Nutrition Research Reviews (2024), 37, 141–168 doi:10.1017/S0954422423000124

Post-natal muscle growth and protein turnover: a narrative review of current understanding

D. Joe Millward
Department of Nutritional Sciences, School of Biosciences & Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK

Abstract

A model explaining the dietary-protein-driven post-natal skeletal muscle growth and protein turnover in the rat is updated, and the mechanisms involved are described, in this narrative review. Dietary protein controls both bone length and muscle growth, which are interrelated through mechanotransduction mechanisms with muscle growth induced both from stretching subsequent to bone length growth and from internal work against gravity. This induces satellite cell activation, myogenesis and remodelling of the extracellular matrix, establishing a growth capacity for myofibre length and cross-sectional area. Protein deposition within this capacity is enabled by adequate dietary protein and other key nutrients. After briefly reviewing the experimental animal origins of the growth model, key concepts and processes important for growth are reviewed. These include the growth in number and size of the myonuclear domain, satellite cell activity during post-natal development and the autocrine/paracrine action of IGF-1. Regulatory and signalling pathways reviewed include developmental mechanotransduction, signalling through the insulin/IGF-1–PI3K–Akt and the Ras–MAPK pathways in the myofibre and during mechanotransduction of satellite cells. Likely pathways activated by maximal-intensity muscle contractions are highlighted and the regulation of the capacity for protein synthesis in terms of ribosome assembly and the translational regulation of 5-TOPmRNA classes by mTORC1 and LARP1 are discussed. Evidence for and potential mechanisms by which volume limitation of muscle growth can occur which would limit protein deposition within the myofibre are reviewed. An understanding of how muscle growth is achieved allows better nutritional management of its growth in health and disease.

Key words: **Protein synthesis: Myonuclear domain: Satellite cells: Mechanotransduction: IGF-1: Signalling pathways**

(Received 21 February 2023; revised 1 June 2023; accepted 27 June 2023; accepted manuscript published online 3 July 2023)

Introduction

The regulation of skeletal muscle's growth in childhood and its maintenance in adult life is of obvious importance given its primary motor function, but is also key to understanding human health and wellbeing throughout the life cycle. Low muscle mass and strength during childhood contributes to several adverse health outcomes[1]. Muscle mass and strength in adolescent men is inversely associated with cardiovascular disease (CVD) events and CVD mortality in middle age[2], and with risk of all-cause mortality and mortality from CVD and cancer, explaining the obesity paradox of increasing mortality risk associated with low BMI[3]. One potential mechanism for these relationships is the inverse relationship between muscle mass and insulin resistance[4] and the development of diabetes.

The phenomenology and potential mechanisms controlling post-natal growth of muscle in children and the interactions between growth in stature and in muscle mass have been recently reviewed[5], drawing on a large and diverse literature. This included a model linking dietary protein intake to the regulation of post-natal growth suggested some years ago[6] and identified as a Protein-Stat model of growth, developed from nutritional studies of muscle growth in the rat over its first year of life. At its core is the concept that dietary protein stimulates growth, especially appendicular growth, through its joint action on bone length growth and on muscle weight growth, with their growth interrelated via mechanotransduction mechanisms. The growth of most other organs and tissues, especially the viscera, adipose tissues and skin, was largely a passive consequence of muscle and bone growth influencing energy expenditure and food intake which was regulated at least in part by an aminostatic appetite mechanism.

Several gaps in the knowledge base were indicated, and those addressed here in this narrative review include the physiological and mechanistic basis of mechanotransduction during growth, the relationships between myonuclear domain size and protein turnover, the role of satellite cells specifically in post-natal muscle growth, the role of IGF-1, especially its auto and paracrine action, and detailed mechanisms of the key processes involved. An updated summary model of dietary protein and appendicular muscle–bone interactions is presented in the context of those protein turnover studies which provided the evidence base for the main interactions identified in the model. The signal transduction pathways mediating these processes are reviewed, including insulin/IGF-1 and MAPK

Corresponding author: D. Joe Millward, email: d.millward@surrey.ac.uk

pathways, and those acting on and within satellite cells. In addition, studies of the responses to maximal-intensity contractions are included which throw light on the component of mechanotransduction generated through muscle force generation in response to gravitational loading during normal post-natal growth. Also given the importance of the ribosomal capacity for the regulation of muscle protein synthesis (MPS), identified in early studies, a recently discovered mTORC1-mediated regulatory mechanism involved is also included. Finally, the concept that muscle mass is regulated at a fixed capacity due to constraints exerted by the extracellular matrix is examined. The literature search was limited to the main subheadings listed in this paper and was completed up to 21 February 2023 with some updating during the review. Whilst the overall focus is on physiological mechanisms of post-natal growth as observed in the rat, where appropriate, work on muscle growth in mouse and other animal models, on responses to injury and on work-induced hypertrophy in human adult muscle has been referenced, where such work provides potentially important mechanisms relevant to post-natal growth.

Concepts and processes

Proteostasis and turnover during skeletal muscle growth: the evidence base for the growth model. The growth model consistent with the current evidence base is shown in Fig. 1, and some of the experimental basis for the model, that is, early studies of developmental growth and protein turnover within hind limb muscles of the rat over a year[7] are shown in Fig. 2.

The growth of an appendicular muscle reflects the combined influence of nutrition, especially dietary protein driving both muscle and bone length growth, and mechanotransduction exerted both by stretching, consequent to length growth of the associated bone, and by internal work against gravity.

Growth is identified in the left panel of Fig. 1 under two headings. The first is stem cell activation, extracellular matrix (ECM) remodelling and myonuclear accumulation allowing myofibre expansion, under control of IGF-1. The second, under the heading of proteostasis, involves myofibrillar protein turnover and accretion resulting in myonuclear domain enlargement under the control of insulin. In this section these key features will be elaborated, including a brief review of the experimental work which allowed the model development.

Proteostasis is an umbrella term describing all aspects of the maintenance of an intact proteome within cells, acting through a proteostasis network (PN). Thus, for each protein the PN exerts strict control of the initial production (protein synthesis), folding, conformational maintenance, abundance, subcellular localisation and disposal by breakdown[8]. Various molecular chaperones and co-chaperones are of central importance ensuring correct *de novo* folding and maintenance of a soluble, non-aggregated state as well as targeting misfolded proteins for degradation or spatial sequestration to protect the rest of the proteome from aberrant interactions. Some of the PN is concerned with environmental stress responses, (thermal, mechanical, pathogen and oxidative stress), which can be triggered and coordinated by proteostasis transcription factors.

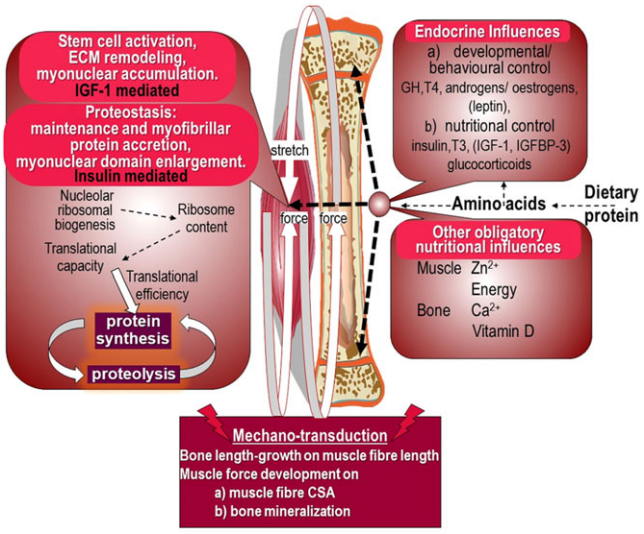

Fig. 1. Dietary protein and appendicular muscle–bone interactions in the rat. The control of the growth of the major appendicular muscles is directly related to the lengthening of the associated bone which occurs by endochondral ossification in the growth plate[5,30]. This latter process is regulated through endocrine and nutritional influences, of which dietary protein plays a dominant role, and by a paracrine/autocrine system of bone growth factors[323,324] within which nutrients including amino acids and zinc have direct roles in signal transduction[325–329], although specific sites of their action in the growth plate have yet to be described. Bone lengthening stretches muscle, inducing growth in myofibre length through addition of sarcomeres. Force development through muscle contractile activity in the lengthening muscle in response to gravitational loading induces growth in cross sectional area. This mechanotransduction involves mechanosensitive pathways[330,331] at the cell–matrix interface[332,333], which activates satellite cells, fibroblasts and other cell types, enabling increases in the synthesis of collagen[78,334,335], proteoglycans[80,85], and other components of the ECM essential to its remodelling. This enables an increase in myofibre volume. The activation of satellite cells also induces myogenesis and fusion with the myofibre, adding new myonuclei to myofibers. This sets the capacity for muscle growth in terms of the number of myonuclear domains within the myofibre and this is in part mediated by the autocrine/paracrine action of IGF-1[81,84,85]. Proteostasis is managed within these myonuclear domains through provision of sufficient protein translational capacity in terms of ribosomal RNA[291,336]. Maximal translational efficiency is under nutritional control via amino acids from dietary protein, insulin and T3 which optimise myofibre protein synthesis[26,37–39]. Although dietary protein induces increases in circulating IGF-1 and IGFBP-3 which reflect insulin concentrations, it is not clear if these endocrine changes in IGF-1 influence MPS in addition to any insulin-mediated stimulation. Bone osteocytes, dependent on calcium and vitamin D for their mineralisation, are mechanosensitive to forces exerted by muscle[337,338]. Thus, muscle and bone growth are intimately connected in a bidirectional relationship in which bone length growth regulates muscle mass and muscle growth regulates bone strength.

