when the catalytic subunit α is phosphorylated by one or several AMPK kinases on Thr 172, located in the "T loop" of the catalytic domain. Once activated, AMPK phosphorylates many substrates, the purpose of which is to maintain the intracellular ATP level. When the ATP concentration falls, AMPK prevents the consumption of ATP by inhibiting key enzymes of anabolic pathways. AMPK also increases ATP synthesis by stimulating for example the rate of fatty acid oxidation and the uptake of glucose by the cell (Yonezawa 2003). It has been reported that, when activated, AMPK could either phosphorylate TSC2 on Thr 1,227 and Ser 1,345 thereby enhancing its activity (Inoki et al. 2003b), or phosphorylate mTOR on Thr 2,446 resulting in its inhibition (Cheng et al. 2004). However, the latter results need to be confirmed, and it is more likely that AMPK does not inhibit mTOR directly but through the phosphorylation of TSC2 (Inoki et al. 2003b).

mTOR and AA

Protein synthesis is an energy consuming process. During translation elongation, four ATP equivalents are used for each AA added to the nascent peptide chain (Browne and Proud 2002). Energy consumption by protein synthesis is small when compared to muscle contraction, but it can contribute significantly to the energy consumption of a resting muscle. If AA are lacking, the cell should conserve its energy and avoid expensive anabolic processes. On the other hand, if AA availability is sufficient, the cell has to increase the rate of protein translation to ensure cell growth. There is evidence for AA playing a role in the translational regulation of protein synthesis. In skeletal muscle of rats in the fasted state, the mTOR-mediated signalling is reduced compared with the fed state. In a study with fasted animals, 4E-BP1 was highly present in hypophosphorylated forms (Yoshizawa et al. 1997, 1998), sequestering eIF4E and inhibiting the formation of the initiation complex eIF4F. In contrast, when the rats received a protein-containing meal, 4E-BP1 became hyperphosphorylated and promoted the formation of the eIF4F complex. This positive effect on muscle protein synthesis can also be observed with oral leucine administration alone (Anthony et al. 2000). In addition to promoting eIF4F assembly, either feeding a complete meal or oral administration of leucine promotes phosphorylation of $p70^{s6k}$ (Anthony et al. 2000).

Types of AA

AA are known to stimulate protein synthesis, but the mechanisms of activation differ according to the AA studied (Meijer and Dubbelhuis 2004). AA can be classified into two major categories. AA in the first category induce cell swelling because of an increase in the intracellular osmolarity following Na^+-dependent AA transport across the membrane, and because some products of

their metabolism accumulate in the cell (e.g. glutamate). Cell swelling per se induces protein, glycogen and lipid synthesis. The second category of AA is represented by leucine which does not induce cell swelling and which essentially regulates protein metabolism by stimulating protein synthesis and by inhibiting autophagy.

A group of AA, the branched chain AA (BCAA), including leucine, valine and isoleucine, seem to be particularly effective anabolic agents, especially leucine which has the most potent effect on the phosphorylation state of $p70^{s6\ k}$ in the skeletal muscle (Anthony et al. 2000) and in various cell lines (Patti et al. 1998; Xu et al. 1998). It is possible that AA activate mTOR through a permissive action of insulin (Jefferson and Kimball 2003). Even low levels of insulin could maintain a minimal activation level of the PI3K/PKB pathway by stimulating the PI3K class 1 and subsequently allowing AA to activate the downstream targets of mTOR in many different cell types (hepatocytes, adipocytes, myocytes and pancreatic cells).

Mechanisms involved in AA regulation

The way by which AA stimulate mTOR is not fully understood, but they do not act like growth factors or insulin which modulate PI3K and its downstream effector PKB (see Roles of mTOR). Indeed, activation of mTOR via AA does not require activation of PKB (Jefferson and Kimball 2003). Several hypotheses have been proposed to explain the activation of mTOR by AA (Fig. 3). AA could stimulate a protein kinase (other than PKB) acting on mTOR, or they could inhibit a protein phosphatase, such as PP2A. This protein phosphatase could act on mTOR or on the downstream proteins. AA could also interact with proteins associated with mTOR (raptor) or acting on mTOR (TSC1/TSC2, AMPK) (Meijer and Dubbelhuis 2004). Some authors have hypothesized that AA increase the phosphorylation on Ser 2,448 by facilitating the dissociation of the complex TSC1/TSC2 from mTOR and allowing PKB to access on Ser 2,448 (Kanazawa et al. 2004). Finally, a direct activation of mTOR by AA could be possible but remains controversial. In conclusion, the molecular nature of AA sensing and the regulation of TSC1/TSC2, Rheb and mTOR by AA remain poorly understood.

