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The Importance of mTOR Trafficking for Human Skeletal Muscle Translational Control

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Abstract

The mechanistic target of rapamycin (mTOR) is a central regulator of muscle protein synthesis, and its activation has long been attributed to its translocation to the lysosome. Here we present a novel model of mTOR activation in skeletal muscle where the translocation of mTOR and the lysosome toward the cell membrane is a key process in mTOR activation.

Key Points

- Cell and murine models suggest that movement of mechanistic target of rapamycin (mTOR) to the lysosome is a fundamental processes enhancing mRNA translational capacity.
- Recent work from our laboratory suggests that translocation of mTORC1/lysosomal complexes towards the cell membrane is a key event in mTORC1 activation following resistance exercise and amino acid ingestion in human skeletal muscle.
- mTORC1/lysosomal complex translocation facilitates mTORC1 interaction with upstream activators (Rheb), translation initiation factors (eIF3F) and the microvasculature during a period of increased protein synthesis.

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This review will critique cell, rodent, and human models of mTOR regulation to discuss why mTOR trafficking may represent a novel and physiologically relevant model of regulation in skeletal muscle.

Key Words: mTORC1; Lysosome; Resistance Exercise; Amino acids; skeletal muscle.

INTRODUCTION

This paper presents the novel hypothesis that the translocation of mTORC1/lysosomal complexes to the cell membrane is a critical factor driving the initial phase of protein translation in human skeletal muscle following resistance exercise or amino acid ingestion.

The importance of protein balance for skeletal muscle regulation.

Skeletal muscle is a highly plastic tissue, displaying an ability to both grow and decrease in size and structure regularly throughout lifespan. The control of skeletal muscle homeostasis is provided through the balance of two dynamic processes, skeletal muscle protein synthesis (MPS) and breakdown (MPB), with each varying significantly on a day-to-day basis (1, 2). Following ingestion of amino acids/protein, the greater amounts of substrates available for MPS, and the activation of signalling pathways, causes MPS to rise (2), while the insulinogenic effects of amino acids and other constituents of meals (carbohydrates) elicit a slight suppression of MPB (3). This culminates in a time period where MPS exceeds MPS and net protein balance (NPB) is positive. In these periods muscle proteins will be accreted. In postabsorptive states, when substrates for MPS aren't readily available, MPS will lower and MPB (ubiquitin-ligase and autophagic systems) will increase in order to provide any needed amino acids and remove damaged/dysfunctional proteins (4). During these periods, MPB will surpass MPS causing a negative NPB and muscle protein loss. In individuals who have a reasonably active lifestyle and a healthy, balanced diet, these periods of net muscle gain and loss, throughout a day, will be equal therefore causing muscle mass maintenance.

An exercise stimulus, both aerobic and resistive in nature, also stimulates MPS (5, 6) although the extent of this is much greater following resistance exercise. In addition, such stimuli elicit increases in MPS such that exercise conducted in the fasted/postabsorptive state, with no post-

exercise nutrients ingested, only serves to allow NPB to become less negative (6). However, exercise does serve to sensitise the muscle to nutrients, and an increase in the amount of amino acids available post-exercise, i.e. via a protein beverage, will cause a rise in MPS that is greater than that stimulated by either exercise or nutrients alone (7). Again, a slight suppression of MPB is elicited via the effects of insulin (3) causing NPB to become positive and the muscle enters a state of protein accretion. If this process is repeatedly regularly such that daily NPB is frequently positive then, over time, skeletal muscle hypertrophy will likely occur (8).

mTOR is a central regulator of MPS in skeletal muscle

At the centre of the regulation of skeletal muscle MPS is the mechanistic target of rapamycin (mTOR). This evolutionary conserved serine/threonine kinase resides in two complexes, each exhibiting unique roles (9). mTOR complex 1 (hereafter referred to as mTORC1) is comprised of mTOR, Regulatory associated protein of mTOR (RAPTOR), DEP domain-containing mTOR-interacting protein (DEPTOR), Proline-rich AKT substrate 40kDa (PRAS40) and G-protein beta subunit-like (GβL) (9) and is involved in cellular growth (10). In contrast, mTOR complex 2 (mTORC2) contains the rapamycin insensitive companion of mTOR (RICTOR), mammalian stress-activated protein kinase interacting protein 1 (mSIN1), PROTOR-1/2, DEPTOR and GβL and coordinates actin cytoskeleton dynamics and glucose uptake (9). Due to its roles in growth regulation, mTORC1 is the mTOR complex which is believed to be primarily associated with the control of skeletal muscle NPB (11). Intensive research has therefore focussed on understanding mTORC1's downstream activity and how this complex can stimulate skeletal muscular hypertrophy in response to anabolic stimuli (resistance exercise and protein feeding).