The accumulation of protein aggregates, such as occurs in the brain with ageing, most likely represents a failure of proteostasis. Protein turnover, the breakdown and resynthesis of cellular proteins, can now be described as the consequence of the action of the ~2000 components of the PN specifically involved in protein synthesis, chaperone-mediated folding and proteolysis mainly by the lysosomal–autophagic system and ubiquitin–proteasomal systems[9–11].

The relative importance of protein synthesis and breakdown in muscle growth control was identified in early nutritional studies. Precursor–product methods[12], as distinct from studies of the decay rates of tracer-labelled proteins[12–14], proved most

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

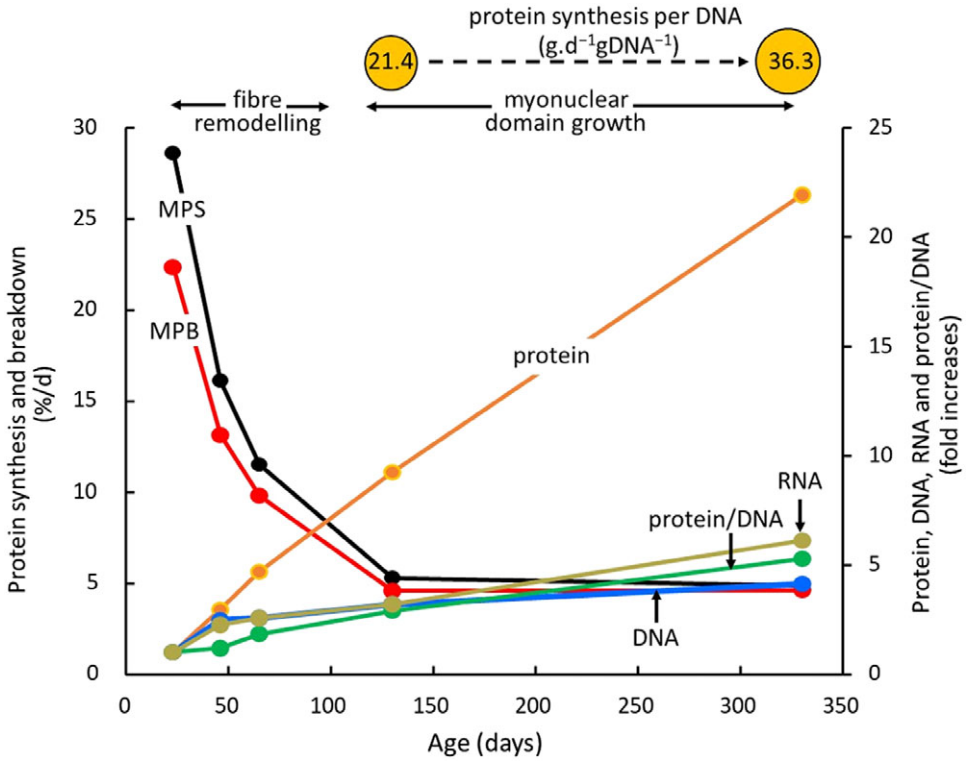

Fig. 2. Developmental changes in protein turnover in rat skeletal muscle.
Muscles were the combined gastrocnemius and quadriceps muscle of male littermates of a hooded strain[7]. Growth is shown as the increases in total muscle non-collagen protein, deemed to be that within muscle myofibres. Muscle myofibre protein synthesis, (MPS, %/d) was measured *in vivo* by the constant intravenous infusion of [^{14}C]tyrosine, with the rate of protein breakdown (MPB) calculated as the difference between MPS and the growth rate of muscle protein. DNA and RNA were total extractable nucleic acids. The early growth phase up to 60 d, with a high rate of turnover, corresponds to intense myogenesis and myofibre remodelling associated with new fibre formation[16]. During the subsequent growth, turnover fell, and after 120 d growth involved mainly enlargement of the myonuclear domain approximated by measurement of the total muscle protein/DNA ratio, with no further change in turnover. However, protein synthesis per unit DNA increased by >50% during this phase of growth mainly through an increase in ribosomal capacity per nucleus (RNA/DNA).

useful, allowing accurate measurement of muscle protein synthesis (MPS) *in vivo* from which the rate of muscle protein breakdown (MPB) can be determined as

$$\text{MPB} = \text{MPS} - \text{the growth rate of the muscle protein mass}$$

Early studies of the developmental changes in muscle protein turnover in the rat obtained in this way are shown in Fig. 2[7]. Thus, turnover was intense at weaning at 23 d (MPB >20% d^{-1}), falling to about 5% d^{-1} by 4 months, which was maintained after this. Furthermore, because intervention studies in young rats which inhibited growth always reduced MPS and often MPB, this identified MPS rather than MPB as the primary site of acute regulation of muscle mass[15]. MPB emerged as important for mediating muscle fibre remodelling associated with its rapid growth, both during early development[16] and during stretch-induced hypertrophy in avian muscles[17–21]. In addition, the MPB rate was clearly important in mediating the different rates of turnover in muscle with different fibre types (discussed further below in 'The myonuclear domain: growth in number and size during development, and relationship with protein turnover').

Figure 1 shows MPS to reflect translational capacity as ribosome content consequent to nucleolar ribosomal biogenesis, and translational control determining translational efficiency. Following initial studies focused on ribosomal activity and polysome profiling[22,23], it proved useful to identify changes in MPS in terms of translational capacity (total RNA, of which ≥85% is ribosomal) and ribosomal efficiency (protein synthesis per unit RNA). Thus, in malnourished rats, the reduced MPS mediating growth suppression involved reductions in both translational capacity and efficiency, with the former mediating the major change during longer-term deficiency[24]. An important newly discovered mechanism involved in the regulation of ribosomal capacity is reviewed below in 'Regulation of the capacity for protein synthesis', and the developmental changes in capacity shown in Fig. 2 are discussed below in 'The myonuclear domain: growth in number and size during development, and relationship with protein turnover'.

Within the growth model, dietary protein is shown to control both muscle and bone growth through the combined effects of amino acids and endocrine responses under behavioural/developmental and nutritional control. Muscle and length growth in the male rat continues throughout its lifespan (Fig. 2), since epiphyseal growth plate fusion does not occur. Their dietary demand for protein is so strong that they can become hyperphagic when given marginally protein-deficient diets[25,26], a response which led to the protein leverage theory[27]. The multiple targets of dietary protein's influence

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

include endochondral ossification in the bone growth plate mediating bone length growth, and myonuclear domain enlargement through myofibre protein deposition. As shown in Fig. 1, other key nutrients are obligatory for muscle growth, of which zinc is most important[28–30], in part to enable dietary protein's anabolic influence.

In early post-natal life, protein-containing food increases circulating insulin and amino acids with a minimal response to a protein-free meal[31], and each of these responses is important for the stimulation of MPS[32]. The role of insulin was established in early studies[23,32–37] emerging as the main acute, nutritionally sensitive regulator of muscle protein synthesis[38,39]. The interaction of amino acids with insulin and the extent of their independent influence on MPS in mediating the feeding response initially proved difficult to establish. When they were both controlled independently, it was demonstrated that insulin was permissive for the maximum influence of amino acids, especially leucine[35,40–42], with insulin, at low physiological levels, and amino acids acting through independently upstream pathways, (see 'Insulin/IGF-1 signalling') to activate an mTORC1-mediated stimulation of MPS. Leucine signals through the Rag GTPase via the cytosolic sensors sestrins[32,43–45], probably sestrin1[46]. In human adult muscle, MPS is regulated by amino acids with no discernible influence of insulin[47–49], at least within the physiological range[47], although insulin does mediate an inhibition of MPB with no obvious role for amino acids[50,51]. However Atherton *et al.*[47] have pointed out that insulin is important in mediating postprandial increases in microvascular recruitment and overall blood flow into muscle, increasing amino acid supply and therefore contributing to the postprandial increase in MPS. One suggestion is that insulin's stimulation of MPS is unique to the immature muscle[32], but there may be a species differences since insulin appears to regulate rat MPS irrespective of age[52].

Dietary-protein-mediated changes in thyroid hormones also emerged as important in the regulation of both MPS[53], at the level of the ribosomal capacity[54–56], and MPB[56], so that reductions in both insulin and thyroid hormones, (specifically circulating free T_3), were shown to mediate protein-deficiency-induced reductions in muscle growth and protein turnover[26]. Insulin influences T_3 activity by activating type 2 iodothyronine deiodinase to generate T_3 in muscle from T_4[57,58]. The role of thyroid hormones in growth regulation has been briefly reviewed elsewhere[5] and by Pascual and Aranda[59].