Although the mTOR pathway plays a major role in the regulation of protein synthesis, it should be mentioned that AA can stimulate cell growth independently of mTOR, and that the involvement of mTOR varies greatly among cell lines. In L6 myoblasts, eIF2B (eukaryotic initiation factor 2B), which is not under the control of mTOR, appears to be more involved in the activation of protein synthesis by AA than eIF4E (Kimball et al. 1998). In isolated rat ventricular myocytes, protein synthesis is acutely activated by insulin (Wang et al. 2000) and this effect is blunted by only around 50% by rapamycin. In skeletal muscle, the activation of protein synthesis by leucine is only partially inhibited by rapamycin (Anthony et al. 2000). Thus it is likely that leucine stimulates protein synthesis via an mTOR-dependent and an mTOR-independent pathway(s) (Proud 2002).

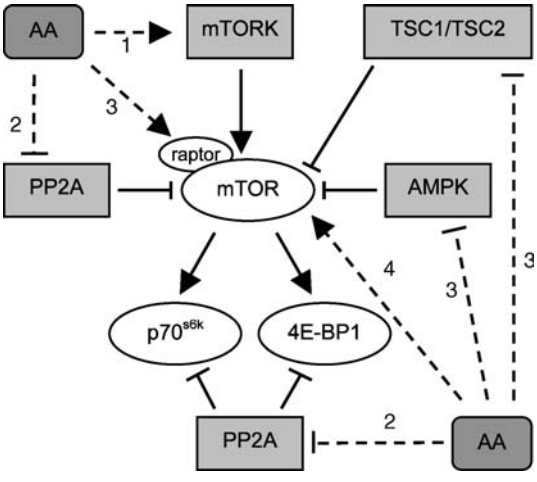

Fig. 3 Hypotheses for activation of mTOR by amino acids (*AA*) (see text for details). (1) AA could stimulate a protein kinase (*mTORK* mammalian target of rapamycin kinase) acting on mTOR. (2) AA could inhibit a protein phosphatase such as *PP2A* (protein phosphatase 2A) which could act on mTOR or on the downstream proteins of mTOR. (3) AA could interact with proteins associated with mTOR (*raptor* regulatory associated protein to mTOR) or acting on mTOR (TSC1/TSC2, *AMPK* AMP-activated protein kinase). (4) AA could activate mTOR directly

Autophagy

The presence of AA not only stimulates protein synthesis but also represses autophagy. Autophagy is the only proteolytic system that can be regulated by the nutritional state. When the nutritional state is low, eukaryotic cells degrade their proteins to scavenge AA. The first step of this autophagy consists in the sequestration of a portion of the cytoplasm inside a double membrane structure called autophagosome. This structure fuses then with lysosomal membranes where the cytoplasmic components can be degraded (Raught et al. 2001). AA and insulin are the main physiological regulators of autophagy in mammals, but it seems that they use different ways to control it. The pathway used by insulin to regulate autophagy is partially the same as for protein synthesis. On the contrary, it seems that the signalling used by AA in autophagy is independent of protein synthesis and that AA have their own signal transduction mechanisms (Kanazawa et al. 2004). In a perfused liver, autophagy is controlled by the extracellular concentrations of phenylalanine and leucine. Leu8-MAP, an analogue of leucine that cannot be transported inside the cell, mimics the effect of leucine. It can be speculated that AA have their own recognition site on the surface of the membrane and their own signal

transduction mechanisms up to the formation site of the autophagosome in the cell (Kanazawa et al. 2004).

