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Review

Intracellular signalling pathways regulating the adaptation of skeletal muscle to exercise and nutritional changes

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Summary. The focus of the present review is to assimilate current knowledge concerning the differing signalling transduction cascades that control muscle mass development and affect skeletal muscle phenotype following exercise or nutritional uptake. Effects of mechanical loading on protein synthesis are discussed. Muscle growth control is regulated by the interplay of growth promoting and growth suppressing factors, which act in concert. Much emphasis has been placed on understanding how increases in the rate of protein synthesis are induced in skeletal muscle during the adaptive process. One key point to emerge is that protein synthesis following resistance exercise or increased nutrient availability is mediated through changes in signal transduction involving the phosphorylation of mTOR and sequential activation of downstream targets. On the other hand, AMPK activation plays an important role in the inhibition of protein synthesis by suppressing the function of multiple translation regulators of the mTOR signalling pathway in response to cellular energy depletion and low metabolic conditions. The effects of exercise and/or nutritional uptake on the activation of signalling molecules that regulate protein synthesis are highlighted, providing a better understanding of the molecular changes in the cell.

Key words: mTOR, Protein synthesis, Myostatin, Exercise, Nutrition

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Introduction

Skeletal muscle is the single largest tissue in the human body. Contraction of skeletal muscle not only allows the body to perform locomotory functions but is also essential for life since it is required for breathing. Skeletal muscle is also a highly adaptable tissue, responding to environmental and physiological challenges by changing its size and as well as its composition, outcomes that are brought about by changes in gene expression, biochemical and metabolic properties. Mechanical load (defined as the force exerted by a weight or gravity on skeletal muscle) is considered as a major regulator of skeletal muscle mass, given that

Abbreviations: AICAR, 5-amino-4-imidazolecarboxamide riboside; AMPK, adenosine monophosphate-activated kinase; BCAA, Branched-chain amino acids; CHO, carbohydrates; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; eIF, eukaryotic initiation factor; GßL, G-protein ß-subunit-like protein; GS, glycogen synthase; GSK-3, glycogen synthase kinase; HF, high-frequency stimulation; IGF-I, Insulin-like growth factor I; IGF-IR, IGF-I receptor type I; IRS, insulin receptor substrate; LF, low-frequency stimulation; MHC, myosin heavy chain; mSin1, mitogen-activated protein kinase-associated protein 1; mTOR, mammalian target of rapamycin; mVps34, mammalian vacuolar protein sorting subunit 34; PDK-1, phosphoinositide-dependent kinase 1; PGC-1α, peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α; PI3K, phosphatidylinositol-3 kinase; PKB, protein kinase B also known as Akt; PPARδ, peroxisome proliferator-activated receptor δ; PRO, protein; p70S6k, 70-kD S6 protein kinase; RAPTOR, regulatory-associated protein of mTOR; Rheb, RAS homolog enriched in brain; RICTOR, rapamycin-insensitive companion of mTOR; mTORC1/2, mTOR complex 1/2; TSC, tuberous sclerosis complex; TSC1, also known as hamartin; TSC2, also known as tuberin; YY1, transcription factor yin-yang 1.

the maintenance of muscle mass significantly contributes to athletic performance, the quality of life and disease prevention (Hornberger et al., 2006) and is a good example of an environmental and physiological challenge that brings about changes in the internal composition and function of the tissue. For instance, skeletal muscle adaptation to contractile activity induced by exercise training is elicited by changes in the transcription of structural genes, changes in protein synthesis, altered myofibrilar protein profiles and changes in metabolism. Furthermore, recent work has shown that the response of muscle to mechanical work can be significantly modulated by the availability of nutrients (see Table 1).

One of the major goals for muscle biologists is to understand the molecular mechanisms that allow muscle to change its phenotype in response to external stimuli. Recent work suggests that mechanical load applied on skeletal muscle activates various signalling pathways leading to enhanced activation of ribosomal machinery (Spangenburg et al., 2008). Therefore a great deal of work has been performed to identify the roles played by differing signalling pathways that become activated following the application of mechanical load to muscle and how this results in an adaptive response as well as how these processes are moderated by nutrient availability. In the first of two reviews, we aim to summarise our current understanding of how specific signalling pathways control muscle mass development. We ultimately hope to describe how exercise and or changes in diet modulate the signalling pathways that control skeletal muscle mass development.

An adaptive response by muscle resulting in muscle mass gain must ultimately alter the rate of protein turnover. Much emphasis has been placed on understanding how increases in the rate of protein synthesis are induced in skeletal muscle during the adaptive process. The molecular mechanisms behind protein synthesis are currently being intensively researched and have been reported to involve the activation of a group of enzymes called kinases, a type of protein that transfers phosphate groups (a process known as phosphorylation) from a donor molecule to specific substrates that can subsequently regulate translation initiation.

Current knowledge indicates that one of the best understood signalling pathways which regulates protein synthesis in skeletal muscle is that promoted by either insulin or insulin related molecules (IGF-I or MGF). These secreted molecules evoke an intracellular response through the activation of the insulin-signalling pathway culminating in a sequential phosphorylation and activation of downstream targets such as phosphatidylinositol-3 kinase (PI3K), phosphoinositide-dependent kinase 1 (PDK-1), Protein Kinase B (Akt/PKB), mammalian target of rapamycin (mTOR) and 70-kD S6 protein kinase (p70S6k also designated in the literature as pS6k, Glass, 2005). In this work we start by reviewing this signalling pathway in some detail. Then we introduce data showing how other signalling molecules also have a major role to play in the development of muscle mass and seem to act by influencing the activity of specific components of the IGF-I pathway.

The molecular basis of skeletal muscle protein synthesis and changes in response to exercise

Skeletal muscle cells have an intrinsic capacity for sensing mechanical signals and converting this information into biochemical events that regulate growth. For example, mechanical stimuli can regulate protein synthesis by changing translational efficiency (initiation, elongation and termination) primarily at the stage of initiation (Hornberger et al., 2006). Changes in muscle mass and function also take place in response to aging, nutritional intervention, positive physiological responses and even disuse and disease. This extreme plasticity of skeletal muscle is in part genetically regulated.

Skeletal muscle mass changes leading to hypertrophy or atrophy are regulated by differences in the balance between protein synthesis and degradation. Hypertrophy takes place during the process of normal growth, but also following resistance exercise, and occurs as a consequence of increased protein synthesis. In particular, an increased rate of protein synthesis is the most important acute response to resistance exercise (Baar et al., 2006). However, protein synthesis is a major cellular (and energy-consuming) process regulated by multiple intracellular and extracellular conditions such as growth factor action, amino acid availability and cellular energy status. Hence, during exercise-induced mechanical muscle loading, amino acid oxidation and protein breakdown increase and at the same time whole-body protein synthesis is suppressed (Rennie et al., 2006). A large body of literature indicates that insulin-like growth factors (IGF-I and IGF-II), working through the type I IGF receptor (IGF-IR), are key mediators of skeletal muscle fibre growth and hypertrophy. It is well-known that increases in muscle load stimulate the expression of IGF-I, whose activity is sufficient to promote skeletal muscle hypertrophy by activating signalling pathways that regulate protein synthesis. IGFs bind the extracellular domain of this receptor inducing the phosphorylation of the insulin receptor substrate (IRS) inside the cell, which functions as an adaptor protein to transduce the signal to downstream molecules. Upon phosphorylation IRS recruits and activates PI3K with a concomitant phosphorylation and subsequent activation of key molecules of the Akt pathway (see Fig. 1; Hornberger and Chien, 2006).

Akt/PKB protein kinase is a serine/threonine protein kinase member of the cAMP-dependent protein kinase AGC superfamily that functions as a critical regulator of mammalian cell signalling. In mammals, there are three Akt/PKB isoforms with conserved domain structure; Akt1, that acts as a key signalling protein in the cellular

Table 1. Effect of nutrients and/or mechanical loading on phosphorylation of skeletal muscle proteins involved in protein synthesis signaling.