In vitro studies have comprehensively characterised mTORC1 substrates and their roles in cellular growth. The most well-known mTORC1 targets are p70 S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1), both of which are phosphorylated in an mTORC1-dependent manner in many cell types under nutrient-rich conditions (12, 13). The phosphorylation of S6K1 activates its kinase capabilities, allowing it to phosphorylate ribosomal protein S6 (rpS6), a component of the 40S ribosomal subunit, and activate protein translation (14). S6K1 is also known to have other targets for phosphorylation including S6K1 Aly/REF-Like Target (SKAR) and eukaryotic translation initiation factor 4B (eIF4B) (15), both of which also enhance protein translation. mTORC1-dependent phosphorylation of 4EBP1 exhibits inhibitory effects, removing this protein from its association with eukaryotic translation initiation factor 4E (eIF4E) permitting translation initiation to occur (15). These molecular events are corroborated in studies of rodent and human skeletal muscle displaying mTORC1s regulatory role in MPS (10, 16). In fact, the vital importance of mTORC1 activity to MPS has been elegantly shown in humans through the use of the mTORC1-specific inhibitor rapamycin. Ingestion of this compound prior to commencing resistance exercise or the ingestion of amino acids can completely ablate the effects of these anabolic stimuli on MPS, suggesting mTORC1 to be critical in these processes (17, 18).

In addition to stimulatory effects on protein translation/accretion, mTORC1 also exerts inhibitory effects on the second component of skeletal muscle NPB, protein catabolism. When active, mTORC1 phosphorylates proteins involved in the autophagic (UV radiation resistance-associated gene protein (UVRAG), unc-51 like autophagy activating kinase (ULK1) & transcription factor EB (TFEB)) and ubiquitin-proteasome (extracellular signal-regulated kinase 5 (ERK5)) systems (15), inhibiting their effects on autophagosome formation and proteasome

assembly respectively. Such events act to reduce protein breakdown in cells allowing NPB to shift toward net protein accretion. These processes are less well characterised *in vivo* with varying findings reported, however, constitutively active mTORC1 seems to cause myopathy due to autophagy inhibition through the phosphorylation of ULK1 (19). Taken together, these data demonstrate the bi-directional control of NPB, by mTORC1, and show how this kinase complex is essential in the regulation of this dynamic process in skeletal muscle.

mTOR association with the lysosome is essential for mTORC1 activation

Lysosomes are spherical organelles which contain a variety of hydrolytic enzymes which digest redundant components of the cell i.e. damaged proteins, with these digested materials then available to be utilised in the production of new cellular components (20). Seminal work from the Sabatini lab (21) using immuno-fluorescence microscopy, displayed that mTORC1, when activated under nutrient-rich conditions, localised with late endosomes/lysosomes (Ras-related protein Rab7 positive staining). This work suggested that mTORC1 requires lysosomal association in order to become maximally activated. Further reports have since reinforced this notion. For example, forced targeting of mTORC1 to the lysosomal surface in HEK293 cells continuously activates the kinase, renders this pathway amino acid insensitive and results in cellular hypertrophy (22). Such protocols have also been utilised *in vivo*, with the mutant RAPTOR-Rheb (Ras homolog enriched in brain) construct, which anchors mTORC1 to the lysosome, transfected into rodents (23). Here similar observations were reported, as this process elicited a 5-fold elevation in $S6K1^{T389}$ phosphorylation, a commonly used readout of mTORC1 activation. The disruption of normal lysosomal function also inhibits mTORC1 activation in response to anabolic stimuli *in vitro* (24), as well as altering gene expression profiles in response to essential amino acid ingestion *in vivo* (25). As such, there is strong evidence to suggest that

mTORC1-lysosomal association is essential to this complex's activation both *in vitro* and *in vivo*.

In addition to being a docking site for mTORC1, the lysosome also supports two known activators of mTORC1, Rheb and phosphatidic acid (PA), which have been shown to be enriched in lysosomal membranes (26, 27). These mTOR activators can bind directly to domains on the mTOR kinase and increase mTORC1 phosphorylation of downstream targets (28, 29). In addition to the presence of these activators, the lysosome also contains an abundance of amino acids produced through the digestion of unwanted cellular proteins. These amino acids both activate mTORC1 (13) and are used as substrates in protein translation. Thus, it seems the lysosome is an ideal location for mTORC1 to become optimally activated.