Catabolic influences on proteostasis in muscle occur in response to energy deficiency and stress mediated in part by increases in corticosterone which exert an increase in MPB[38,39,60,61]. Such changes are observed with severe energy deficiency in zinc-deficient rats[28,29], and in response to endotoxaemia[62]. The role of glucocorticoids in the regulation of muscle mass has been reviewed by Braun and Marks[63] which includes a useful summary of the other main growth inhibitory factor, myostatin. Myostatin (GDF8), the best-known ligand of the TGF-β superfamily expressed in muscle, acts through ActRII receptors to suppress early developmental muscle growth, induce muscle atrophy and antagonise the actions of muscle growth promoters[64–69]. Myostatin is therefore described as acting as a molecular brake that prevents excessive muscle hypertrophy: that is, its inhibition allows for muscle growth and its deletion results in massive hypertrophy[70]. It signals via the activin receptors to phosphorylate responsive Smad proteins which form a transcriptional complex with Smad4 to transcribe poorly defined target genes associated with proteolysis and other growth-inhibitory factors[66,67,71,72]. Recently, an interaction between the MAP kinase JNK and myostatin pathway has been described in relation to exercise-induced muscle hypertrophy. Mechanical stress activated JNK, which initiated muscle growth via phosphorylation of the transcription factor SMAD2, which prevented its nuclear translocation and otherwise activation of growth suppression factors[73]. JNK2 was highly phosphorylated after maximal-intensity contractions[74]. Atwaters and Hughes suggest myostatin is involved in neurotrophic signalling in muscle[75]. Taken together, this literature suggests that, although the TGFβ/myostatin system is unlikely to be involved in the nutritional regulation of muscle growth, it may well play a role in mechanotransduction pathways.

The mechanotransduction shown in Fig. 1 involves bone length growth acting to mediate muscle fibre length through passive stretching and muscle force development acting to increase both muscle fibre cross-sectional area (CSA) and bone mineralisation. The targets in muscle are activation of satellite cells (SCs), remodelling of the extracellular matrix and myogenesis, which increases the capacity for myofibrillar protein accretion. Passive stretch mediates rapid and marked skeletal muscle hypertrophy in avian models in which the wing is weighted, stretching the anterior latissimus dorsi muscle supporting the wing, and was investigated by Laurent in the adult fowl[20,21,76], and by others in the adult quail[17,77]. The treatment induced myogenesis, ECM remodelling as indicated by collagen synthesis[78], increases in ribosomal capacity and activity and transient increases in MPB.

Protein-mediated bone and linked muscle growth was demonstrated in rat studies of growth regulation of the tibial length and the associated gastrocnemius muscle weight[79–82], with the observed relationship informative about likely physiological control. In theory, given that post-natal muscle growth must enable it to acquire the necessary strength to support increasing body weight against gravity, the relationship between increase in muscle weight (W) and tibial length (L) is predictable[1] as $W \propto L^4$[6], and in well-fed rats a similar relationship is observed: that is, $W \propto L^{3.85}$[79]. This is similar to that observed in boys and girls for quadriceps weight and height ($W \propto H^4$)[83]. Mechanotransduction of muscle growth by gravitational loading in response to increasing body weight is an obvious mechanism explaining this relationship between muscle weight and tibial length growth.

Stretching by tibial length growth was suggested by the time course of growth inhibition in young rats with protein deficiency. Body weight growth stopped more or less immediately, but inhibition of tibial length growth was markedly delayed during which time some muscle growth occurred as the rats got longer

[1] Given that muscle strength mainly varies as the cross-sectional area, and that body volume and weight vary with length^3, then for strength to increase in proportion to body weight, cross sectional area of muscle should vary with bone length^3, with muscle weight varying as length^4.

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

and leaner[79]. Taken together, these relationships are consistent with the model shown in Fig. 1: that is, the mechanotransduction of muscle growth derives from both stretching consequent to bone length growth and force development within muscle during its action against the gravitational loading. Subsequent more recent work (discussed below in 'Mechanotransduction during post-natal growth as indicated by animal models') has been able to separate and better characterise these two influences.

Finally, remodelling of the extracellular matrix, necessary for increases in muscle cross-sectional area and length during growth, is identified in Fig. 1 as a target of mechanotransduction. Proteoglycan synthesis is active in growing rat muscle and is reduced to very low levels as growth slows and ceases with protein deficiency[80]. Measurements of the time course of changes in insulin, plasma IGF-1, muscle IGF-1, (i.e. IGF-1 extracted from muscle) and rates of myofibrillar and proteoglycan synthesis in muscle during the growth inhibition by protein deficiency[80,81,84–86] pointed to potential mechanisms: the reductions in insulin, (and plasma IGF-1, highly correlated with insulin[81,87]), were consistent with insulin mediating the reduced MPS, whereas the delayed reductions in both muscle IGF-1 and proteoglycan synthesis suggested an autocrine/paracrine action of IGF-1 associated with ECM remodelling[81,84,85,87]. As discussed in more detail below, local IGF-1 production is now known to be part of the mechanotransduction of stem cell activation and myogenesis.

The myonuclear domain: growth in number and size during development, and relationship with protein turnover. Given that myofibres form a syncytium containing hundreds or thousands of post-mitotic myonuclei beneath the sarcolemma, with no discernible structural compartmentation between them, the myonuclear domain, (MND), is a necessary concept. It is a volume of cytoplasm within which proteostasis is controlled by a single nucleus. The concept remains valid even with the recent suggestion that in some but not all mouse muscles there may be endoreplication of a very small fraction of myonuclei resulting in some polyploidy[88]. Polyploidy is a strategy which allows a nucleus to manage a larger volume of cytoplasm, and is best understood for the liver[89].

Our early studies of turnover and growth of the mainly fast type II muscles, (gastrocnemius and quadriceps), shown in Fig. 2, based on total muscle DNA and protein/DNA ratios as measures of MND number and size indicated a complicated two-phase relationship between turnover and growth in MND size during development[7]. The very high turnover at weaning (day 23), and in early post-natal life, most likely related to intense muscle fibre remodelling[16], fell in concert with some growth in apparent MND size. In later life (days 130–330), turnover was low and stable and MND size growth occurred mediated by a >50% increase in the rate of protein synthesis per myonucleus, (per unit DNA), associated with a comparable increase in myonuclear ribosomal capacity indicated by the RNA/DNA ratio. This response enabled the MND size to increase without any further reduction in turnover. Measurements in the adult animals showed that muscles with mainly slow oxidative fibres had faster turnover rates and lower protein/DNA ratios than fast oxidative or glycolytic muscles[12,90–92].

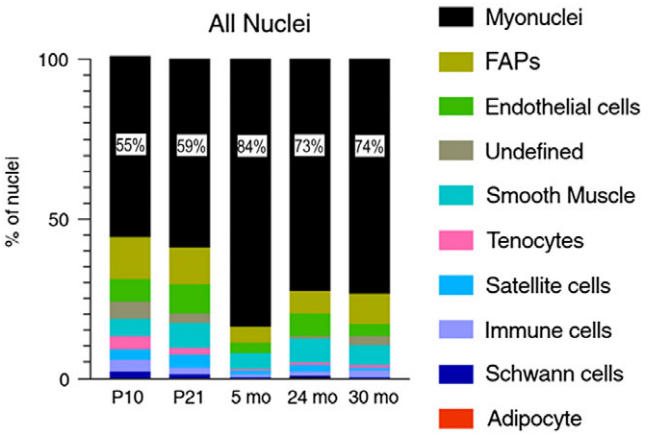

Fig. 3. Distribution of cell types in the tibialis anterior hind limb muscle across the life span of the mouse.
Cell types were identified by their pattern of gene expression measured by single-nucleus RNA sequencing. Myonuclear number varied from 55% in early life to 73–84% in adult and aged animals, with satellite cells ranging from 3–5% in early life to 1–2% in adult and aged muscle. FAPS, fibro-adipogenic progenitor cells are a muscle interstitial mesenchymal cell population, which support satellite cell differentiation during muscle growth. Modified from Petrany *et al.* 2020[94] under a Creative Commons Attribution BY-NC-ND License.

These interpretations were made on the assumption that total muscle DNA could serve as a measure of muscle myonuclei and hence MND number. In fact, in adult skeletal muscle at least ten mononucleated cell types are present in addition to the myonuclei in the multinucleated myofibre[93]. This means that our observations about changes in MND number and size based on total muscle DNA could have been artefacts of changes in numbers of other cell types in muscle. Recently, the changes in the cellular sources of nuclei in muscle during development have been quantified[94]. Myonuclei account for 59% or less, up to weaning, rising to over 80% in the adult (Fig. 3). This means that the early, post-weaning increase in total DNA which we took to indicate intense myogenesis is, if anything, an underestimate of the actual accumulation rate of new myonuclei, given the increasing proportion of myonuclei among the other cell types. Nevertheless, calculation of changes in MND size during the intense fibre remodelling occurring in this early growth phase[16] cannot be made from the whole muscle protein/DNA ratio with any certainty. However, changes in MND size and number during the subsequent growth phase after turnover had stabilised were likely to be a reasonably accurate representation of the reality.

As for the inverse MND size and turnover relationship with fibre type, the higher protein/DNA ratios of fast compared with slow muscle were consistent with early histological studies of fibre cross-sectional area and myonuclear number in different muscle types. In fast compared with slow muscle fibres, half the myonuclear number per fibre was observed in fibres of the same breadth, resulting in a 75% greater CSA per nucleus[95]. Furthermore, more recent discussions of the determination of MND size based on isolated fibre studies indicate that the fast and slow fibre type–MND size relationship has become a more

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

widely known feature, including the inverse relationship between MND size and turnover rate[96]. Direct measurement of MND size calculated from three-dimensional reconstructions in single muscle fibre segments[97] confirmed that actual MND volumes do correspond to our measured protein/DNA ratios. The largest MND size was observed in muscle fibres expressing fast myosin heavy chain (MyHC) isoforms. For the rat at 6 months, the soleus muscle, with 92% type 1 MyHC isoforms, had a MND size which was 60% of the weighted mean value of the gastrocnemius with type IIX (62.5%) and IIXB (37.5%) MyHC isoforms. For comparison, the relative protein/DNA ratios of these muscles indicated that MND size in the soleus was 54% that of the gastrocnemius muscles[12].