Intervention	Phosphorylation states	Reference
Supplementation with CHO-PRO mixture or placebo after one bout of cycling in humans.	Exercise alone: ↑ p70S6k (Thr412), ↑ rpS6 (Ser235/236); Exercise and supplementation: ↑ Akt (Ser473), ↑ mTOR (Ser2448), ↑ p70S6k (Thr412), ↑ rpS6 (Ser235/236) 45 min post-exercise	Ivy et al., 2008
Administration of 10g essential amino acids in young and elderly humans.	↑ mTOR (Ser2448), p70S6k (Thr389), 4E-BP1 (Ser37/46) 3h after amino acid injection in the elderly and further more significant increase in young individuals	Cuthbertson et al., 2005
Administration of a leucine-enriched essential AA-CHO mixture in humans .	1h after supplementation: ↓ AMPK (Thr172), - TSC2 (Thr1462), ↑ Akt (Ser473), ↑ mTOR (Ser2448), ↑ 4E-BP1 (Thr37/46), ↑↑ p70S6k (Thr389), ↓ eEF2 (Thr56)	Fujita et al., 2007
One bout of resistance exercise with or without a CHO-PRO mixture administered 1-hour post-exercise in humans. Measurements obtained before, 0-, 1- and 2-hours post-exercise.	↑ (1h) Akt (Ser473), - (0-2h) TSC2 (Thr1462), ↑ (0-2h) mTOR (Ser2448), ↑ (0h) 4E-BP1 (Thr37/46), ↑ (0-2h) p70S6k (Thr389), ↓ (1-2h) eEF2 (Thr56); Supplementation: ↑ (2h) Akt (Ser473), - (2h) TSC2 (Thr1462), ↑↑ (2h) mTOR (Ser2448), ↑ (0h) 4E-BP1 (Thr37/46), ↑↑ (2h) p70S6k (Thr389), ↓ (2h) eEF2 (Thr56)	Dryer et al., 2008
Supplementation with CHO and/or PRO after one bout of resistance exercise in humans. Measurements obtained before, 0-, 1- and 4-hours post-exercise.	Exercise and CHO: ↓↓ (0h), ↑ (1-4h) 4E-BP1 (Thr37), ↑ (0-4h) p70S6k (Thr421/Ser424), - (0-4h) p70S6k (Thr389), ↓ (0h), - (1-4h) rpS6 (Ser235/236), Exercise and CHO-PRO: ↓ (0h), ↑ (1-4h) 4E-BP1 (Thr37), ↑ (0-4h) p70S6k (Thr421/Ser424), ↑↑ (0-4h) p70S6k (Thr389), ↑↑ (0-4h) rpS6 (Ser235/236)	Koopman et al., 2007
One bout of exercise (shortening vs. lengthening) combined with 45g essential amino acids and 135g CHO administered 2-hours post-exercise in humans. before, 3-, 6- and 24-hours post-exercise.	↑ (3-24h) Akt (Ser473/Thr308) and p70S6k (Thr389). No effect of the mode of contraction	Cuthbertson et al., 2006
One bout of resistance exercise with or without administration of BCAA in humans. Measurements obtained before, 0-, 1- and 2-hours post exercise.	↑ (0-2h) p70S6k (Ser424/Thr421) with further increase upon BCAA treatment, ↑↑ (1-2 h) p70S6k (Thr389) and rpS6 (Ser235/236) only with BCAA	Karlsson et al., 2004
One bout of resistance exercise with or without creatine administration for 5 days in humans.	↓ Akt (Thr308), 4E-BP1 (Thr37/46) immediately after exercise independent of creatine and 24 h post exercise with creatine	Deldicque et al., 2008
One session of resistance exercise with BCAA supplementation or placebo in humans.	Placebo: ↓ (0h) Akt (Ser473), - (0-2h) mTOR, (Ser2448), ↑ (0-2h) p70S6k (Ser424/Thr421), - (0-2h) p70S6k (Thr389), BCAA: ↓ (0h), ↑ (1-2h) Akt (Ser473), ↑ (1h) mTOR, (Ser2448), ↑ (0h), ↑↑ (1-2h) p70S6k (Ser424/Thr421), ↑↑ (1-2h) p70S6k (Thr389)	Blomstrand et al., 2006
Concentric and eccentric contractions in humans. Measurements obtained before, 0-, 1- and 2-hours post-exercise.	- (0-2h) Akt (Ser473), - (0-2h) mTOR (Ser2448 and Ser2481), ↑ (0-2h) p70S6k (Ser424/Thr421), ↑ (0-2h) p70S6k (Thr389), ↑ rpS6 (Ser235/236) after maximal eccentric but no concentric contractions	Eliasson et al., 2006
One bout of cycling or strength training in endurance and strength trained individuals. Measurements obtained before, 0- and 3-h post-exercise.	Strength: ↑ (0h) AMPK (Thr172), ↓ (0-3h) TSC2 (Thr1462), ↑ (3h) p70S6k (Thr389), ↑ (0h) rpS6 only in endurance trained individuals, - Akt (Ser473), - eIF2B (Ser540) Endurance: ↑ (0) AMPK (Thr172), ↓ (0-3h) eIF2B (Ser540) only in strength trained individuals, ↑ (0h) ↓ (3h) Akt (Ser473) only in endurance trained individuals, - TSC2 (Thr1462), - p70S6k (Thr389), - rpS6	Coffey et al., 2006
One bout of cycling exercise for 1 hour in humans. Measurements obtained before, 0-, 0.5-, 1-, 2- and 3-hours post-exercise.	↑ (1-2h) Akt (Ser473), ↓ (30min-3h) eEF2 (Thr56), ↑ (0-30min) p70S6k (Ser424/Thr421), - (0-3h) p70S6k (Thr389), ↑ (0h, 30min, 2h) mTOR, (Ser2448), - (0-3h) mTOR (Ser2481), ↑ (30min-3h) GSK-3 (Ser21), ↑ (0-3h) GSK-3 (Ser9)	Mascher et al., 2007
One bout of resistance exercise in humans. Measurements obtained before, 0-, 1- and 2-hours post-exercise.	↓ (1h) TSC2 (Thr1462),↑ (1h) PKB (Ser473), ↑ (1-2h) mTOR (Ser2448), ↓ (0h) 4e-BP1 (Thr37/46), ↑ (2h) p70S6k (Thr389), ↓ (1-2h) eEF2 (Thr56)	Dreyer et al., 2006
One bout of contractile activity by means of high-frequency electrical stimulation in rat muscles (P: plantaris, TA: tibialis anterior, S:soleus). Measurements obtained before, 0- and 6-hours post-stimulation.	Fibre type-dependent mTOR phosphorylation results: - (0-6h in P and TA) Akt (Thr308), ↑ (0-6h in P), ↑ (6h in TA), - (0-6h in S) mTOR (Ser2448), ↑ (0-6h in P), ↑ (6h in TA), - (0-6h in S) p70S6k (Thr389)	Parkington et al., 2003
Administration of CHO, CHO-soy or CHO-whey protein 1-hour after a bout of treadmill exercise in rats.	↑ mTOR, (Ser2448) with both soy and ↑↑ with whey protein; ↓ 4E-BP1 with CHO, - 4E-BP1 with any protein; ↑ p70S6k with whey protein	Anthony, 2007
Pharmacological (AICAR) activation of AMPK with high-frequency (HF) electrical stimulation of rat EDL muscle. Measurements obtained 0-, 20- and 40-min post-stimulation.	AICAR treatment: ↑ (0-40min without HF, ↑↑ (0-40min with HF) AMPK (Thr172), ↑↑ (0min with HF), ↓ (20min independent of HF), - (40min) Akt (Ser473), ↑ (0-40min with HF) p70S6k (Thr389), ↑ (0-40min with HF) p70S6k (Ser424/Thr421), ↑ (0-40min) ↓ (40min without HF) 4E-BP1,↑ (0min independent of HF), ↓ (20-40min with HF) eEF2 (Thr56) Saline treatment with HF: ↑ (0min), - (20-40min) Akt (Ser473), ↑↑ (0-40min) p70S6k (Thr389), ↑ (0min), ↑↑ (20-40min) p70S6k (Ser424/Thr421), ↑ (0min), ↑↑ (20-40min) 4E-BP1, ↓↓ (20-40min) eEF2 (Thr56)	Thomson et al., 2008
One bout of endurance or resistance training-like electrical stimulation (low- and high-frequency stimulation respectively) in rats. Measurements obtained before, 0- and 3-hours post-stimulation.	LF: - (0-3h) Akt (Ser473), ↓ (0-3h) TSC2 (Thr1462), - (0-3h) mTOR (Ser2448), ↓ (0-3h) p70S6k (Thr389), ↓ (0-3h) 4E-BP1 (Thr37/46), ↑ (0h) GSK-3ß (Ser9), ↑ (3h) eIF2B (Ser535), ↑↑ (0-3h) eEF2 (Thr56), ↑ (0-3h) AMPK (Thr172) HF: ↑ (0h) Akt (Ser473), ↑ (0h) TSC2 (Thr1462), ↑ (0h) mTOR (Ser2448), ↑↑ (0-3h) p70S6k (Thr389), ↑↑ (0h) 4E-BP1 (Thr37/46), ↑↑ (0h) GSK-3ß (Ser9), ↓↓ (0-3h) eIF2B (Ser535), ↓ (0-3h) eEF2 (Thr56), ↑ (3h) AMPK (Thr172)	Atherton et al., 2005
Enzymes phosphorylation time course (15-, 30-, 45-, 60- and 120-min) changes after leucine administration compared to placebo in rats.	↑ (15-60min) 4E-BP1, ↑ (15-45min) p70S6k (Thr389), ↑ (15-120min) rpS6	Anthony et al., 2002