The mechanism by which mTORC1 is translocated to the lysosome appears to involve an interplay with Rag family of small GTPase proteins. Co-immunoprecipitation experiments in HEK293 cells have shown mTORC1 to associate with Rag GTPase proteins in cells upon amino acid introduction (21, 22). These proteins reside at the lysosome in a heterodimer of RagA/B bound to RagC/D (21), and are only able traffic mTORC1 to the lysosome when RagA/B is guanosine-triphosphate (GTP)-loaded and Rag C/D guanosine-diphosphate (GDP)-loaded through association with the RAPTOR component of mTORC1 (22). Expression of dominant-negative mutants of these proteins prevent mTORC1-lysosome association and abolish mTORC1 activity even if amino acid availability is high (21). This status is governed by the activity of other proteins situated at the lysosome, such as the ragulator complex, which acts as both a scaffold for Rag proteins to the lysosome (22) and a guanine nucleotide exchange factor (GEF) to Rag A/B, initiating the change from GDP- to GTP-loading upon activation by amino acids (30). The GTP-loading status of RagC/D is controlled by folliculin, a tumor suppressor protein

which has GTPase activating protein (GAP) activity toward RagC/D (31) and increases its GDP-loaded status upon amino-acid activation. The intricate network of mTORC1 trafficking to the lysosome in HEK293 cells has been recently proposed to be coordinated by the activity of the v-ATPase, a vacuolar-associated ATP hydrolase which senses intra-lysosomal amino acids and signals to the ragulator-Rag complex to ultimately associate mTORC1 to the lysosomal surface (32). *In vivo* work has supported this mechanism in rodent skeletal muscle, where eccentric contractions enhanced mTOR-lysosome colocalisation in concurrence with enhanced $S6K1^{T389}$ phosphorylation (27). This model of mTORC1 activation is depicted in Figure 1, displaying the dissociation of mTORC1 from the lysosome when nutrient levels are low (Fig. 1A) and subsequently the translocation of mTORC1 to the lysosome upon nutrient introduction/mechanical stimulation (Fig. 1B). Overall, these data suggest mTOR-lysosomal association is essential for the optimal activation of mTORC1 to both nutrients and mechanical loading.

Is the cellular localisation of mTOR-lysosomal complexes biologically relevant?

As discussed, several cell and rodent-based investigations have suggested that the recruitment of mTORC1 to the lysosome is a critical factor in mTORC1 activation in response to elevated amino acid availability (21, 22, 30, 31). However, more recently, a role for the translocation of mTORC1-lysosome complexes has been proposed as an additional layer of mTORC1 activation (33). Korolchuk et al. (33) first proposed this hypothesis following the observation that physiologically relevant amino acid deprivation, milder in nature to that previously utilised (21, 32), did not result in mTOR disassociation from the lysosome yet reduced mTORC1 activity in HeLa cells. This notable observation suggests that under physiological nutrient-deprived

conditions, i.e. the post-absorptive period in human skeletal muscle when autophagy and protein breakdown increase to maintain intracellular amino acid concentration (34, 35), mTORC1 activity may not be governed by lysosomal association. Following this, the authors reported an association between the number of cells with predominantly peripheral lysosomes, measured through immunofluorescence microscopy, and the extent of mTORC1 activation following amino acid stimulation. This notion was then tested more directly through the use of nocodazole, a drug which depolymerises microtubules and prevents lysosomal movement. When this drug was administered to cells, the response of mTORC1 activity to nutrient reintroduction following deprivation was abolished (33). Further investigation through the use of siRNA targeting a protein implicated in lysosomal movement, ADP-ribosylation factor-like protein 8B (ARL8B), confirmed these observations. The knock down of this protein fixed lysosomes close to the nucleus of HeLa cells, and prevented $S6K1^{T389}$ phosphorylation irrespective of intracellular amino acid levels (33). Moreover, when this protein was overexpressed in HeLa cells, the amount of peripheral lysosomes were raised by 350% but mTORC1 activity was only elevated when amino acid concentrations were sufficient (33). This data has been replicated in the osteosarcoma cell line U2OS (36) expressing an increased activity of the transcription factor E2F1. Upon activation of E2F1, lysosomal associated membrane protein 2 (LAMP2)-positive structures (lysosomes) were seen to translocate to the cell membrane and once again this movement coincided with an increase in mTORC1-dependent S6K1 phosphorylation. Furthermore, this movement was displayed to be a result of a v-ATPase-dependent mechanism as the use of siRNA targeting V0 subunit C of ATP-ase resulted in a reduction in mTORC1 activity and peripheral lysosome content. These data, taken together, suggest that the cellular localisation of mTOR-lysosome complexes, rather than the trafficking of mTOR to the lysosome,

could be the fundamental regulator of mTORC1 activation. We believe these differing findings are a result of the divergent nutrient-deprivation models utilised (complete vs. milder and more 'physiologically relevant'). Therefore, this potential mechanism of mTORC1 activation is more likely to relate to the physiological processes occurring in human skeletal muscle, and consequently deserved further investigation.