Most recent discussions of the myonuclear domain have focused on whether and/or how MND size is limited[96,98–100], and the linkage of MND size and number to the role of SCs during induced muscle hypertrophy rather than post-natal development. Murach *et al.*[98] note the evidence that type 2 fibres can undergo hypertrophy without myonuclei acquisition as evidence of myonuclear domain flexibility. The evidence base in these discussions comes from the synergist ablation model of hypertrophy of the plantaris muscle in which, normally, DNA accumulation occurs from SC activation. However, hypertrophy still occurs in the absence of SCs in transgenic mice[101], through increased myonuclear transcriptional activity resulting in an increase in MND size. This is consistent with our observations[7] shown in Fig. 2 of an apparent increase in MND size and myonuclear transcriptional and translational activity during the adult growth phase in the male rat as a feature of normal growth and development of muscles with predominantly type 2 fibres.

As for the differences in turnover rate in muscles characterised by fast or slow fibre types identified above which determine MND size, the assembly and turnover of the contractile apparatus remains largely mysterious apart from a much better understanding of the proteolytic systems which are likely to be involved[8]. We demonstrated a heterogeneity of turnover of individual myofibrillar proteins[102], and suggested a turnover mechanism in which exchange of protein subunits within the sarcomere occurred prior to the interaction of subunits in peripheral myofilaments with the proteolytic system[103]. We speculated that subunit exchange could be mediated by the cytoplasmic flow during each contraction cycle, and this is supported by recent observations. Contracting sarcomeres do change volume with flow into and out of the myofilament lattice, enabling delivery of ATP and other metabolites, that is, 'advective flow'[104]. We also speculated that the less regular structures of type 1 slow muscle could aid subunit exchange and interaction with the proteolytic systems. Recent work on muscle structure and force transmission has demonstrated that within myofibres there is a continuous myofibrillar matrix linked together by frequently branching sarcomeres. Furthermore, the frequency of myofibril branching and splitting in mouse muscles is three to four times higher in slow fibres (soleus) than fast fibres (gastrocnemius) and higher in early compared with late post-natal development[105]. These structural differences and the need for sustained contractions in type 1 slow fibre muscles compared with type 2 fast muscles with a consequent more sustained cytoplasmic flow could explain the higher turnover rates of tonic slow muscles.

Satellite cell activity during post-natal development. Although the myonuclear accumulation from SCs was verified by the radiographic–histochemical determination of SC-to-myonuclei conversion in growing rat soleus and extensor digitorum longus (EDL) muscles[106], the issue of whether SC addition is/is not obligatory for work-induced skeletal muscle hypertrophy was debated in a series of point–counterpoint articles[107–109]. Moreover, the issue was reviewed again more recently[110–112]. Whilst it appears clear that any work-induced hypertrophy, (e.g. progressive weighted wheel running), occurring in the absence of SCs results in functionally impaired muscle[113], there is a consensus that post-natal muscle growth requires the activation, proliferation and fusion of SCs as the source of new myonuclei which enable an increased number of MNDs. Bachman and Chakkalakal have recently reviewed the role of SCs in growth of mouse muscle from birth to puberty onset to young adulthood[114]. They argue that the mouse is an appropriate model for human muscle growth given that, in terms of myofibre size and myonuclear number, human myofibres scale similarly to that of mice. In fact, whilst active accretion of new myonuclei from SCs from birth to weaning has been widely accepted following early observations from Moss and Leblond[115], the developmental stage when myonuclear accretion finally ceases and SC quiescence is established has been controversial. For example, in our rat muscle data, total muscle DNA present at weaning had increased by 2.5 fold at day 46 and by 4.1 fold at 330 d (Fig. 2)[7]. However, White *et al.* reported that, in mice, myonuclear addition ceased after weaning at day P21[116]. Nevertheless, Bachman and Chakkalakal argue that evidence from a variety of approaches, including lineage tracing, (where actual transfer of SC DNA into myonuclear DNA is traced), indicates that relatively robust SC activity and progression to myonuclei occurs up to puberty or even into young adulthood. They also document much higher accumulation of new myonuclei in myofibres of slow oxidative soleus muscle compared with faster glycolytic EDL muscle, as previously also observed by Schultz[106]. This is consistent with our assumptions from total DNA measurements that the MND size is smaller in the slow than in fast muscles[90].

As discussed in more detail elsewhere[5], after the activation of SCs, their transformation into myogenic cells and eventual myonuclei is mediated by a series of transcription factors, of which the best known[117] are the MyoD family of four myogenic regulatory factors. Myf5 and MyoD are early markers for myogenic commitment to the myogenic programme, myogenin is a direct target of MyoD, and both myogenin and Mrf4 control expression of the terminal differentiation genes in differentiated myocytes. These fuse with the myofibre and express muscle genes such as myosin heavy chain (MHC). A detailed account of these developmental changes in SCs isolated from mouse hind limb muscles at different ages between birth and adulthood at 49–56 d as revealed by flow cytometry[118] is shown in Fig. 4. During this time the composition of the myogenic population changed from a mixture of myogenic cells in various stages to an

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

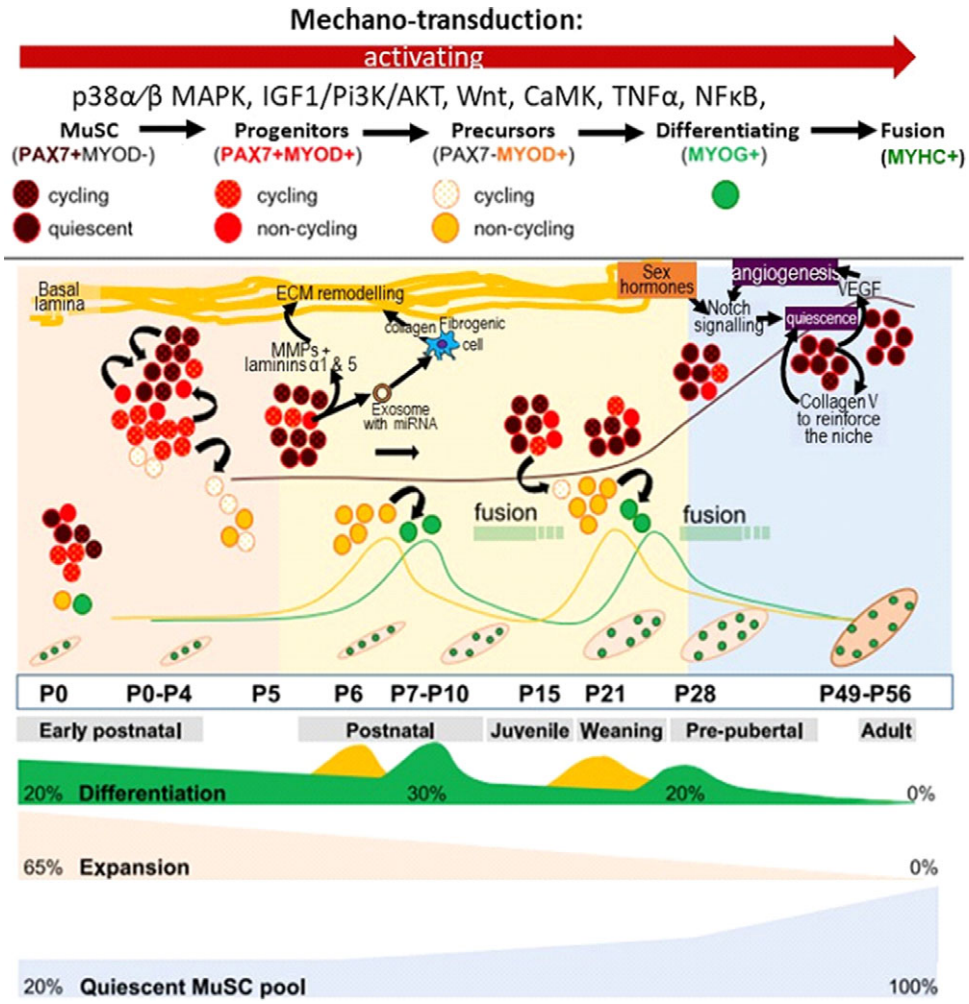

Fig. 4. Schematic representation of the mechano-transduction of skeletal muscle myogenic cell dynamics during muscle post-natal growth.
Post-natal myogenesis is mediated by forces imposed on and generated by muscle, acting on SCs in their niche. Signalling pathways known to be activated include p38 mitogen activated protein kinase (MAPK)[258,262], IGF1/Pi3K/AKT[339], Wnt[340], Ca^{2+}/calmodulin-dependent protein kinase (CaMK)[341], TNFα[342] and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)[121]. The myogenic programme of SC activation and myogenesis is mediated by a group of specific transcription factors, the myogenic regulatory factors. The sequence shown was identified in mouse muscle[118] on the basis of the expression markers shown. Myogenesis commences with the activation of SCs expressing PAX7 in their niche, which then express MYOD as progenitors and cease to express PAX7, allowing progression towards differentiation as precursors ($PAX^{-}MYOD^{+}$). These differentiate expressing MYOG and start to express muscle genes such as myosin heavy chain while fusing with the myofibre to provide a new myonucleus. At each of these stages these cells can enter the cell cycle and replicate, recognised by expression of Ki-67 protein. Also the progenitor cells can return to quiescence as $PAX7^{+}MYOD^{-}$ cells. Two successive phases of expansion and differentiation were identified: an early post-natal phase and a post-weaning/prepubertal phase, when precursor production, differentiation and myofibre fusion is most rapid. Activated SCs mediate remodelling of the basal lamina, secreting both metalloproteinases and laminin proteins as well as communicating with fibrogenic cells to regulate an appropriate amount of collagen production. In adulthood, activation and differentiation ceases with SCs converted to quiescence, a transition mediated in part by sex hormones through Notch signalling. Notch also regulates production of collagen V by the SCs to stabilise the niche. SCs secrete vascular endothelial growth factor mediating angiogenesis which also helps to maintain quiescence.
Modified from Gattazo *et al.* 2020[118] under the CC BY-NC-ND license with additions as described in the text.

essentially single population of quiescent cells with two peak periods of terminal differentiation at the early post-natal and prepubertal phases of muscle growth.