The phosphorylation residue is given in parenthesis. ↑/↓: denote increase and decrease respectively, ↑↑/ ↓↓: denote more potent increase and decrease respectively, -: no significant changes, CHO: carbohydrates, PRO: protein, BCAA: Branched-chain amino acids, LF: low-frequency stimulation, HF: high-frequency stimulation.

pathways promoting protein synthesis that leads to skeletal muscle hypertrophy and general tissue growth, Akt2 that acts as an important signalling molecule in the insulin signalling pathway and glucose transport induction and Akt3 that is mainly expressed in the brain whose function has yet to be elucidated. All three Akt isoforms contain a conserved amino terminal pleckstrin homology domain, which interacts with membrane lipid components and can then be phosphorylated at two regulatory sites Thr308 (partial activation of the kinase) and Ser473 (phosphorylation of both sites is necessary for full activation). It is activated either by PDK-1 or the mTORC2 complex or by distinct serine kinases such as integrin-linked kinase, or even by autophosphorylation. In addition, a probable role of tyrosine (e.g. Tyr315, Tyr326, Tyr474) phosphorylation in Akt/PKB regulation has been postulated and is under current investigation, implying a novel regulatory mechanism for Akt (Song et al., 2005). Over the past few years, PKB/Akt has been considered a crucial molecule in the signal transduction pathways of cell survival (promoting growth factor-mediated cell survival both directly and indirectly), cell metabolism (regulating glucose transport and promoting glycogen synthesis through GSK-3ß inhibition) and cell proliferation (e.g. angiogenesis and tumour development, Song et al., 2005; Somanath et al., 2006).

Overall, the PI3K/Akt pathway is considered the major intracellular pathway activated by IGF-I stimulation during myogenesis and muscle mass maintenance. Activation of Akt has been shown to induce remarkable increases in the cross sectional area of skeletal muscle fibres, as a result of activated protein synthesis pathways (Glass, 2005). Furthermore, stimulation of the PI3K/Akt pathway by IGF-I promotes protein synthesis and blocks atrophy pathways (Glass, 2005).

mTOR signalling pathway

Intracellular signalling initiated by IGF-I leads to the

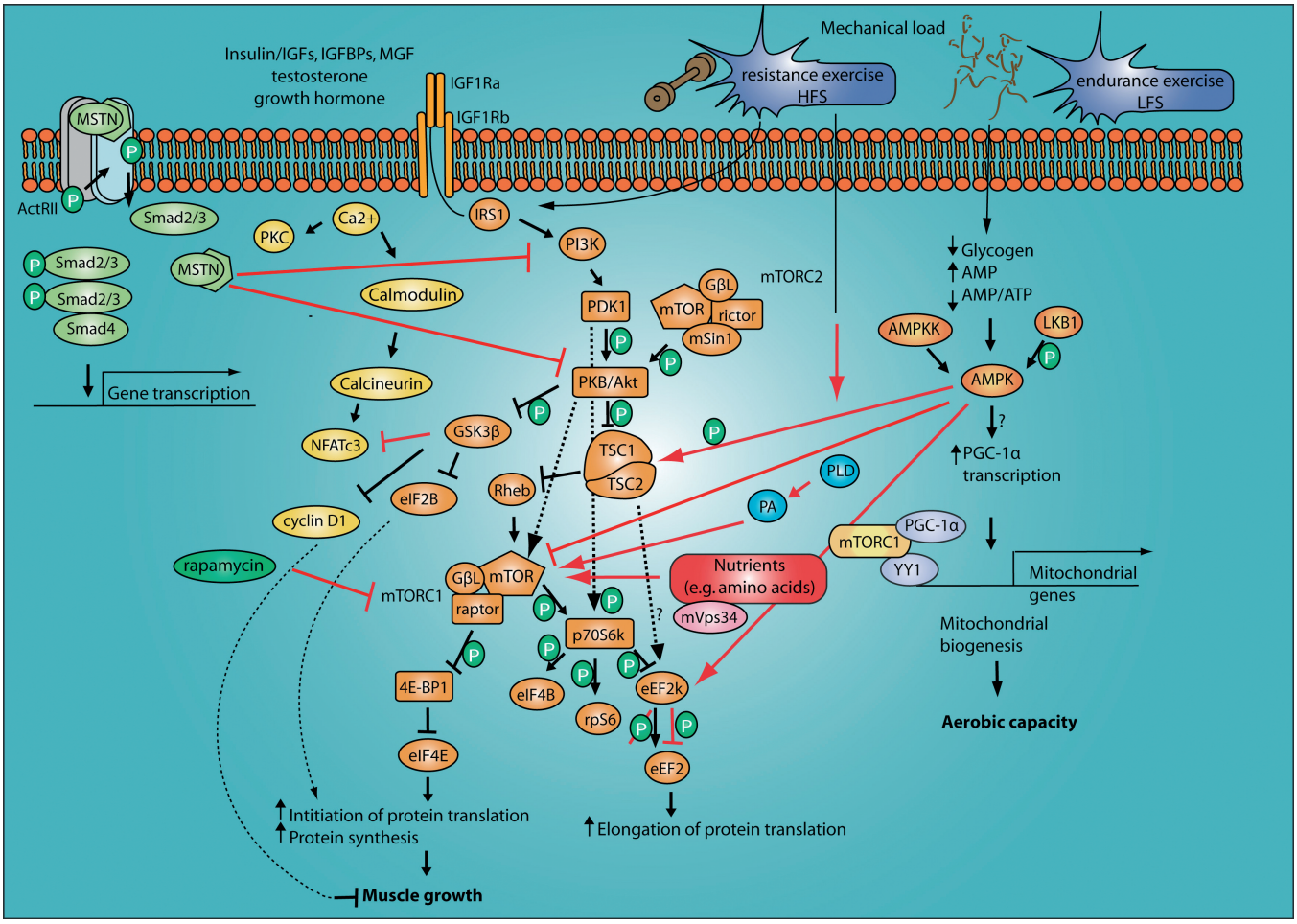

Fig. 1. Schematic representation of the main events from signalling pathways regulating skeletal muscle protein synthesis, cell growth and energy status. Red arrows indicate interactions between different signalling pathways. Dashed lines denote alternative actions of the signalling molecules.