mTOR could be a central component of the signal transduction of autophagy (Fig. 1), whereas downstream effectors of mTOR are not well known. In yeast, autophagy induced by fasting is controlled by TOR via a complex Apg1–Apg13, which plays a role in the initial formation of the phagosome (Kamada et al. 2000). In isolated hepatocytes, inhibition of autophagy by AA occurs via the phosphorylation of the ribosomal protein S6 (Blommaart et al. 1995). As described above, phosphorylation of S6 is activated by $p70^{s6k}$, a direct target of mTOR. However some studies do not support the hypothesis of mTOR involvement in autophagy. In mouse C_2C_{12} myotubes, mTOR signalling pathway was not involved in the regulation of autophagy by leucine (Mordier et al. 2000). Furthermore rapamycin had no effect on the suppression of autophagic proteolysis by AA in isolated hepatocytes (Kanazawa et al. 2004). The discrepancy between these studies and the study of Blommaart et al. (1995) could be explained by methodological considerations which are beyond the scope of this review. Clearly more research is warranted to clarify if and how mTOR regulates autophagy in skeletal muscle.

mTOR and exercise

Acute resistance exercise is known to increase skeletal muscle protein synthesis during the recovery phase. Both concentric and eccentric contractions seem to be effective in promoting protein synthesis (Phillips et al. 1997, 1999; Wong and Booth 1990). Two major pathways leading to protein synthesis have been proposed to induce this increase: the PI3K/PKB/mTOR and the calcineurin/NFAT (nuclear factor of activated T cells) pathways. The PI3K/PKB/mTOR pathway could be involved mainly in skeletal muscle growth (Pallafacchina et al. 2002), whereas the calcineurin/NFAT pathway, and to a lesser extent the Ras/MAPK (mitogen-activated protein kinase) pathway, control muscle fibre type (Fig. 4) (Serrano et al. 2001). Indeed, inhibitors of calcineurin, cyclosporin A and FK506, do not blunt muscle hypertrophy, as shown by increases in plantaris muscle mass and fibre size of rats after overload (Bodine et al. 2001). In contrast, specific inhibition of mTOR with rapamycin leads to a 95% blockade of hypertrophy, indicating that mTOR and its downstream targets, $p70^{s6k}$ and 4E-BP1, are crucial regulators of muscle hypertrophy (Bodine et al. 2001).

Protein kinase B

No information is available to date on the activation of PI3K following skeletal muscle overload. However, a series of studies have demonstrated upregulation of PKB activity. PKB phosphorylation on Ser 473 was almost tripled immediately after high-frequency electrical stimulation (Nader and Esser 2001), and was higher in rat plantaris muscle after a few days overloading compared to control conditions (Bodine et al. 2001). PKB activity increased 2.5-fold from day 3 to day 10 in regenerating innervated soleus muscle after injury. In the same study, overexpression of active PKB increased fibre size in regenerating soleus and in adult extensor digitorum longus (EDL) and soleus muscles (Pallafacchina et al. 2002).

Some results suggest that PKB phosphorylation is fibre type specific. Indeed, contraction- or stretch-induced activation of PKB was only observed in isolated fast-twitch EDL but not in isolated slow-twitch soleus (Sakamoto et al. 2003, 2004). These data provide evidence that the different contractile and/or metabolic properties of slow or fast fibres confer distinct signalling properties to mixed skeletal muscles. Because no activation of PKB by exercise has been observed in isolated soleus muscle but it occurs well in vivo, it is likely that systemic factors and/or oxidative stress mediate the activation of PKB by exercise (Cooper et al. 2002; Shaw et al. 1998).

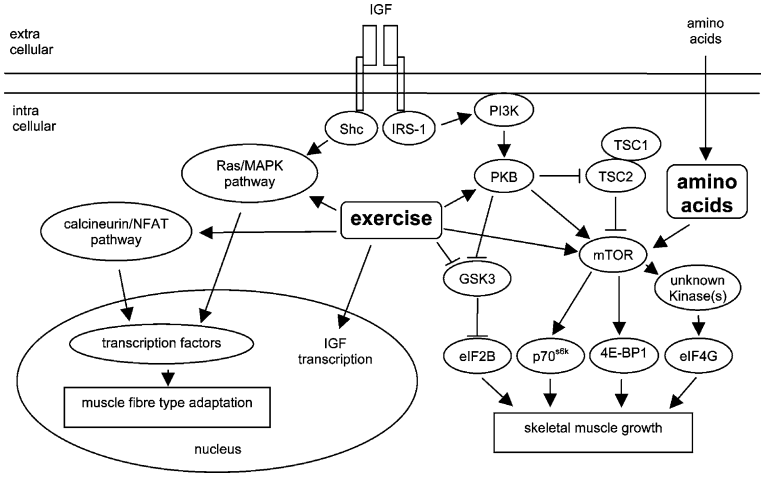

Fig. 4 AA and exercise signalling. AA stimulate skeletal muscle growth by activating mTOR and its downstream effectors (for details see Fig. 3), whereas exercise acts upstream of mTOR via PKB to promote hypertrophy. Exercise also activates the calcineurin/*NFAT* (nuclear factor of activated T cells) and Ras/*MAPK* (mitogen-activated protein kinase) pathways leading to muscle fibre type adaptation. *IGF* Insulin-like growth factor, *Shc* Src-homology and collagen homology, *GSK3* glycogen synthase kinase 3, *eIF2B* eukaryotic initiation factor 2B