mTOR-Lysosomal Trafficking Following Resistance Exercise

Based on the observations from Korolchuk et al, (33) our lab investigated the physiological relevance of mTORC1 localisation in human skeletal muscle following resistance exercise in the presence or absence of protein-carbohydrate feeding (37). To do this, we utilised immunofluorescence microscopy approaches to identify mTOR-positive structures and lysosomes (LAMP2-positive) and analysed colocalisation through the correlation of fluorescence signals. Similar to the findings of Korolchuk and colleagues (33), and contrary to complete starvation protocols (21, 30, 32), our results display no changes in colocalisation of mTOR and the lysosome between the initial post-absorptive period and any post-exercise/feeding time point (Fig. 2A & B), reinforcing the notion that during physiological states of nutrient deprivation, mTOR localisation at the lysosome is unaffected. We also observed a change in mTOR and LAMP2 colocalisation with a marker of the muscle plasma membrane (wheat germ agglutinin (WGA)) following resistance exercise with or without feeding. Specifically, a ~20% increase in both mTOR and LAMP2 colocalisation with WGA was noted immediately post exercise (both with and without feeding), and this elevation remained for a further three hours (Fig 2A & C). This translocation of mTOR/LAMP2-positive structures was accompanied by a significant increase in S6K1 activity in both subject cohorts (37), with a greater increase apparent in

subjects consuming a protein-carbohydrate beverage post-exercise (Fig. 2D), a finding consistent with previous data in the field revealing a synergistic effect of exercise and feeding (7). An increase in S6K1 activity is suggestive of a greater phosphorylation status in response to mTOR activation and results from these kinase assays are proposed to be comparable to immunoblotting techniques targeted toward mTOR activity ($S6K1^{Thr389}$) (38).

In an attempt to further elucidate the role of mTOR-lysosomal trafficking, we next utilised a within-subject unilateral exercise model to remove any effects of inter-individual variability on the findings reported (37). This protocol allowed the comparison between feeding alone and feeding following resistance exercise within an individual simultaneously. Here, we again reported no alteration in mTOR-lysosome colocalisation from baseline in either condition, however a greater colocalisation was noted in the FED condition 3h following exercise/feeding (39). We propose this may be a result of an increase in lysosomal biogenesis following resistance exercise (40) however, this notion requires further research. mTOR-WGA colocalisation increased 1h post-exercise/feeding in both conditions, returning to baseline at 3h in the FED condition whilst continuing to rise in the FED+EX condition. Moreover, a significant condition effect was the observed in mTOR-WGA colocalisation suggesting that, across the entire timecourse, mTOR localisation to the cell periphery was greater in the FED+EX condition (39), thus implying a synergistic effect of resistance exercise and feeding. A trend toward greater colocalisation in the FED+EX condition was also observed at the 3h time point further reinforcing this synergism, and LAMP2-WGA colocalisation mirrored this response. Again, these alterations in mTOR-LAMP2 translocation to the cell periphery were accompanied by changes in S6K1 activity suggesting the two processes are somewhat related. It is however important to state here that mTOR-LAMP2 translocation, in our work, is not directly associated

with S6K1 kinase activity. No difference in mTOR-WGA colocalisation, between the two conditions, was observed at 1h post-exercise/feeding whilst a large difference in S6K1 kinase activity was noted. We believe this is because mTOR-LAMP2 translocation is not the only factor implicated in mTORC1 activation in human skeletal muscle. Other factors, such as amino acid availability and mechanical transduction, which are likely to be greater in FED+EX conditions, would also affect mTORC1 activity no matter where mTOR-lysosome complexes reside. It is however possible that at later time points (i.e. 3h post-exercise/feeding), when these additional factors are less prominent, mTOR-lysosome translocation may play a greater role in mTORC1 activation as we have previously suggested (39). Nevertheless, we believe this mTOR-lysosome translocation is a key event in the process of mTORC1 activation, supporting work previously reported *in vitro* (33).