activation of PKB/Akt. However, there are a number of key intermediary molecules downstream of PKB/Akt before a cell can respond by altering its protein synthesis programme. One of the key molecules is mammalian Target of Rapamycin, commonly known as mTOR. This molecule is a highly conserved checkpoint serine/threonine protein kinase that has emerged as a major regulator of cell growth and proliferation through the regulation of protein synthesis, by enhancing the initiation phase of protein translation (Hay and Sonenberg, 2004). The mTOR pathway is not only important in muscle mass development but has recently emerged as a critical factor in the etiology of cancer and metabolic diseases, including diabetes and obesity as well as in cell growth. Accumulating evidence over the past few years suggests that growth factor-induced activation of mTOR is mediated by PI3K. mTOR is considered as a downstream effector of the insulin/IGF-I receptor, usually activated by a variety of stimuli including hormones (i.e. insulin), growth factors (i.e. IGF-I) and amino acids (i.e. leucine), through sequential activation of PI3-kinase, PDK-1 and Akt/PKB. The latter acts to phosphorylate and activate mTOR (Fig. 1).

mTOR is found in two differing multiprotein complexes, mTOR complex 1 (mTORC1) and mTORC2, each exhibiting different cellular functions. The mTORC1 complex consists of mTOR, a protein called raptor (the regulatory associated protein of mTOR) and mLST8/G-protein ß-subunit-like protein (GßL). This complex is sensitive to rapamycin. mTORC1 regulates muscle mass development by controlling protein translation initiation via its two major downstream targets: the p70S6k and the eukaryotic initiation factor 4E binding protein 1 (4E-BP1). mTORC1 activity leads to the inhibition of 4E-BP1 which normally inhibits the action of eIF4E. When eIF4E becomes active it binds to eIF4G and forms the active eIF4F complex, which is needed for ribosomal assembly. mTOR not only initiates protein synthesis, by it also leads to an increase in the capacity of the cell to synthesize protein. This function is played out by the ability of mTOR to activate p70S6k which then phosphorylates rpS6 leading to enhanced translation (Ivy et al., 2008). The phosphorylation of 4E-BP1 and p70S6k, are often used as an in vivo readout of mTOR activity (Hay and Sonenberg, 2004). Insulin regulates the phosphorylation states of these proteins in a rapamycin-sensitive manner, implying that mTOR activity is required. Indeed, very recent evidence suggests that insulin increases the kinase activity of intact only (as opposed to disrupted mTORC1, where mTOR and raptor dissociate) dimeric mTORC1, a structure consisting of two heterodimers of mTOR, raptor and GßL. This insulin-induced mTORC1 kinase activity stimulation is associated with increased binding of substrate (e.g. 4E-BP1) to raptor, although the precise molecular mechanisms linking 4E-BP1 and p70S6k to mTOR remain to be fully elucidated (Wang et al., 2001, 2006).

mTOR also exists in another complex called mTORC2 which also contains mLST8/GßL (like the mTORC1 complex) but instead of raptor, it contains the rapamycin-insensitive companion of mTOR (rictor) and mitogen-activated protein kinase-associated protein 1 (mSin1). mTORC2 is required for the activation of PKB/Akt, which mediates insulin signalling and cell survival (Bodine et al., 2001; Sabrassov et al., 2005).

Both insulin and Akt induce the phosphorylation of mTOR in vivo. In particular, Akt activates mTOR, at least in part, through the phosphorylation and subsequent inactivation of TSC2 (Hay and Sonenberg, 2004). TSC1 (known as hamartin)/TSC2 (known as tuberin) protein heterodimer, are encoded by the tuberous sclerosis complex 1 (TSC1) and tuberous sclerosis complex 2 (TSC2) respectively, have been recognised as an upstream negative regulator of mTOR. TSC2 phosphorylation has been reported to disrupt TSC1/TSC2 heterodimer formation and accelerates degradation of TSC1 and TSC2, but the precise mechanism remains to be fully elucidated. One of the proposed major cellular functions of TSC1/TSC2 is to inhibit translation by inhibiting the phosphorylation of p70S6k and 4E-BP1 (Inoki et al., 2003). This can be achieved through Rheb, a small GTPase initially isolated as a Ras homologue enriched in brain, which appears to be a direct target for TSC2 and is capable of regulating mTOR signalling.

One of the key targets for mTOR, p70S6k, is considered to be essential for the hypertrophic process. There are two similar p70S6k phosphoproteins (a group of proteins which are chemically bonded to a substance containing phosphoric acid), isoforms 1 and 2 are encoded by different genes, but contain conserved phosphorylation sites between them. These serve as direct targets for mTOR and their respective phosphorylation state regulates their kinase activity towards the rpS6 protein. p70S6k1 has been mostly used in the vast majority of studies purely because of its earlier discovery than p70S6k2 and often referred to as p70S6k phosphorylation state based on the use of an antibody against p70S6k1 (Hay and Sonenberg, 2004; Wang and Proud, 2006). For the sake of consistency we will use the term p70S6k in this review. p70S6k plays a prominent role in the regulation of cell growth, proliferation and translational upregulation of mRNAs encoding for components of the protein synthetic apparatus, for example ribosomal proteins and elongation factors (Hornberger et al., 2006; Stewart and Rittweger, 2006). Its activity is dramatically increased through multisite phosphorylation in response to insulin. In particular, p70S6k is necessary for the progression through G1 phase of the cell cycle and disruption of the p70S6k gene or inhibition of p70S6k with the drug rapamycin results in smaller muscle cell size (Hornberger et al., 2006).

mTOR activity and energy

A significant volume of work has shown that the

ability of skeletal muscle to undergo anabolic development is linked to the energy status of the tissue. Recent work has now established links between the cellular energy and signalling pathways that lead to muscle protein synthesis. The ability of insulin and probably IGF-I to activate mTOR is impaired either by a reduction in cellular ATP levels, or by reduced glucose availability or the inhibition of mitochondrial respiration, suggesting that the levels of cellular energy impact mTOR activity (Kimura et al., 2003; Hay and Sonenberg, 2004).

A key player linking energy status of the cell and muscle growth is Adenosine Monophosphate-Activated kinase (AMPK) whose activity is regulated by even moderate changes in ATP levels and can sense the AMP/ATP ratio (Hardie and Sakamoto, 2006). This is important for muscle growth since AMPK activation leads to a decrease in mTOR activity. Research data provide evidence for a link between intracellular ATP levels, AMPK, and mTOR activity, whereby AMPK senses a decrease in cellular ATP and becomes activated to phosphorylate and activate effectors such TSC2, which normally inhibits mTOR activity (Kimura et al., 2003; Hay and Sonenberg, 2004). AMPK induced phosphorylation of TSC2 at Thr1227 or Ser1345 which improves the ability of TSC2 to inhibit mTOR activity, independent of the possibility that mTOR activity may be regulated by additional non TSC2-associated mechanisms (e.g. nutrients, see Fig. 1; Inoki et al., 2003; Dreyer et al., 2006). Hence, increased energy demands depress protein synthesis through an interaction between AMPK and mTOR signalling pathways (Fig. 1). Activated AMPK is involved in numerous cellular functions such as fuel metabolism.