Mammalian target of rapamycin

In contrast to the above described results, some experiments did not reveal an enhanced activity or phosphorylation of PKB after exercise (Brozinick and Birnbaum 1998; Markuns et al. 1999; Parkington et al. 2003; Sakamoto et al. 2002; Sherwood et al. 1999) or sciatic nerve stimulation, despite mTOR activation (Parkington et al. 2003). These observations suggest a PKB-independent activation of mTOR signalling by contractile activity. In the study of Parkington et al. (2003), mTOR phosphorylation on Ser 2,448 increased in tibialis anterior muscle after 6 h recovery, and in plantaris immediately after and 6 h after the stimulation. mTOR was not altered in soleus muscle at either time point. This implies that similar to PKB, mTOR phosphorylation depends on muscle fibre type. Indeed, mTOR phosphorylation in tibialis anterior is preferentially localized in type IIa fibres, suggesting that this class of fibres may be more responsive to hypertrophy (Parkington et al. 2003).

p70 Ribosomal S6 kinase

The first report on the phosphorylation state of $p70^{s6k}$ after exercise in rats was published a few years ago (Baar and Esser 1999). The phosphorylation of $p70^{s6k}$ was unchanged immediately after tibialis anterior and EDL muscle stimulation, but was tripled 3 h and 6 h post-exercise. This elevated phosphorylation was maintained until 36 h of recovery. The changes in $p70^{s6k}$ phosphorylation after a single bout of resistance exercise could be a good marker for the long-term increases in muscle mass. A later study confirmed that the activity of $p70^{s6k}$ is elevated between 6 h and 24 h after acute resistance exercise but not immediately after exercise (Hernandez et al. 2000).

Glycogen synthase kinase 3

Glycogen synthase kinase 3 (GSK3) is another effector of PKB that can regulate muscle hypertrophy, independently of the mTOR-mediated pathway (Bodine et al. 2001). Although GSK3 is controlled by PKB, other kinases can modulate its activity, such as MAP kinase-activated protein kinase-1 (also known as RSK2 or $p90^{rsk}$) and $p70^{s6k}$ (Cross et al. 1995; Sutherland et al. 1993). The phosphorylation of GSK3 results in its inhibition and in activation of eIF2B, which stimulates global protein synthesis. Indeed, eIF2B activity has been shown to coincide with increased protein synthesis several hours following resistance exercise (Farrell et al. 1999). Moreover, exercise itself could inhibit GSK3, thus increasing the effect of enhanced protein synthesis (Fig. 4). Stretch-induced activation of PKB in EDL muscle of rats was fully prevented by wortmannin, an inhibitor of PI3K, whereas GSK3 phosphorylation was only partially reduced (Sakamoto et al. 2003). These results suggest that exercise also regulates GSK3 by a PKB-independent pathway. In humans, phosphorylation and deactivation of GSK3 by exercise is less clear (Sakamoto et al. 2004; Wojtaszewski et al. 2001).

Time course of activation of the mTOR-mediated pathway

Some authors have investigated immediate responses of the mTOR-mediated signalling after resistance exercise in skeletal muscle of fed rats (Bolster et al. 2003). Their results revealed that the PKB/mTOR pathway was induced after a few minutes following resistance exercise. The phosphorylation state of PKB, mTOR, $p70^{s6k}$, ribosomal protein S6, 4E-BP1 and the association of eIF4E to eIF4G peaked between 5 min and 10 min of recovery. These early responses may serve as a priming event favouring the growth response by the cell during exercise recovery. It should be noted that most variables measured in this study showed a "bell-shaped" curve. Thus, the components of the PKB/mTOR pathway could be phosphorylated in an oscillatory fashion during the beginning of recovery. The summation of these transient activations could be critical in inducing muscle hypertrophy with repeated training sessions (Bolster et al. 2003). The oscillatory fashion of activation of the PKB/mTOR pathway after exercise makes its study very difficult, and the time points at which the samples are taken following exercise may lead to opposing results, as illustrated by the previously cited studies.