One main limitation with this initial research was that the antibody used targeted the mTOR kinase protein that is present in both mTOR complexes. This resulted in an inability to directly distinguish between the two complexes in colocalisation analysis, meaning conclusions relating these measures to mTORC1 activity may not be entirely valid. To combat this, antibodies targeting specific proteins in each mTOR complex (RAPTOR– complex 1, RICTOR – complex 2) were validated and utilised (39). Colocalisation analysis of these proteins revealed that it is indeed mTORC1 which is the complex seemingly translocating in human skeletal muscle. This was concluded as RICTOR positive structures were visualised close to the cell periphery at baseline, and this remained unchanged throughout trials in either condition (39). Moreover, RAPTOR-WGA colocalisation increased slightly at 1h post-exercise/feeding in both conditions, returned to baseline in the FED+EX condition at 3h yet dropped significantly below baseline values in the FED condition (39). The difference between conditions at this 3h time point was

also noted as significant, suggesting RAPTOR-WGA colocalisation was greater in the FED+EX condition similar to the findings noted with mTOR-WGA colocalisation. RAPTOR-mTOR colocalisation remained unchanged throughout suggesting these proteins moved in unison, as a complex (mTORC1), toward the cell periphery. These data thus suggests mTORC1 is the most likely candidate of mTOR translocation in skeletal muscle and validates conclusions made relating mTOR-lysosome translocation to mTORC1 activity. Of note, only one other study has investigated these processes in human skeletal muscle (41). Here, mTOR-LAMP2 colocalisation was noted to increase at 3h following a resistance exercise bout, only in type II fibres. This contrast to our reported findings is most likely due to the analysis method used. In this study, authors disregarded peripheral regions of muscle fibres during analysis despite stronger immunofluorescent staining apparent in these areas compared to intracellular regions. Therefore, it is possible that the intracellular colocalisation of mTOR and LAMP2 was observed to increase because only a small proportion of these proteins were actually included in analysis. Furthermore, as any analysis regarding the translocation of mTOR toward the sarcolemmal membrane was not conducted, we are unable to conclude whether this study's findings are congruent with our hypothesis. Nevertheless, the purported fibre type difference in mTOR colocalisation is an area that merits future investigations, especially any possible variation in movement toward the cell periphery between differing fibres.

An important detail to discuss here is our use of WGA as a membrane marker. WGA recognises many glycosylated proteins which are found on the sarcolemmal membrane of skeletal muscle (42), and is noted as a valid sarcolemmal membrane marker (43). Although not the most specific and sensitive marker of this membrane, we believe the colocalisation of this marker and mTOR/LAMP2 is a valid measure we are not suggesting direct association of these constructs.

This readout of colocalisation is used predominantly as an inference of closer association of the proteins investigated allowing us to display an increased translocation of mTOR-LAMP2 constructs toward the sarcolemmal membrane.

Overall, our recent work proposes that mTORC1/lysosomal translocation toward the cell periphery is a principal event regulating mTORC1 activation following both resistance exercise and nutrient availability in human skeletal muscle.

Why do mTOR/Lysosomal complexes translocate in skeletal muscle?

As mTORC1-lysosome trafficking toward the cell periphery seems to be important for mTORC1 activation in response to several anabolic stimuli, an important question then becomes as to why this process might occur? Our lab has begun to investigate this through the use of immunofluorescence microscopy to identify mTOR protein-protein interactions. We identified mTOR to translocate close to skeletal muscle microvasculature (identified by ulex europaeus agglutinin 1 (UEA-1) staining) following resistance exercise (37), both with and without protein-carbohydrate feeding. As such, mTOR appears to move closer to blood vessels following resistance exercise and protein-carbohydrate ingestion, and the influx of the substrates needed for MPS (amino acids) originating from this area (44) may provide a partial explanation as to why the translocation of mTORC1 controls its activity.

The tuberous sclerosis complex 2 (TSC2)-Rheb axis of mTORC1 activation has previously been studied *in vivo* by immunofluorescence microscopy techniques, with eccentric contractions eliciting the translocation of TSC2 away from Rheb at the lysosome membrane in rodent skeletal muscle (27). TSC2 displays GAP activity towards Rheb (45) and, when associated, maintains Rheb in a GDP-loaded state which cannot bind to the catalytic domain of mTOR and influence

its activity (28). Our lab studied the mechanism of mTORC1 activation in human skeletal muscle and, intriguingly, we were able to visualise both Rheb and TSC2 close to the sarcolemmal membrane (37). Furthermore, in response to anabolic stimuli (resistance exercise & protein feeding) we report a reduction in Rheb-TSC2 colocalisation along with a reciprocal increase in mTOR-Rheb colocalisation. These alterations in the cellular location of such proteins indicates a mechanism by which mTORC1 activity could be directly modulated at the cell periphery.