Overall, AMPK is responsible for inhibiting anabolic processes that require energy and for stimulating catabolic processes that generate energy. Treatment of rats with an AMPK-activator (AICAR) resulted in reduced skeletal muscle protein synthesis, accompanied by a downregulation of PKB-mTOR and its downstream targets p70S6k and 4E-BP1 (Rennie et al., 2004). At present, it is accepted that energy metabolism and protein synthesis are tightly coupled. AMPK seems to mediate this coupling via phosphorylation and activation of TSC2 (upstream inhibitor of mTOR) or alternatively by directly phosphorylating and inactivating mTOR at Thr2446, which can prevent the Akt phosphorylation of mTOR at Ser2448, resulting in inhibition of translation initiation. However, the mechanism by which TSC2 activity is affected by AMPK-mediated phosphorylation is under current intense investigation and remains to be yet fully elucidated (Cheng et al., 2004; Hay and Sonenberg, 2004). In accordance, AMPK may inhibit translation elongation through a direct phosphorylation of eEF2k (Horman et al., 2002; Wang and Proud, 2006; Fig. 1).

Apart from its central role in the regulation of protein synthesis, mTOR has been recently reported to be necessary for the maintenance of mitochondrial oxidative function by promoting mitochondrial gene expression and oxygen consumption in an Akt-independent manner (Cunningham et al., 2007). These authors demonstrated decreased gene expression levels of the mitochondrial transcriptional regulators peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), estrogen-related receptor and nuclear respiratory factors followed by impaired mitochondrial gene expression and oxygen consumption in skeletal muscles and myotubes, in response to treatment with the mTOR inhibitor rapamycin. Interestingly, the transcription factor yin-yang 1 (YY1) was identified as a common target for both mTOR and PGC-1α, meaning that YY1 is able to bind directly to mitochondrial gene promoters and PGC-1α functions as a transcriptional coactivator for YY1 in an mTOR-dependent manner. The transcriptional function of YY1-PGC-1α is regulated by mTOR through a direct alteration of their physical interaction. Thus, mTOR inhibition leads to a failure of YY1 to interact with and be co-activated by PGC-1α (Cunningham et al., 2007). The facts that activated AMPK may inhibit the mTOR signalling pathway and thus suppress protein synthesis but on the other hand mTOR appears to be required for mitochondrial gene expression seem to represent a paradox. However, one should bear in mind that the AMPK-induced mTOR inhibition is a rather short-term effect driven by low cell energy status (i.e. in response to endurance exercise or food deprivation), while during the recovery phase or upon nutrient availability mTOR might serve to increase PGC-1α function in order to maintain cellular energy levels. However, this speculation remains to be fully examined in vivo.

Nutrient infusion

Nutrient availability, growth factors as well as energy metabolism and stress, all regulate mTOR activity (Hay and Sonenberg, 2004; Wullschleger et al., 2006). mTOR plays an important role, not only in insulin and mitogen signalling, but also in the regulation of cellular function in response to nutritional cues (Rennie, 2007). In mammalian cells, growth factors and cytokines, in addition to regulating nutrient uptake, also activate signalling pathways that act in parallel or in concert with nutrients (Hay and Sonenberg, 2004). Amino acids, hormones and physical activity are considered major short-term physiological regulators of muscle mass, mainly through affecting protein synthesis or breakdown (Rennie, 2007).

Dietary supplementation with essential amino acids and carbohydrates was recently found to selectively ameliorate the deleterious effects of prolonged bed rest in human soleus but not vastus lateralis muscle (e.g. fibre atrophy and loss of force and power as a result of impaired protein synthetic rate), indicating that dietary supplementation could preserve muscle function during inactivity, a finding that remains to be further investigated (Fitts et al., 2007). In addition, amino acid

administration has been shown to stimulate protein synthesis 30-120 minutes after infusion (Blomstrand et al., 2006). However, there is evidence that despite a continued availability of amino acids, muscle protein synthesis seems to have a limited duration exhibiting an upper limit directly related to the amount of protein that can be incorporated into the muscle over a certain period of time (Rennie et al., 2006). Hence, excess amount of amino acids that have not been incorporated into tissue protein within 2-3 hours after infusion are likely to be removed through the urea pathway.

Although exogenous nutrients have been reported not to be required for mechanically induced rapamycin signalling, it appears that in their presence, p70S6k phosphorylation and hence the process of elongation of protein translation is optimised (Hornberger and Chien, 2006). Amino acids have been proposed to activate mTORC1 via inhibition of TSC1-TSC2 or probably via stimulation of Rheb and they pass nutrient information to the protein synthesis machinery through phosphorylation of p70S6k and its downstream target rpS6 (Wullschleger et al., 2006). A change in the configuration of the mTOR-Raptor complex, which is mediated by nutrient conditions such as amino acid availability, affects the ability of mTOR to interact with and phosphorylate its substrates. In the absence of amino acids, the mTOR-mLST8-Raptor complex prevents mTOR from binding to its substrates. On the other hand, in the presence of amino acids, a conformational change promotes an efficient interaction between Raptor and mTOR substrates.

Hornberger and Chien (2006) demonstrated that stimulation of skeletal muscle with nutrients such amino acids induces signalling to p70S6k, as a part of the rapamycin pathway. However, these authors provided evidence that unlike growth factors, amino acid stimulation does not produce any detectable increase in PKB phosphorylation, in accordance with a previous finding that amino acid-induced rapamycin signalling is independent of PI3K activation. Nevertheless, these authors do not rule out the possibility that amino acid stimulation can cause a transient increase in PKB phosphorylation in a short time frame that they did not measure. The existence of two distinct pathways that promote rapamycin signalling has been proposed by the same authors (Hornberger and Chien, 2006). One core pathway is utilized by growth factors and nutrients in a PI3K-dependent manner, while the other core pathway is activated by mechanical stimuli in a PI3K-independent manner that is not yet fully understood.

Essential amino acids stimulate protein synthesis via a nutrient-sensitive complex of the proteins Raptor and mTOR. Hence, increased availability of essential amino acids is sensed by the Raptor-mTOR complex and activates mTOR as well as downstream translational regulators, under the involvement of the positive effector GßL, which upon binding to mTOR strongly stimulates downstream targets such p70S6k, eIF2 and 4E-BP1 (Rennie et al., 2004). Meal feeding stimulates two signal pathways in skeletal muscle, elevating the eIF4G-eIF4E complex through increased phosphorylation of eIF4G and decreased association of 4E-BP1 with eIF4E (Vary and Lynch, 2007). As mentioned above, amino acids may activate protein synthesis through mTOR and its downstream effector activation. However, amino acids can also indirectly phosphorylate 4E-BP1 and p70S6k via an mTOR independent pathway, resulting in the inhibition of 4E-BP1 and activation of p70S6k (Kimball and Jefferson, 2004). Administration of protein with carbohydrate provides an option to further increase phosphorylation of these enzymes and thus enhance protein synthesis (Ivy et al., 2008).

Branched-chain amino acid (BCAA-leucine, isoleucine and valine) administration has been shown to increase the rate of protein synthesis by inducing mTOR phosphorylation on Ser2448 and further activation of p70S6k through phosphorylation on several residues such as Thr389 in human muscle via an Akt-independent pathway (reviewed by Blomstrand et al., 2006). In addition, increased phosphorylation of p70S6k on Ser424/Thr421 after BCAA treatment has been reported without mTOR activation. BCAA uptake has been shown to play a role in central fatigue. It has been hypothesized that the administration of BCAA is involved in central fatigue delay, probably through a mechanism that decreases brain tryptophan uptake and subsequently impairs the neurotransmitter 5-hydroxytryptamine synthesis and release or even by means of a probable action of BCAA as neuro-transmitters per se or influencing other biochemical pathways (Newsholme and Blomstrand, 2006).