The signalling pathways involved and the molecular mechanisms at the level of transcriptional and epigenetic regulation of the transitions shown in Fig. 4 have been recently reviewed by Segalés *et al.*[119,120] and by Rugowska, Starosta and Konieczny[121], and are discussed further below in 'Satellite cell signalling'.

After emerging in embryogenesis, a key property of SCs like all true stem cells is their ability to survive long term in their 'niche' between the myofibre sarcolemma and the basal lamina, in a cell-cycle-arrested specific state of low cellular activity called quiescence[122–126]. This allows for any subsequent growth and regeneration late in life through activation, replication and differentiation into a fully competent myonucleus which can direct myofibre reconstruction. Notch signalling is particularly important for the maintenance of quiescence[126]. Notch signalling promotes cell–cell communication through the interaction between transmembrane ligands on one cell and the Notch protein receptor on the other. Unlike activated myogenic cells, quiescent SCs express high levels of the Notch receptors and intracellular mediators of Notch nuclear activation of quiescence

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

genes[127,128]. Endocytosis of the Notch ligand allows the intracellular domain of Notch receptors to translocate into the nucleus and activate Notch target genes which maintain quiescence. This sequence is initiated by E3 ubiquitin ligases, one of which, Mib1, has been shown to be induced by the sex hormones within the myofibre face of the SC niche[129]. This activates Notch signalling in cycling juvenile satellite cells, causing them to be converted into adult quiescent satellite cells. Notch also regulates the expression of collagen genes. Autocrine production of collagen V by SCs is used as a feed-forward mechanism to stabilise the niche and reinforce the quiescent state[130]. Finally, Notch signalling in SCs is involved in angiogenesis within the niche. Quiescent SCs secrete the vascular endothelial growth factor (VEGF) to attract endothelial cells and bind the endothelial-cell-derived Dll4 Notch ligand to maintain quiescence[122]. Some of these mechanisms around notch signalling are shown in Fig. 4.

Another important issue related to MND expansion is the need for associated ECM remodelling to allow the increased CSA of muscle fibres (i.e. 'bag' enlargement as discussed below in 'Evidence for limitation of muscle growth through a 'bag full' signal'). The maintenance of this complex connective tissue structure (see Purslow, 2020[131]) is essential to enable force transmission from contracting sarcomeres within muscle fibres to the adjacent tendon and bone[132,133]. Increased collagen synthesis was a very early event in our weighted wing muscle studies[78], and SCs play an important role in regulating this process. Early after activation they secrete both metalloproteinases (MMPs), which can degrade the ECM, and also substrates for remodelling such as the laminin glycoproteins[134]. Activated SCs also interact with interstitial fibrogenic cells, the source of most of the ECM proteins, which require regulation to prevent excessive collagen synthesis and fibrosis. Fry and colleagues[135] showed that this involves release of exosomes by activated SCs into the extracellular niche containing specific microRNA molecules which regulate ribosomal binding protein 1 (RBP1). This is a master regulator of collagen biosynthesis, preventing excessive collagen production by fibrogenic cells. This group subsequently showed SC-derived exosomes can also transfer microRNAs to the myofibre which regulates MMP synthesis[136]. The pivotal role of extracellular matrix remodelling during muscle hypertrophy has recently been reviewed with the suggestion that ECM remodelling is a rate-limiting step in load-induced hypertrophy[137], and this may also be true for post-natal growth.

Taken together, the evidence base for a central role for SC-derived myogenesis and ECM remodelling in post-natal muscle growth is now overwhelming.

Role of IGF-1. It is commonly accepted (e.g.[138]) that the primary action of IGF1 in mammals is to promote structural growth, with the primary action of insulin limited to regulation of postprandial nutrient utilisation: and in the present context, nutrient utilisation includes protein deposition within the myofibre. While there is no dispute about insulin's role[42,44,139–143], equivalent detail is, however, lacking for the role of IGF-1, especially in relation to whether any role in proteostasis can be identified which is distinct from that of insulin. Furthermore, whilst the GH–IGF-1 axis has become much better understood in relation to bone growth[144], and despite the considerable evidence from gene knockout studies, of both IGF-1 and its receptor, of its importance for post-natal growth[138,145–148], details of the action of IGF-1 in post-natal muscle growth have been difficult to untangle and controversial for several reasons. Firstly, the biology of the two hormones, their receptors and the hormone receptor interactions is extremely complex[149,150]. Secondly, the IGF-1 literature is controversial with some issues not entirely resolved. Thirdly, although IGF-1 and insulin are generally identified as 'mitogenic' and 'metabolic', respectively, they each signal through common pathways and it was argued in 2012[151] that no evidence had yet emerged for signalling mechanisms that are specifically engaged by insulin receptors but not IGF receptors or vice versa, with only limited evidence for differential activation of signalling mechanisms that are common to both receptors. What this means is that the differences between IGF-1 and insulin must relate to the ligand–receptor interactions and the cellular context in which they occur, and this will be explored here.

The IGF/insulin system consists of three primary ligands (IGF-1, IGF-2 and insulin), six ligand-binding proteins, (IGFBP1–6), and five receptors, IGF1R, IGF2R, IR-A and IR-B, and a hybrid IGF-1/insulin receptor (HybR)[152]. Furthermore, unlike insulin, which comprises mainly a single species acting in an endocrine role to mediate postprandial responses to food intake, the IGF-1 ligand exists in multiple isoforms[149,153] and can exert both endocrine and autocrine/paracrine actions, with the latter assumed to be particularly important for muscle growth. The isoforms of IGF-1 result from alternate splicing during transcription of the six exons of the IGF-1 gene, which gives rise to a pre-pro-IGF-1 transcript comprising the 70-amino-acid IGF-1 peptide, preceded by one of two signal peptides, which direct its secretion, and followed by one of three different C-terminal E-peptide extensions[154,155]. After cleavage of the signal peptide, the pro-IGF-1, either IGF-1A, IGF-1B or IGF-1C, can be cleaved to give the IGF-1 and E-peptide, or secreted as the pro IGF-1 without cleavage[156]. All three isoforms are expressed in human muscle (A>>B>C)[157], with the A and B isoforms predominant in rodent skeletal muscle, although the A isoform is by far the most abundant[156,158]. To increase the complexity, N-glycosylation in the E-peptide of the predominant IGF-1 isoform (IGF-1A) can occur. Glycosylation reduces the potency of activation of the IGF-1 receptor but protects the ligand from degradation by the proteasome[159]. This may enable it to be stored in the ECM until needed.

As for the relative biological significance of the different isoforms of IGF-1, there is a contentious literature relating to whether the B (rodent)/C (human) isoform is a specific factor involved in the mechanical stimulation of muscle growth identified as mechano-growth factor (MGF)[160]. MGF was also used to describe the biological activity of a peptide encoded within the C-terminal E-peptide reported to have an independent mitogenic biological activity[161]. However, the independent biological activity of this peptide was questioned[162], and an editorial entitled 'The Fall of Mechanogrowth Factor?' published in 2014 called for experiments that critically and

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

objectively test the MGF hypothesis[163]. It was subsequently showed that viral expression in mouse muscle of the A and B IGF-1 isoforms in which the IGF-1 component was inactivated demonstrated that the B peptide did induce muscle hypertrophy that was IGF-1 receptor dependent but the increased mass was associated with a loss of muscle strength[164]. This raised questions about its physiological relevance. More recently, overexpression of the proIGF-1A in mice induced a pronounced hypertrophic phenotype in mice at 6 months, which was not observed with proIGF-1B overexpression[165]. In these mice, the EDL muscle exhibited a shift to larger fibres and was capable of production of >30% higher tetanic force. Also, most of the IGF-1 in the IGF-1A transgenic muscle was the mature peptide, whereas overexpression of the IGF-1B pro peptide resulted in mainly unprocessed or partially processed isoforms in muscle. Barton's group investigated the relative roles of the IGF-1 isoforms[153] and concluded that, although it appears beneficial for muscle to accumulate glycosylated pro-IGF-1Ea within a local muscle reservoir, glycosylation of IGF-1A reduces its activity. They speculated that, although multiple strategies exist to eliminate the glycosylated form, the expression of a non-glycosylated form as IGF-1B would be simpler when a non-glycosylated form was needed, for example after mechanical stimulation of muscle. Overall, the physiological importance of the IGF-1 isoforms remains by no means clear, and in any case it is most likely that E-peptides are removed in the extracellular milieu, leaving the mature and active IGF-1 to interact with the receptor[149].