Given that mechanisms of mTORC1 activation appear to converge close to the cell periphery it is plausible to hypothesise that downstream substrates of mTORC1, or related pathways, may also be located in such areas. As mTORC1 controls cellular translational capacity, we investigated whether mTOR increased interaction with eukaryotic translation initiation factor 3 subunit F (eIF3F), a translational initiation factor which is a component of the ribosome pre-initiation complex believed to be essential for the stimulation of protein translation (46). Positive eIF3F puncta were identified close to the cell periphery and mTOR's colocalisation with this protein was elevated immediately following resistance exercise (37). Interestingly, the interaction between mTOR and eIF3F was greater if resistance exercise was followed by a protein-carbohydrate beverage, compared to the exercise bout in isolation. The overall process of MPS has also been displayed to occur in regions close to the cell periphery, through the use of the antibiotic puromycin (47). Here, positive puromycin staining, indicative of increased protein synthesis, was apparent close to cell borders (48). Overall, these observations provide further clarification as to why mTORC1 translocates following these stimuli yet also elucidates a possible mechanism as to why skeletal muscle loading alongside nutrient provision is able to enhance mTORC1 activity/MPS to a greater extent than either stimulus alone (7).

There are also several other potential candidates for mTOR interaction close to the sarcolemmal membrane, which require further investigation. Protein kinase B (PKB/AKT), a kinase which phosphorylates TSC2 in response to growth factors, removing its inhibition of Rheb and therefore activating mTORC1, has been visualised at the periphery of HeLa cells (33). Further, the mechano-transducer focal adhesion kinase (FAK), implicated in the conversion of mechanical stimuli to mTORC1 activation, is expressed at the periphery of muscle fibres, close to blood vessels (49), an area where mTORC1 is noted following anabolic stimuli (37). Amino acid transporters, as catalysts of the transport of systemic amino acids into muscle, are believed to reside close to blood vessels allowing the efficient transport of these MPS substrates. Our group has recently confirmed this through the visualisation of the primary leucine transporter L-type amino acid transporter 1 (LAT1) close to the skeletal muscle vasculature (50). Finally, there is also an abundance of ribosomal RNA (rRNA) close to the sarcolemma (51) and a sub-sarcolemmal pool of ribosomes has been identified (52), showing that the site of translation is most likely to be in the region which mTORC1 is located. All of these possible targets of mTORC1 translocation require further investigation in order to extend the myriad of protein-protein interactions already identified by our group (37) and fully understand the influence of mTORC1-lysosome translocation in human skeletal muscle.

Future directions

We believe the identification of mTORC1-lysosome translocation as a mechanism of mTORC1 activation in human skeletal muscle provides novel insight into the molecular regulation of MPS. Currently, to our knowledge there are only 3 studies investigating mTOR/lysosomal translocation in human skeletal muscle, and of these, only two measure this complex's

translocation toward the sarcolemmal membrane. Therefore, more research is needed to fully characterise this process and understand how varying feeding/exercise protocols may affect it. Of these studies, all have been performed in young, healthy male participants, so the relevance of these findings outside this cohort demographic has yet to be determined. Furthermore, the concurrent use of stable isotope methodology in future studies of these mechanisms is required to confirm the role of this cellular process in the control of MPS. Further research should also be aimed at understanding the mechanisms which drive this mTOR translocation. Currently, it is unclear whether this cellular process contributes to, or is a result of, mTOR activation. Previous work inhibiting lysosomal trafficking (33) reported a complete removal of mTOR activation in response to amino acids, suggesting this process contributes to the activation of the kinase. However, as we have identified several downstream targets in areas close to where mTOR is translocating towards (37), it is plausible that this intracellular movement may be a result of mTOR activation itself. It will also be of interest to examine the localisation of mTORC1/lysosomal complexes in populations who exhibit skeletal muscle anabolic resistance i.e. elderly individuals (53), to determine whether the inhibit MPS observed in these scenarios is due to impaired mTORC1 trafficking following an anabolic stimulus. If this phenomenon is observed, drugs and interventions which influence mTORC1 translocation could be developed as a means to counteract skeletal muscle atrophy.