Acute administration of a leucine-enriched essential amino acids and carbohydrate mixture has been reported to both enhance Akt/mTOR anabolic cell signalling (as judged by increased phosphorylation of Akt/PKB at Ser473, mTOR at Ser2448, 4E-BP1 at Thr37/46, especially p70S6k at Thr389 and decreased phosphorylation of eEF2 at Thr56) and protein synthesis, while decreasing AMPK phosphorylation at Thr172, implying that AMPK might serve as an important mechanistic link between the intake of nutrient and protein synthesis (Fujita et al., 2007; Thomson et al., 2008). It is worth mentioning that the role of eEF2 is to promote the translocation step of elongation, in which the ribosome moves by the equivalent by one codon relative to the mRNA and the peptidyl-tRNA migrates from the ribosomal A site into the P site (ribosome sites where aminoacyl-tRNAs can bind during translation initiation), giving rise to a new peptide bond (Wang and Proud, 2006).

Leucine seems to be the most potent BCAA acting as a signal for accelerating protein synthesis. It brings about a response within 20 minutes after oral intake and it appears that signalling through mTOR is important for mediating, at least in part, the effects of leucine feeding on protein translation in muscle, as judged by leucine administration and subsequent mTOR inhibition. In vitro data indicate that in the absence of other regulatory

agents, leucine modulates distinct steps of translation initiation and protein synthesis directly in skeletal muscle through a signalling pathway independent of mTOR. Hence it has been suggested that leucine alone can substitute for a meal in stimulating signal transduction pathways, leading to a stimulation of protein synthesis by enhancing mRNA translation initiation (Vary and Lynch, 2007). Although the sensor protein in mediating the effects of leucine on mTOR remains to be identified, novel data illustrate a probable candidate (see below).

Nutrients and exercise

A potential candidate for controlling muscle protein synthesis in response to resistance exercise appears to be the mammalian vacuolar protein sorting mutant 34 (mVps34), a widely distributed protein found in most tissues which controls protein degradation and amino acid sensitivity and plays an important role in the ability of cells to respond to changes in nutrient conditions. An interesting mechanism whereby mVps34 might act as an internal amino acid sensor to mTOR after resistance exercise has been recently proposed, since an acute bout of exercise has been reported to activate mVps34 3-6 hours post-exercise (MacKenzie et al., 2007; Backer, 2008). Both fuel depletion (i.e. glucose and amino acid starvation) and AMPK activation inhibit mVps34, which is required for insulin-stimulated mTOR activation under nutrient-replete conditions. Thus, inhibition of mVps34 blocks insulin-stimulated phosphorylation of both p70S6k and 4E-BP1, while overexpression of mVps34 activates p70S6k in the absence of insulin stimulation. Conversely, addition of amino acids to starved cells is followed by increased mVps34 activity. Thus, mVps34 plays paradoxically opposing roles in nutrient sensing, as a positive effector of autophagy, and as a positive effector of mTOR, which inhibits autophagy (Fig. 2). Subsequently, mTOR is recognised both as a growth factor and a nutrient-sensitive molecule that controls the translational machinery and cell growth (Hornberger and Chien, 2006). However, although mVps34 is required for the activation of the mTOR pathway, a direct effect of mVps34 on mTOR kinase activity remains to be established (Backer, 2008).

Increased IGF-I mRNA and protein expression in response to mechanical load is well established in the literature (Adams and Haddad, 1996). Although IGF-I is a potent activator of skeletal muscle growth and is believed to act through both the activation and proliferation of satellite cells as well as the enhancement of protein translation, resulting in an increased rate of protein synthesis (Adams, 2002), recent data provide evidence that the IGF-I receptor is not necessary for the induction of skeletal muscle growth in response to mechanical loading. Mice deficient in the IGF-I receptor were shown to retain the capacity to induce muscle growth in response to functional overload and components of the Akt-mTOR pathway, critical for the induction of muscle growth, were found to be activated (Spangenburg et al., 2008). In addition, these authors concluded that although exogenous IGF-I may induce muscle hypertrophy, the production of endogenous IGF-I by skeletal muscle and activation of the IGF-I receptor are not necessary for the activation of the Akt signalling pathway or the induction of muscle growth in response to mechanical load. In support of this hypothesis, another study has reported increased mTOR signalling in a fibre-type dependent manner, after a single bout of muscle contractile activity independent of Akt/PKB phosphorylation (Parkington et al., 2003). In particular, these authors demonstrated that one session of high-

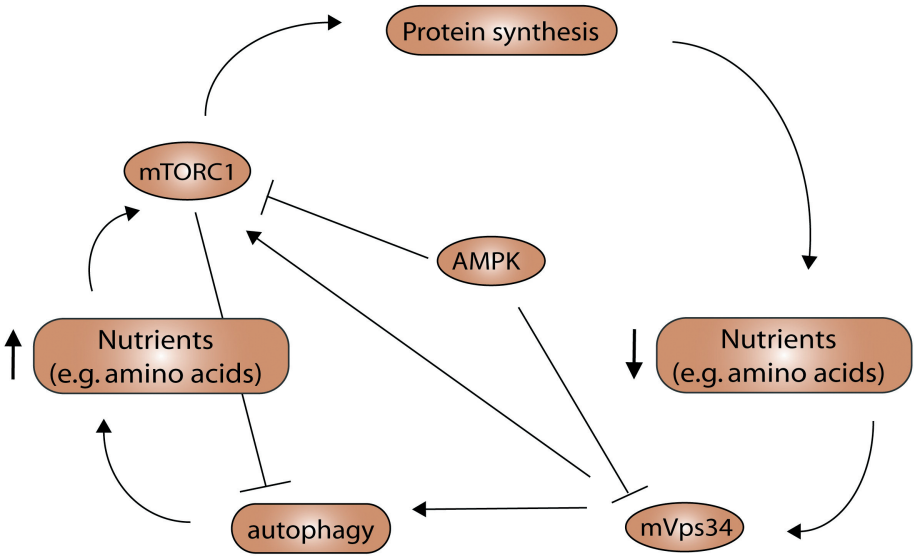

Fig. 2. The potential role mVps34 in skeletal muscle protein synthesis.

frequency electrical stimulation in rat plantaris, tibialis anterior and soleus muscles led to increased phosphorylation of both mTOR (at Ser2448) and p70S6k (at Thr389) in plantaris and tibialis anterior muscles (despite a potent but delayed response) but not in soleus (Table 1). Interestingly, mTOR phosphorylation in tibialis anterior muscle was found to be specifically localised in a subset of fibre types characterized as IIa fibres.

On the other hand, muscle-specific overexpression of the IGF-IR can mediate skeletal muscle hypertrophy in cultured rat skeletal myotubes in a myoblast-independent fashion by PI3K, but not calcineurin signalling. Cultured rat skeletal myoblasts overexpressing the IGF-IR accumulated significantly higher levels of myosin heavy chain (MHC) and α-actin, accompanied by increased protein synthesis. However, the stimulation of muscle hypertrophy was independent of myoblast contributions, based on the fact that these cultures did not exhibit increased levels of myoblast proliferation or differentiation, indicating that IGF-I may have myoblast-independent effects on skeletal muscle protein dynamics and muscle hypertrophy (Quinn et al., 2007).