Knowledge of the regulation of the autocrine/paracrine expression of the IGF-1 gene in muscle is limited. While expression can be mediated in response to growth hormone which signals through the JAK/STAT pathway[166], it is not exclusively dependent on GH[147]. It is likely that myogenic regulatory factors recognise translation initiation sites on the IGF-1 gene. It has been shown in C2C12 myocytes that an IGF-1 promotor contains a response element to an nuclear factor of activated T cells (NFAT) transcription factor which is responsive to calcineurin signalling[167]. As discussed elsewhere[5], changes in free Ca^{2+} in response to stretch-induced Ca^{2+} influx into satellite cells is an important mechanism for regulating gene expression. Calcium binds to calmodulin and then activates calcineurin, the calcium- and calmodulin-dependent serine/threonine protein phosphatase which dephosphorylates NFAT. Also in mouse myoblasts GH induced the nuclear localisation of NFATc2 and IL-4 expression: IL-4 is a cytokine essential for myoblast recruitment[168]. There is very little information about any nutritional regulation of muscle IGF-1 expression, but in non-proliferating rat L6 muscle cells, increased amino acid levels increased expression of IGF-1 mRNA[169].

As for the cellular source of IGF-1 within muscle, it appears to derive from the muscle fibres as well as from satellite cells and other cells within the extracellular matrix including fibro-adipogenic progenitor cells[170], endothelial cells of the micro-vasculature[171], and the nervous system[153]. However, regardless of its source, IGF-1 in the extracellular matrix of muscle occurs as a complex with an IGF-1 binding protein so that expression of IGF-1 activity requires binding protein cleavage by protease action allowing the IGF-1 to bind to its receptor.

Of the six IGF-1 binding proteins, all of which bind IGFs with an affinity that is one order of magnitude higher than the binding of IGFs to their receptors,[149], IGFBP-3 is the most abundant in the circulation where, together with an acid-labile subunit glycoprotein, it forms a stable ternary complex with IGF-1[172]. Thus, any endocrine action of circulating IGF-1 requires cleavage of the ternary complex prior to IGF-1 receptor action. This means that, in contrast to insulin, serum IGF-1 is less influenced by the immediate fed state. However, the hepatic, GH-mediated IGF-1 expression pathway, the source of most circulating IGF-1, is regulated by insulin, enabling hepatic IGF-1 secretion in the fed state[173,174]. This explains the mainly parallel changes in serum insulin and IGF-1 in response to feeding and protein deficiency which we observed in our early studies[81,84,85]. Release of the circulating IGF-1 from the ternary complex is achieved by the action of a highly specific metalloproteinase, binding tightly to glycosaminoglycans present on the surface of cells[175]. By cleaving the IGFBP, the protease can function within tissues as a growth-promoting enzyme, releasing bioactive IGF in close proximity to the IGF receptor[172].

Within muscle the biology of the IGFBPs is by no means understood, but IGFBP 3, 4, 5 and 6 are expressed, and deletion of IGFBP 3, 4 and 5 reduces post-natal muscle growth[153]. Thus, these IGFBPs are required to coordinate IGF-1 bioavailability within muscle, both maintaining stored or recently secreted IGF ligands away from receptors and making them available when required through cleavage by proteinases within muscle.

As for IGF-1 signalling, it can be assumed that the 'anabolic' signalling of IGF-1 and metabolic signalling of insulin are mediated through their respective highly homologous tyrosine kinase receptors, although, as indicated above, once signalling has been initiated it may well be that similar target processes can be activated[151]. Of the five receptors available to IGF-1 within muscle, IGF-1R, IGF-2R, IR-A and IR-B, and a hybrid IGF-1/insulin receptor, HybR[152,176], the latter hybrid receptors present the major form of IGF-1 receptors, with which IGF-1 has high affinity and insulin has a lower affinity[177,178]. IGF-1 has low affinity for the two insulin receptors, IR-A and IR-B. These result from alternative splicing of the primary IR transcript, with IR-B, containing an extra twelve amino acid residues, most likely acting as the functionally important insulin-specific receptor in the main insulin target tissues in post-natal life. Although the IGF-1R and IR are very similar in the kinase domains and respond similarly to ligand bindings in terms of autophosphorylation of tyrosine residues, the IGF-1R C-terminal tail differs with only 44% of sequence identity with IR and may signal differently from the IR[179]. Thus, the tyrosine kinase activity of the IGF-1R is normally inhibited by GSK-3β-mediated serine phosphorylation in the IGF-1R C terminus, but IGF-1 binding reverses this, allowing kinase activity.

In mouse muscle, the relative amounts of the insulin and IGF-1 receptors change from a 4:1 excess of IGF-1 receptor in myoblasts (day −2) and myotubes (day +7) to a 4:1 excess of the insulin receptor at 8 weeks[180]. Various knock-out studies suggest that IR and IGF-1R have overlapping anabolic and metabolic roles in skeletal muscle[153,181–183], and at high concentrations, insulin and IGF-1 can bind and initiate signalling

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

with the opposite receptor. In each case, intracellular signalling occurs via similar cascades, beginning with tyrosine phosphorylation of insulin receptor substrates (IRSs), which leads to activation of the phosphatidylinositol 3-kinase PI3K/Akt pathway, as well as other downstream signals (see 'Insulin/IGF-1 signalling' and Fig. 5 for more discussion). Mice with single-receptor deletions show only a modest fall in muscle mass for the $IR^{-/-}$, no change with the IGF-$1R^{-/-}$ but a marked fall with both deletions[180,184]. Similarly, Spangenburg *et al.*[185] reported that, in a transgenic mouse model expressing a dominant negative IGF-1 receptor in skeletal muscle and with the insulin receptor also dysfunctional, muscle growth was impaired. However, in these animals, muscle hypertrophy and Akt–mTOR signalling induced by functional overload was not impaired, suggesting that growth in response to functional overload must involve mechanisms other than the insulin/IGF-1 receptor.

As for any endocrine action of IGF-1 on MPS on muscle, no influence which is distinct from insulin, long identified as the primary regulator of MPS in rodent muscle[33,37,44,141], has been unequivocally established. Experimental studies involving the infusion of recombinant IGF-1 with *in vivo* measurement of MPS have either failed[186] or succeeded to show an influence[187], although in the latter case it was not shown whether the action of IGF-1 was achieved via the insulin receptor.

The cross-receptor action of insulin and IGF-1 and the role of IGF-1BPs in regulating IGF-1 signal transduction at its receptor *in vivo* makes for difficulties in interpreting *in vitro* studies of IGF-1 action on muscle cells. For example, a widely cited cell culture study with C2C12 myotubes showing hypertrophy in response to added IGF-1[188] could well have achieved the effect of IGF-1 on proteostasis through its signalling via the insulin receptor. This would have been shown if the response of these cells to insulin had also been examined. As already referred to above, rat L6 muscle cells respond to increased amino acid concentrations by increasing expression of IGF-1 mRNA[169], and this is associated with an increased rate of MPS. However, the investigators showed that the increased IGF-1 was not responsible for the increased MPS because neither inhibition of IGF-1 signalling by a variety of inhibitors nor knockdown of the IGF-1 gene altered stimulation of protein synthesis by amino acids. In fact, the increased IGF-1 expression was shown to be related to myogenesis within the L6 muscle cells. Thus, these studies imply that the signalling induced by the amino acids not only activates MPS but also induces expression of IGF-1, which mediates satellite cell proliferation and myogenesis.

The importance of IGF-1 for exercise-induced muscle growth was the subject of a vigorous debate. This involved a proposal by Stewart and Pell[189] that IGF-1 was the main driver of muscle growth, which was disputed[190,191]. With hindsight it is difficult to understand much of the debate which in part was semantic in terms of the meaning of muscle growth (work-induced hypertrophy as distinct from post-natal growth) and in part was related to the relevance of MGF as an important mediator of IGF-1 action work induced hypertrophy[192]. In fact, at the time an autocrine/paracrine role for IGF-1 in the regulation of SC activity following intense damaging muscle contractions in adult human muscle was known[193], and subsequently IGF-1 was shown to co-localise with muscle satellite cells following acute exercise in human adults[194]. Furthermore, there was no disagreement about the importance of IGF-1 for embryonic growth, and physiological myofibre hypertrophy during post-natal development (e.g.[195]) through its paracrine/autocrine role in the stimulation and proliferation of satellite cells. Early studies showed muscle IGF-1 to be regulated by muscle stretching[196–199], and by responses to injury when monocytes and macrophages in muscle secrete IGF-1 probably through a GH-independent mechanism[200].

As indicated in 'Proteostasis and turnover during skeletal muscle growth: the evidence base for the growth model', our early studies of the responses of bone and muscle growth to graded protein deficiency pointed to the inhibition of MPS reflecting reductions in insulin, and the inhibition of muscle connective tissue synthesis reflecting reductions in muscle IGF-1. Although these were only associative studies, the findings are consistent with the separate regulation during post-natal growth of IGF-1 within muscle and circulating IGF-1, and with muscle IGF-1 being a driver of ECM remodelling in response to mechanotransduction associated with bone length growth[79–81,84–86].