Conclusions

The importance of mTORC1 activity in stimulating MPS, and therefore skeletal muscle hypertrophy, is widely accepted and characterised. The current, most widely accepted, model of mTORC1 activation suggests the recruitment of mTORC1 to the lysosome is essential to activate

this kinase and stimulate protein translation. Alternatively, we propose that in addition to altered mTOR/lysosomal interaction, mTORC1-lysosome complex trafficking toward the sarcolemmal membrane may be a fundamental process involved in mTORC1 activation in human skeletal (Figure 3). We believe this intra-cellular translocation occurs in order to position mTORC1 close to the sarcolemmal membrane where an abundance of upstream activators and downstream substrates of mTORC1 seem to reside i.e. Rheb, AA transporters and translation initiation factors (Fig. 3). These findings are important to the field of skeletal muscle physiology as they identify a novel process by which MPS may be co-ordinated following resistance exercise and amino acid provision.

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Figure Legends

Figure 1. Depiction of the current, mostly widely accepted model of mechanistic target of rapamycin complex 1 (mTORC1) activation. When nutrient levels within a cell are low, mTORC1 resides away from the lysosome. As it is not in contact with its direct activators (Rheb and PA), and away from the abundant supply of AAs that the lysosomes provides, its activity is low. This results in low phosphorylation levels of key mTORC1 substrates S6K1 and 4EBP1 and consequently protein synthesis is diminished (A). When nutrients are introduced to the cell, the GTP-loading status of the Rag heterodimer is altered resulting in its recruitment to the lysosome. This in turn recruits mTORC1 to the lysosome, whilst simultaneously TSC2 is phosphorylated and removed from its association with Rheb. As mTORC1 can now bind to its direct activators, its activity increases, elevating phosphorylation of downstream targets and causing an increase in protein synthesis (B).

Figure 2. The effect of resistance exercise, with and without protein-carbohydrate feeding, on the mechanistic target of rapamycin (mTOR) the localisation with the lysosome (LAMP2) and the cell membrane (WGA) in human skeletal muscle. Representative images are provided (A) with mTOR displayed in red, LAMP2 in green, and WGA in blue. mTOR localisation with LAMP2 did not change at any time point in either condition (B). However mTOR localisation with WGA was elevated in both conditions post-exercise and remained elevated for 3h post-exercise (C). These changes in mTOR cellular location coincided with elevations in S6K1 kinase activity (D), a common readout of mTORC1 activity. Scale bars equal 50μm. *[§]Significantly different from resting values ($p<0.05$). ϕ Significant difference between conditions at this time

point ($p<0.05$). All data presented is Mean±SE.[Panel XXXX, Reprinted from SOURCE. No permission required.]

Figure 3. Our proposed model of the mechanistic target of rapamycin complex 1 (mTORC1) activation in human skeletal muscle. In the postprandial, resting state, mTORC1 and the lysosome are associated yet reside in the sarcoplasm of muscle cells. Rheb, situated close to the cell membrane, is associated with TSC2 and therefore GDP-loaded and inactive. This results in low mTORC1 activity and low levels of muscle protein synthesis (A). Upon the introduction of anabolic stimuli i.e. AA ingestion or mechanical stimuli, translocation of mTORC1 (and the lysosome) toward the cell membrane is initiated. Simultaneously, TSC2 becomes phosphorylated and is removed from Rheb allowing it to become GTP-loaded and active. mTORC1-lysosome complexes associate with Rheb, activating mTORC1 and increasing its activity. Active mTORC1 then resides in areas close to blood vessels, AA transporters and translation initiation factors (eIF3F) which allows a more efficient stimulation of protein synthesis which is subsequently elevated (B).