Protein and carbohydrate loading post-exercise promotes protein synthesis and the protein breakdown is blunted in an insulin-related effect, so that the overall effect is a marked increase in the net protein balance of the muscle (Rennie, 2007). Recent data provide evidence that a transient activation of proteins that stimulate protein synthesis takes place during the recovery phase after acute moderate to high-intensity endurance exercise. In particular, Akt, mTOR and GSK-3α/ß phosphorylation is significantly increased at various time points with a different time course in the first 2 hours after an acute bout of endurance exercise, as opposed to eEF2 dephosphorylation, returning to the baseline levels by 3 hours post-exercise (Mascher et al., 2007). The different time course in the changes of Akt and mTOR phosphorylation, as judged by the much earlier phosphorylation of mTOR, indicates that mTOR is activated not only by Akt but also through alternative pathways (Mascher et al., 2007). Inhibition of mTOR by pre-treatment with rapamycin just prior to resistance exercise prevents an increase in protein synthesis 16 hours post-exercise, indicating once more that mTOR activity is important in the hypertrophic response (Kubica et al., 2005; Thomson, 2008). Table 1 summarizes results of recent studies aimed to analyse the effect of mechanical loading e.g. exercise and/or nutritional supplementation on the phosphorylation state of key proteins involved in the skeletal muscle protein synthesis signalling.

Intake of amino acids after a bout of resistance training stimulates protein synthesis, leading to a positive protein balance (Blomstrand et al., 2006). However, acute resistance exercise in the absence of branched-chain amino acids increased p70S6k phosphorylation on Ser424/Thr421, but not on Thr389, suggesting that BCAA supplementation combined with acute resistance exercise leads to increased site-specific phosphorylation and activation of p70S6k in skeletal muscle. It is worth bearing in mind that apart from phosphorylation of Ser424/Thr421, phosphorylation on Thr389 is required to fully activate the kinase (Karlsson et al., 2004; Blomstrand et al., 2006). Subsequently, activated p70S6k enhances mRNA translation through increases in rpS6 phosphorylation (Blomstrand et al., 2006). Thus, it appears that branched chain amino acids, and particularly leucine, which might substitute for a complete protein meal, may stimulate muscle protein synthesis for a short period of time, via signalling pathways involving mTOR in a dose-response manner. This suggests that leucine acts both as a signal as well as a substrate. However the total contribution of BCAA to fuel provision during exercise is minor and insufficient to increase dietary protein requirements (Rennie et al., 2006; Vary and Lynch, 2007).

The type of exercise (i.e. intensity and duration) seems to be critical in the regulation of skeletal muscle protein synthesis. Hence, resistance exercise is expected to increase muscle strength and mass, whereas endurance exercise is followed by increased oxidative and aerobic capacity (increased oxygen consumption, fat oxidation, mitochondrial density, vascularization). Trying to elucidate the molecular mechanisms underlying skeletal muscle adaptability in response to different types of exercise, Atherton et al. (2005) applied both low and high electrical stimulation patterns to isolated rat muscles, in order to mimic endurance and resistance exercise respectively and measured the phosphorylation state of major activity-related sites of various effectors of the AMPK and PKB/Akt pathway. These authors reported a selective activation of the AMPK-PGC-1α signalling and suppression of TSC2 and downstream regulators of translation initiation and elongation with regard to endurance-like low frequency stimulation. On the other hand, resistance-like high frequency stimulation lead to a pronounced activation of PKB-TSC2-mTOR signalling and its downstream translational regulators (Atherton et al., 2005).

Although both acute endurance exercise (e.g. treadmill running, cycling) and low-frequency electrical nerve stimulation do not seem to significantly modulate p70S6k activation, it appears that high-frequency electrical stimulation, resistance exercise and either high force isometric or eccentric contractions increase its activity (Nader and Esser, 2001; Haddad and Adams, 2002; Atherton et al., 2005; Coffey et al., 2006; Mascher et al., 2007). However, apart from the type of exercise (endurance vs. resistance) another critical factor affecting the early signalling responses to divergent exercise stimuli in skeletal muscle is any previous training-specific adaptations and the individuals training history (Coffey et al., 2006). For instance, increased Akt phosphorylation after cycling was found only in endurance- but not strength-trained group, without changes after resistance exercise in either group (for

details see Table 1). Similarly, decreased TSC2 phosphorylation after resistance exercise was reported only in endurance-trained subjects, without remarkable alterations following cycling in either group. In addition, increased p70S6k phosphorylation three hours after resistance exercise was found only in endurance- but not strength-trained men, while remained similar to rest levels after cycling in both groups. Phosphorylation of rpS6 protein (a substrate for p70S6k) was increased immediately after resistance exercise in endurance- but not strength-trained subjects. In a consistent manner, increased AMPK phosphorylation after cycling was found only in strength- but not endurance-trained individuals and on the contrary, elevated AMPK phosphorylation after resistance exercise was reported only in endurance- but not strength-trained men (Coffey et al., 2006).

Similarly, Dryer et al. (2008) aimed to analyse protein synthesis signalling in response to the administration of a leucine-enriched essential amino acid and carbohydrate solution 1 hour after resistance exercise (Table 1). Although the total protein contents of Akt, TSC2, mTOR, 4E-BP1, p70S6k and eEF2 remained unchanged, their phosphorylation states in part increased for Akt at Ser473, mTOR at Ser2448, 4E-BP1 at Thr37/46, most remarkably p70S6k at Thr389 and decreased for eEF2 either immediately after the exercise stimulus and/or 1 hour post exercise and/or remained elevated and were positively affected 2 hours post exercise only in the group that received the amino acids/carbohydrate mixture. Furthermore, while mixed muscle protein synthesis increased at a rate of 41% compared to the pre-exercise state, amino acid/carbohydrate intake lead to a 145% increase, implying a more anabolic additive response when combined than either amino acids or exercise separately (Dreyer et al., 2008).

As mentioned above, the enzyme p70S6k is phosphorylated on several residues including Ser424/Thr421, but phosphorylation of p70S6k at Thr389 is required for full activation of the kinase. Although several studies regarding the effect of exercise on the phosphorylation state of key enzymes involved in muscle protein synthesis failed to show any activation of the Thr389 residue during recovery in the absence of nutrient uptake e.g. BCAA (Karlsson et al., 2004, Table 1), recent evidence suggests that maximal eccentric (but not concentric) contractions cause a significant increase in p70S6k phosphorylation on both Ser424/Thr421 and Thr389 in the absence of nutritional update, implying a full kinase activation and subsequent stimulation of protein translation that persists for 2 hours into recovery (Eliasson et al., 2006). In accordance, activation of the p70S6k is further indirectly supported by the markedly elevated phosphorylation of rpS6, the substrate of p70S6k (Eliasson et al., 2006). However, no effect of the mode of contraction (shortening vs. lengthening) on the magnitude or time course of the signalling protein activation or on rates of myofibrilar and sarcoplasmic protein synthesis have also been reported, indicating that several variables within the experimental design (e.g. voluntary vs. electrical stimulated contraction, the potentially exhausting nature of the exercise regime used and the nutritional status post-exercise) could have accounted for the observed fluctuation (Cuthbertson et al., 2006). Aside from the mode of contraction, it has been postulated that the activation of p70S6k is muscle fibre type-specific, since experimental animal data have shown an activation in fast-twitch muscles (e.g. tibialis anterior and plantaris) as opposed to slow-twitch ones (e.g. soleus, Parkington et al., 2003). Ivy et al. (2008) examined the effect of a carbohydrate-protein supplement on the phosphorylation state of Akt, mTOR, p70S6k, rpS6, GSK3α/ß and glycogen synthase (GS) after one bout of variable intensity endurance cycling in young men (Table 1) and found significantly increased phosphorylation of Akt at Ser473 and mTOR at Ser 2448 only after nutritional supplementation 45 min post-exercise. Although exercise stimulus led to increased phosphorylation of p70S6k at Thr412 in the absence of any additive effect of supplementation and a similar increase in rpS6 at Ser235/236 post-exercise, a further significant increase was obtained following carbohydrate-protein administration. These results may have important implications for training recommendations during rehabilitation, recreational and competitive activity.