Since our early studies, the case for an autocrine/paracrine role of IGF-1 in mediating muscle growth has become very strong, yet major gaps remain. The importance of both insulin and IGF-1 for post-natal muscle growth has been clearly shown by the knock-out models which have also shown the separate roles for insulin, in mediating growth in terms of protein deposition and IGF-1 in terms of myogenesis as shown in Fig. 1. To some extent there has been more confusion over the role of insulin than that of IGF-1 because of failure by some reviewers to recognise that insulin's influence on 'growth', that is, protein deposition within the myofibre, is not different in kind from its 'metabolic' action in relation to glucose disposal and lipogenesis, (e.g.[181]). As for the ligand–receptor interactions which regulate IGF-1 action and make it distinct from insulin, the means for regulation clearly exists in terms of both post-translational processing of the IGF-1 transcript isoforms, and interactions with the various IGF-1BPs known to be active in muscle. However, detail is lacking as to how this works during normal growth. Thus, although we can be reasonably certain that satellite cell activation and myogenesis is a target, we have no detailed map describing target processes for IGF-1 action in muscle. Although there is no unequivocal evidence for any role for IGF-1 in myofibre growth in length and CSA other than through myogenesis, it would be surprising if it played no direct role in proteostasis at the level of MPS or MPB. Furthermore, assuming IGF-1 does play an important role in the physiological mechanotransduction of post-natal muscle growth mediated by bone length growth-induced stretching and gravitational loading (as discussed further below), and seems to be involved in activating satellite cells in human muscle following intense damaging muscle contractions[193] and acute exercise[194], how does this differ from the growth induced by surgically induced functional overload[201]? Growth in the latter case can occur without a functioning IGF-I or insulin receptor[185]. This is a puzzle to be resolved.

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

Regulatory mechanisms and signalling pathways involved in muscle growth

When post-natal muscle growth is considered from a proteostasis perspective, it can be expected to involve the increase in myonuclear capacity for protein synthesis through SC-mediated myogenesis as shown in Fig. 4, and the maximisation of myonuclear transcriptional and ribosomal translational activity as identified[7]. The developmental and nutritionally activated hormones, other mediators such as metabolites[202], including amino acids[203,204], cytokines and the mechanical signals[205] which provoke muscle growth and hypertrophy, exert their influences on both SCs and on the myofibre through either receptors or focal adhesion complexes of one sort or another[206]. Each of these connects to signal transduction pathways which activate or inhibit target processes – transcription, translation or proteolysis, which together regulate proteostasis.

Of these pathways driving growth, the multiprotein complex, mTOR complex 1 (mTORC1), plays a central role as a conduit between growth signals and the metabolic processes underlying growth[204,205,207]. It is now known that its cellular localisation may be fundamentally important in the regulation of mTOR activity in response to physiological stimuli. Thus, after mitogen or amino acid stimulation, mTOR translocates to the lysosome where it associates with GTP-bound RHEB (ras-homologue enriched in brain) to achieve full activation, and this translocation is reversed with the lack of anabolic signals[208,209]. This spatial regulation of mTOR is particularly important in skeletal muscle with mTOR co-localising with the lysosome in basal conditions and then translocating to the cell periphery, in close proximity to capillaries, in response to feeding and resistance exercise[210]. One current suggestion is that mTOR trafficking is important for translational control, allowing close proximity with focal adhesion complexes involved with mechanotransduction, with the microvasculature and associated amino acid transporters, and with the ribosomal translational apparatus which is also close to the sarcolemma[211].

However, in the case of mechanical stimuli, Goodman has argued that, depending on its duration and intensity, rapamycin-insensitive mTORC1-dependent or even mTORC1-independent mechanisms may play a role[212]. Also, as discussed below, Hornberger's group have identified some apparent disconnects between mTORC1-dependent hypertrophy and MPS[213], and shown that some of the signals from maximal-intensity contractions which mediate muscle hypertrophy involve rapamycin-insensitive pathways[74]. Whilst the roles of insulin/IGF-1[180,184,214,215], AKT/PKB signalling[216,217] and the MAPK pathway[120,218,219] have become much better understood, the initiation of mechanotransduction has yet to be described in detail, although plausible focal adhesion-related mechanisms can be suggested[5].

Mechanotransduction during post-natal growth as indicated by animal models. Any mechanism linking muscle growth to bone length growth must mediate increases in both length and cross-sectional area of individual myofibres within the ECM which supports them. As recently reviewed[5], the evidence base now includes substantial information about the mechanotransduction of muscle stem cells in their niche and the role of other stromal cells within the ECM and the muscle myofibre. However, this evidence base is heterogeneous in its origins, in terms of both the experimental models from which it derives, and the type of muscle growth which is the specific focus of investigators. Also, the application of knowledge gained from some models of hypertrophy, such as the avian weighted wing model or synergist ablation has been questioned: both models are often described as supra-physiological in terms of the extent of the muscle overload imposed (see Goh *et al.*, 2019[220]).

Despite the plethora of recent reviews on skeletal muscle hypertrophy[111,221–227], there has been only limited reference to post-natal growth (e.g.[114,228]). Attwaters and Hughes reviewed cellular and molecular pathways controlling muscle size in response to exercise and argued that muscle stretch and force development need to be considered separately as drivers of muscle hypertrophy[75]. They argue that, although muscle stretch is a specific mechanical stimulus for increases in muscle mass as bones elongate during development, this only involves adding nuclei and sarcomeres at the ends of fibres. This cellular mode of growth is distinct from the increase in fibre CSA at a fixed muscle length observed after increased force development during training. Certainly during post-natal muscle growth of limb muscles, any bone length-growth mediated muscle length increase must be matched by sufficient cross sectional growth to enable the increased force development necessary to support the increasing body weight. In fact there is a four- to five-fold increase in human muscle fibre diameters from birth to adulthood[229]. This means that post-natal muscle growth could be considered to reflect both externally applied, bone length-increase stretching and internally generated forces by the increasing work demands made on the muscle by the increasing body weight. Animal models demonstrate each of these separate influences.

Hind limb unloading (hind limbs suspended at the tail) is a model for gravitational unloading as occurs, for example, during spaceflight[230]. Very little muscle contraction and force development can occur. Indeed, if started early, this leads to a lack of development, or in older animals, a reduction in the CSA of limb muscles[231,232]. With suspension of newborn rat pups (day 4) for 3 months continuously, both femur and tibial length growth occurred as usual (although their mineralisation is markedly depressed[233]), and length growth of the hindlimb soleus muscle was unchanged[230]. However muscle weight and fibre CSA was reduced by ~70% and myonuclear number by 60% at 3 months[230]. After removal of the tail suspension with a return to normal ambulation, fibre CSA and myonuclear number increases towards control size. What this model shows is that both bone length growth-induced muscle stretch as well as gravitational loading and tension development are needed for post-natal growth and recruitment of satellite cells.

The importance and consequences of force development during a mechanical load has been demonstrated in many different studies (e.g. see Zhu *et al.*, 2021[234]). One rat muscle training model[235] involved electrical stimulation of the dorsiflexor muscles *in vivo* under deep anaesthesia, with force output induced in a regulated way with either high-load pure isometric contractions or low-load concentric contractions. The trained

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

muscles exhibited up to 15% additional growth and increased myonuclei compared with untrained muscles, demonstrating load-dependent muscle hypertrophy. Other models include non-invasive high-intensity treadmill protocols which induced progressive myonuclear accretion and muscle hypertrophy[220]. Progressive resistance exercise can be induced with mice trained to pull an increasingly weighted cart with their tail[234]. The latter protocol induced a 6–23% increase in mass of muscle from both fore and hind limbs, associated with an overall 50% increase in grip strength, increases in myofibre CSA and myonuclear number, and decreases in interstitial nuclei in several muscles analysed. There is little dispute that, in human adults, resistance training sufficient to induce measurable increases in strength and muscle size (CSA) can activate and increase SCs and myonuclei[236,237]. For example, an 8-week resistance training regime in young men and women increased muscle strength and type II fibre hypertrophy, and increased SC and myonuclear accretion in proportion to the hypertrophy[238].

Taken together, these various animal models show that mechanotransduction of post-natal growth most likely involves not only passive stretching induced by bone length growth but also the separate influence of force generation during muscle contraction in response to gravity-induced load bearing with increasing body weight. This should allow muscle size to increase in response to overall mechanical demands made upon it. One aspect of the signalling in response to force generation is described below in 'Phosphoproteomics of maximal-intensity contractions' and Fig. 6.

Insulin/IGF-1 signalling. It is likely that insulin and IGF-1 post-receptor signalling is similar[151]. Recent increases in our understanding of signal transduction mechanisms driving muscle growth have come from technologies such as transgenics probing specific pathways such as AKT signalling during mouse muscle growth[239], and phosphoproteomics[240–242] which can reveal both targets and kinases involved in phosphorylations of proteins during signal transduction[243]. The latter technology can reveal personalised phosphoproteomic profiles regulated by exercise obtained from single muscle biopsies[242]; during the differentiation of proliferating myoblasts into multinuclear myotubes enabling muscle growth[244]; and following repeated maximal intensity contractions which result in muscle hypertrophy[74,214,245] (see 'Phosphoproteomics of maximal-intensity contractions'). The combination of phosphoproteomics with cellular fractionation into different cellular compartments (spatial proteomics) allows spatio-temporal signalling to be investigated such as the stress-induced cellular relocation of ribosomal proteins in response to the *in vivo* electrical stimulation of muscle contraction[246]. Key results from both transgenics and phosphoproteomics will be highlighted here.