Figure 1

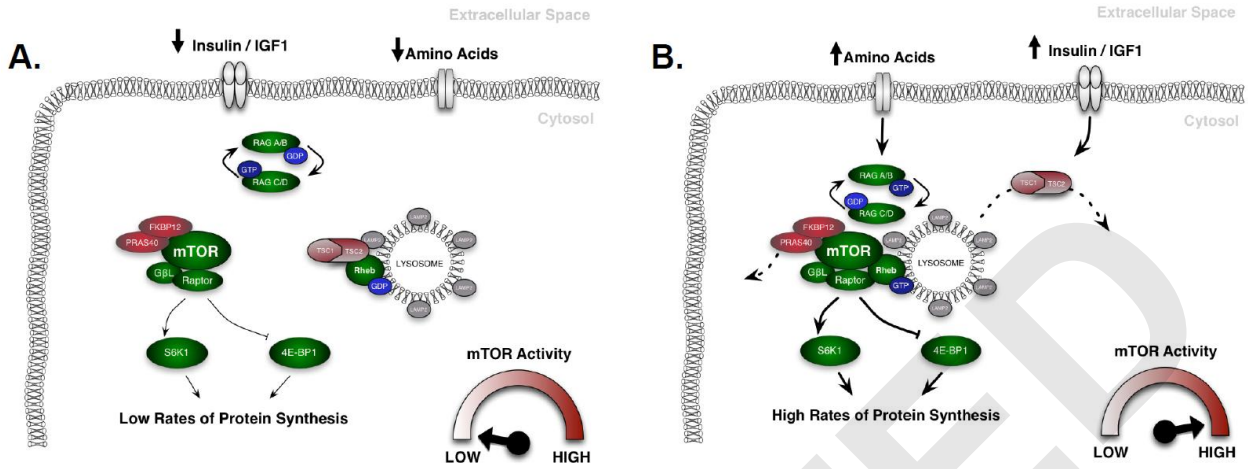

Figure 2

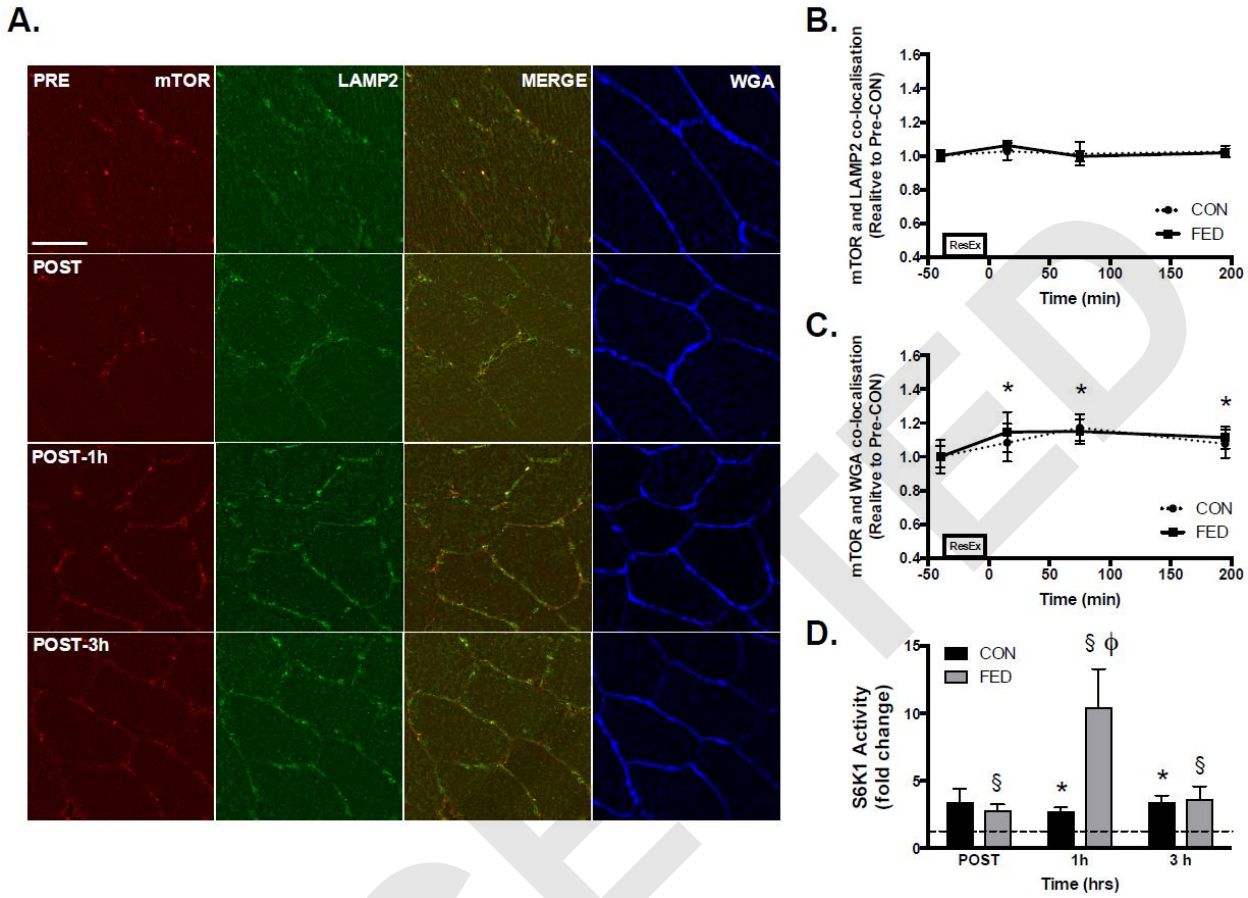

Figure 3