Even if the literature is abundant regarding the study of the PKB/mTOR pathway and exercise, one major question remains unanswered at this time: what is the link between contraction and activation of the PKB/mTOR pathway? Several hypotheses have been proposed to explain the changes in muscle protein synthesis induced by exercise. In the short term, calcium release (Ji et al. 2002) and integrin signalling (MacKenna et al. 1998) are important actors in the responses to stretch in muscle. However, up to now there is no evidence linking one of these factors, exercise and the activation of PKB/mTOR signalling. In the long term, insulin-like growth factors (IGF) and the so-called mechanical growth factor (Goldspink 1999) could stimulate a late sustained response leading to muscle hypertrophy (Rennie and Wackerhage 2003). Indeed, resistance exercise increases the expression of IGF-I, which is known to be a potent activator of the PKB/mTOR pathway. However, the involvement of other, so far unknown, players cannot be excluded. The summation of both short- and long-term processes leads to increased muscle protein synthesis and hypertrophy via activation of the PKB/mTOR pathway (Rennie and Wackerhage 2003).

mTOR, exercise and AA supplementation

All the previously cited studies were carried out on fed animals. Because both AA and exercise use the mTOR

pathway, it is difficult to isolate the effect of exercise on this pathway. A recent study in humans examined the effect of exercise on the phosphorylation state of $p70^{s6k}$ and the S6 ribosomal protein after ingestion of a placebo or a drink containing BCAA. The phosphorylation of the S6 ribosomal protein and of $p70^{s6k}$ on Thr 389, which corresponds to its highest level of phosphorylation and its highest activity, were only slightly higher 1 h and 2 h after exercise in the placebo condition, and were significantly increased at the same time points when the subject received the drink containing BCAA (Karlsson et al. 2004). These data suggest that BCAA could exert a permissive action on the activation of the mTOR-mediated pathway by exercise (Karlsson et al. 2004). Furthermore, as previously discussed, there appears to be a requirement for a minimal concentration of plasma insulin to stimulate muscle protein synthesis in response to resistance exercise. However, the concentration of insulin needed is low, values equivalent to or less than fasting values are sufficient to induce the effect (Kimball et al. 2002).

Summary and conclusion

Protein synthesis is enhanced up to 48 h after the completion of a resistance exercise concomitantly with a lesser increase in protein breakdown, leading to a positive protein net balance (Tipton and Wolfe 1998). The opposite situation occurs during exercise, protein breakdown is greater than protein synthesis resulting in a negative net balance. From a biochemical point of view, we are able to propose the following hypothesis: during acute exercise, AMPK is activated due to an increase in the AMP/ATP ratio (Chen et al. 2003; Kemp et al. 1999; Nielsen et al. 2003), and AMPK inhibits mTOR either directly (Cheng et al. 2004) or via activation of the repressor TSC2 (Inoki et al. 2003b). mTOR is thus less active in promoting protein synthesis and preventing autophagy. Increased breakdown allows for more AA to be available as fuel for exercise. Once exercise is completed, AMPK activity quickly returns to basal values. The inhibition on mTOR is suppressed and the latter becomes able to reduce autophagy and to promote protein synthesis via phosphorylation of $p70^{s6k}$, 4E-BP1 and eIF4G. The role of mTOR could be to preserve AA from being enrolled in protein synthesis during exercise through an inhibition by AMPK and, on the other hand, to favour their utilization and to promote muscle mass accumulation during recovery.

Many recent investigations focus on exercise and its influence on translational control of protein synthesis. Interpretation of the results is complex, since the type, the frequency and the intensity of muscle contractions, and also the type of muscle studied and the training status of the subjects represent factors that may lead to diverse responses. Different models and experiments may induce various degrees of muscle damage, AA availability and hormonal responses, and therefore different adaptations to exercise. The heterogeneity in the protocols could \explain some discrepancies in the literature regarding the exercise signalling. It is, however, very clear that AA ingestion before or immediately after exercise is necessary to fully activate protein synthesis signalling in muscle and therefore to accumulate muscle mass.

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