Overall, amino acid availability is considered as a primary regulator of mTOR signalling and muscle protein synthesis in skeletal muscle, while insulin is likely to play a supportive and permissive role in the regulation of muscle protein synthesis, given that insulin fails to stimulate muscle protein synthesis in humans when amino acid availability is reduced (Fujita et al., 2007). However, an amino acid/carbohydrate supplement combination enables a larger stimulation of skeletal muscle protein synthesis than amino acids alone. As a matter of fact, the role of BCAA in metabolism and physiology has been highlighted in numerous studies aimed to explore the potential mechanisms underlying the regulation of the protein synthetic machinery.

To summarise, several lines of evidence have shown that resistance exercise-induced muscle protein synthesis is stimulated during a post-exercise recovery period of between 1-24 hours depending on previous training history of the individual and may even endure up to 48 hours in untrained individuals (Phillips et al., 1997; Dryer et al., 2008). Nutrient administration may promote protein synthesis by activating components of the mTOR pathway, either via the known insulin-stimulated Akt/mTOR signalling pathway or bypassing it through a novel class of PI3k receptor mVps34 (Byfield et al., 2005; Nobukuni et al., 2005).

The molecular basis of concurrent strength and endurance training

Several studies within the past twenty five years

have addressed the issue of the potential interference of concurrent strength and endurance training, providing evidence both for and against an impairment of strength development when training simultaneously for strength and endurance (e.g. Hickson, 1980; Nelson et al., 1990; McCarthy et al., 1995). More recently, accumulating evidence at the molecular level sheds light on this debate and suggests that strength and endurance training induce antagonistic intracellular signalling pathways, which could in specific situations have a negative impact on the adaptive response of the muscle interfering with the development of strength (e.g. Baar, 2006; Nader, 2006).

Apart from the potential mechanisms enlisted in the literature that could account for the interference of strength development during concurrent strength and endurance training (i.e. reduced muscle glycogen, fibre type transformation, protein turnover), molecular studies regarding the cellular regulatory processes induced by different forms of exercise, proposed that activation of AMPK by endurance exercise may inhibit both the mTOR activity and the anabolic response downstream of mTOR signalling, hence decreasing the protein synthesis signalling network (Fig. 1). This may be achieved through the phosphorylation (on Ser1345) and activation of the TSC2 complex (an upstream regulator of the mTORC1 pathway) by AMPK, a fact which blocks the activation of the mTORC1 and thus inhibits skeletal muscle hypertrophy (given that mTORC1 activation in response to resistance exercise or PKB/Akt activation results in muscle hypertrophy (Bodine et al., 2001; Inoki et al., 2003; Sabrassov et al., 2005)). Hence, the effects of resistance exercise are modulated through increased PKB and TSC2 phosphorylation, while those of endurance exercise through AMPK and inhibition of the signalling effect of TSC2 on mTOR (Rennie, 2007).

Furthermore, calcium fluxes resulting from prolonged contractile activity may inhibit protein synthesis in a calcium-dependent calmodulin manner by inhibiting the elongation step of translation via activation of eEF2k and a subsequent increase in eEF2 phosphorylation, which is inhibitory upon its activity and consistent with an inhibition of protein translation (Nader, 2006). In accordance with this, AMPK activation by AICAR treatment inhibited translational signalling downstream of Akt/mTOR via the suppression of p70S6k, 4E-BP1 and eEF2 signalling responses after electrically stimulated lengthening muscle contractions in the rat extensor digitorum longus (Thomson et al., 2008). In parallel, AMPK seems to activate eEF2k in a less clearly defined mechanism, probably by phosphorylating this kinase at Ser398, which again leads to eEF2 phosphorylation and inactivation, serving to slow protein translation elongation when cell energy levels fall (Browne and Proud, 2002; Wang and Proud, 2006).

Enhanced mTOR-p70S6k signalling activates eEF2 by phosphorylating and inactivating eEF2k. Inactivation of eEF2k is followed by eEF2 dephosphorylation at Thr56 and hence activation of eEF2 and promotion of translation elongation. However, AMPK-induced eEF2k activation (at a stimulatory eEF2k site (e.g. Ser398), different than the inhibitory site phosphorylated by p70S6k (e.g. Ser366)) leads to phosphorylation of eEF2 at Thr56 by eEF2k, which inactivates it by suppressing its activity, not allowing the attachment of eEF2 to ribosomes and thereby inhibiting translation elongation (Wang et al., 2001; Wang and Proud, 2006). Accordingly, a robust activation of AMPK in response to endurance exercise may act as a negative upstream regulator of mTOR, serving to attenuate the translational signalling response downstream to mTOR (regarding to this type of stimulus), in order to reduce the energetic cost of protein synthesis at a time when the cell needs to restore ATP levels. This provides a possible explanation for the impaired muscle hypertrophy and strength gain observed after endurance exercise (Thomson et al., 2008). Hence, it can be assumed that the degree of AMPK activation, as an index of energetic stress, after resistance exercise may be an important factor with an antagonistic role in the regulation of the hypertrophic translational signalling response to this kind of stimulus. Consequently, the aspect of concurrent strength and endurance training should be considered for the design of optimal exercise training programs, mainly when the primary aim of the training contents is to improve strength and induce skeletal muscle hypertrophy.

The role of Myostatin in skeletal muscle protein synthesis

A highly complex network of interacting pathways with great physiological significance precisely controls cell growth. Two differing signalling molecules, IGF-I and MSTN (MSTN), regulate key steps during muscle growth (for review see e.g. Patel et al., 2005; Matsakas and Diel, 2005). IGF-I (growth-promoting factor) and MSTN (growth-suppressing factor) function in an opposing manner to maintain cell homeostasis by cross-talking through the PI3K/Akt/GSK-3ß pathway (Fig. 1). Recent data has provided a direct biochemical evidence for a novel role for MSTN in the regulation of muscle cell growth and proliferation which functions through GSK-3ß activation and PI3K/Akt inhibition independent of the typical Smad2/3 pathway (Yang et al., 2007). More specifically, MSTN inhibits the action of Akt by preventing its phosphorylation at Ser473, which in turn prevents the phosphorylation (at Ser9) and inactivation of GSK-3ß. Unphosphorylated and therefore active GSK-3ß controls cell proliferation. GSK-3ß once activated translocates to the nucleus and phosphorylates cyclin D1, which in turn is exported from the nucleus to the cytoplasm, where the phosphorylated cyclin D1 is degraded through the proteosome-mediated pathway (Yang et al., 2007). Loss of cyclin D1 reduces the proliferation rate of the cell. Overall, although IGF-I and MSTN exhibit opposite functions in cell growth control, they act through the same PI3K/Akt/GSK-3ß signalling pathway. Based on the fact that IGF-I may potentially

induce MSTN expression via the above mentioned pathway, but MSTN does not induce IGF-I expression, one may consider that MSTN functions as a local skeletal muscle cell IGF-I induced chalone, aiming to limit muscle growth induced by growth-promoting factors (Yang et al., 2007). Thus, muscle growth control is regulated by the interplay of growth promoting- and suppressing-factors, acting in concert with one another.

Conclusions and perspectives

Advances in molecular biology and physiology have led to a plethora of recent studies about the phenotypical adaptation of skeletal muscle to various stimuli, providing novel findings about the molecular mechanisms involved in the multicomplexed cellular processes. There is currently an intense research interest in the signalling pathways that control skeletal muscle protein synthesis. mTOR signalling plays a very important role in this process, although it still remains to fully unravel the way that muscle protein synthesis is regulated mainly under physiological conditions in whole systems such as tissues or organisms. Identification of new key molecules or novel functions of already known parameters that play a major role in cellular signalling cascades and interact with different pathways are the basis for further expanding our current understanding about skeletal muscle adaptability and will provide new insights regarding muscle therapeutic strategies or exercise training recommendations.

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