Insulin and IGF-1 receptors are both very similar tyrosine kinases so that ligand binding induces autophosphorylation of the receptor leading to the recruitment and phosphorylation of a cascade of receptor substrates which activate specific pathways[140,215,247]. Those important for proteostasis and growth include both the PI3K–Akt pathway[216,217] and the Ras–MAPK pathway[219]. Proud has specifically addressed the regulation of protein synthesis by these pathways[142]. One scheme showing current understanding of these transduction pathways is shown in Fig. 5[139]. A phosphoproteomic analysis of insulin signalling through this pathway in human myoblasts obtained from cultured human SC cells obtained from adult human skeletal muscle by biopsy indicates that this pathway is expressed in satellite cells[248].

As shown in Fig. 5, insulin/IGF-1 binding with its receptor enables activation of AKT through phosphorylation by the phosphoinositide-dependent protein kinase 1 (PDK1). The importance of insulin–P13K–AKT signalling for muscle growth is illustrated by the marked skeletal muscle hypertrophy induced by the muscle-specific over-expression of an activated form of AKT1[249]. Activated AKT signalling promotes growth and proliferation and regulates proteostasis via a number of downstream substrates, of which tuberous sclerosis complex, (TSC1/2) controls mTORC1 and protein synthesis, and the forkhead box O (FOXO) transcription factors mainly control proteolysis. Phosphorylation of TSC1/2 allows activation of mTORC1 by relieving the TSC-mediated inhibition of Ras homologue enriched in brain (RHEB) which activates mTORC1. This regulates protein synthesis through phosphorylation of its two best-established substrates, S6 kinase 1 and eukaryotic translation initiation factor 4e (eIF4e)-binding protein (4E-BP1). Phosphorylation of FOXO by AKT inhibits their ability to induce proteasomal protein degradation through the muscle atrogenes, Murf-1 and Atrogin-1 (E3 ligases which ubiquitinate target proteins resulting in their proteolysis). As discussed below in 'Regulation of the capacity for protein synthesis' and Fig. 7, the role of mTORC1 in regulating mRNA translation is best understood in relation to the 5′TOP group of mRNAs.

AKT exists in three isoforms in mammals AKT 1–3 with AKT1 (<10%) and AKT2 (~90%) expressed in skeletal muscle. On the basis that whole-body knockout of both AKT isoforms results in organismal growth retardation and early post-natal lethality, Jaiswal and colleagues utilised both congenital and tamoxifen-inducible mouse models of skeletal muscle-specific AKT deficiency to investigate insulin signalling through the AKT pathway[239]. If AKT stimulates protein synthesis via activating mTORC1 and inhibits proteolysis via inhibiting FOXO it might be predicted that deletion of AKT would inhibit, in the fed state, protein synthesis and stimulate proteolysis. In fact, the induced deletion of both AKT1 and AKT2 isoforms reduced muscle size (by 40%), fibre CSA and exercise performance, impaired contractile properties of individual muscles and reduced fed-state protein synthesis. However, it had no effect on fed-state proteolysis, atrogene expression or the expression of key autophagy markers, all of which were increased in the fasted state. This indicated that skeletal muscle AKT deletion reduces muscle mass and fibre size through a reduction in protein synthesis but independent of multiple protein degradation pathways. Restoration of mTORC1 signalling downstream of AKT by deletion of both AKT and TSC1, or removal of any FOXO-mediated inhibitory influence by deletion of AKT and FOXO only partially restored muscle growth. However coordinated inhibition of FOXO1 and activation of mTORC1 pathways by the induced deletion of AKT1/2, TSC1 and FOXO1 not only rescued muscle mass and restored protein synthesis and body performance but also increased both body weight and muscle

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

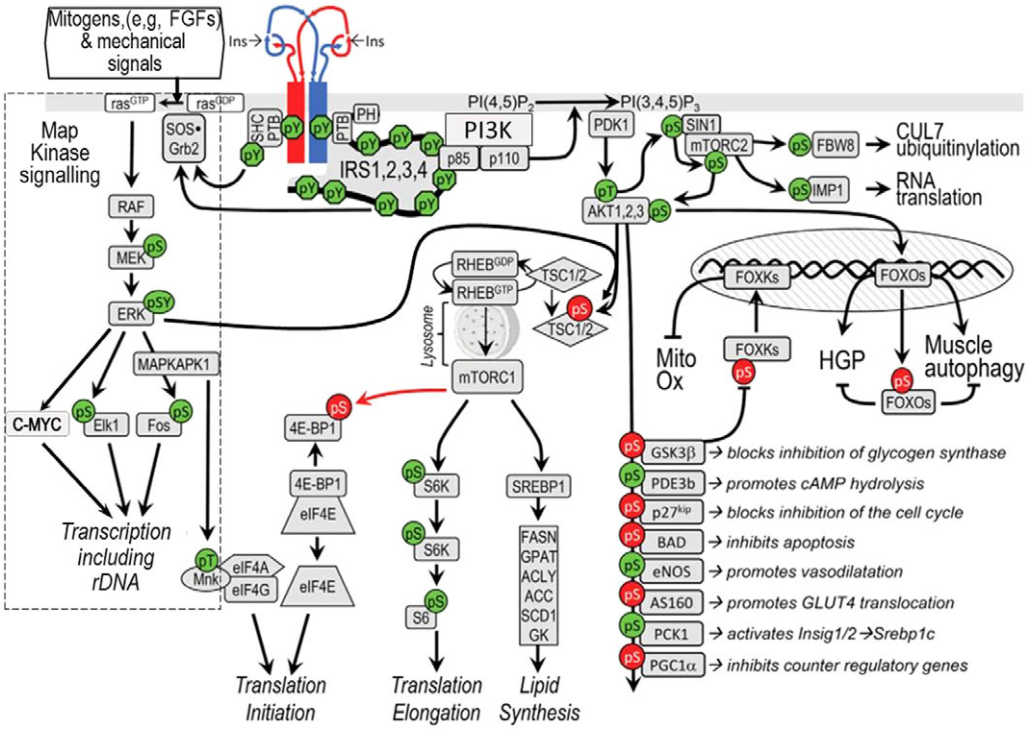

Fig. 5. Cross-talk between insulin/IGF and Ras/Raf/ERK Map Kinase signalling cascade.
The insulin receptor subunits are illustrated at the top in red and blue. Ligand binding triggers tyrosine phosphorylation of the insulin receptor substrate (IRS) or SH3-containing protein (SHC). The IRS protein binds and activates phosphoinositide 3-kinase (PI3K), which generates phosphatidylinositol (3,4,5)-trisphosphate (PIP3) that recruits phosphoinositide-dependent kinase-1 (PDK1), SIN1 (a MAPK-associated protein 1) and AKT to the plasma membrane. AKT is activated upon phosphorylation by PDK1 and by the SIN1–mTORC2 complex. AKT acts on a number of downstream pathways, as illustrated, of which phosphorylation of the Tuberous Sclerosis 1 & 2 Protein (TSC1/TSC2) complex activates mTORC1 via RHEB GTP. mTORC1 mediates phosphorylation of S6K and SREBP1, which promote protein and lipid synthesis, respectively. AKT-mediated phosphorylation of the FOXO transcriptional factors causes their sequestration in the cytoplasm, which inhibits their influence upon transcriptional activity. Insulin and or IGF-1 activation of MAPK signalling by the small GTPase ras, commences by targeting the GTP exchange factor SOS associated with the small adapter protein Grb2 via either phosphorylation of IRS or by the signalling adapter protein SHC. In each case this promotes GDP/GTP exchange on p21ras, which activates the ras/raf/MEK/ERK1/2 cascade. Mitogens such as fibroblast growth factors (FGFs) and mechanical signals also activate Map Kinase signalling at this point. Recent studies suggest effective insulin receptor activity requires its linkage via plakoglobin, a scaffold protein at the sarcolemma, to the dystrophin glycoprotein complex which anchors the myofibre cytoskeleton to the extracellular matrix[343]. It has yet to be shown whether mechanical activation of insulin signalling involves this linkage. Activated ERK can stimulate transcriptional activity by inducing expression of the proto-oncogene C-MYC which activates the ribosomal RNA Polymerase complex (Pol I regulon)[287] (see 'Regulation of the capacity for protein synthesis'). ERK also mediates direct phosphorylation of ELK1 (ETS domain-containing protein) and by indirect phosphorylation of cFOS through MAPKAPK1 (MAPK-activated protein kinase-1). Transcriptional targets of ERK also include mTORC1 and consequent protein synthesis via the TSC1/TSC2 complex[142]. Insulin stimulates protein synthesis by altering the intrinsic activity or binding properties of key translation initiation and elongation factors (eIFs and eEFs, respectively) as well as critical ribosomal proteins (see also Fig. 7). mTORC1-mediated phosphorylation of 4E-BP1 and S6K plays an important role in stimulating translation initiation and elongation[142,304]. Stimulatory phosphorylation sites (tyrosine pY, threonine pT, serine pS and both serine and tyrosine pSY) are highlighted in green, and inhibitory sites are highlighted in red. Copied and modified from White and Kahn[139] under the terms of the Creative Commons Attribution License BY-NC-ND.

mass. They then applied a casting-induced immobilisation model of muscle wasting to stimulate a reduction in muscle mass, the inhibition of protein synthesis and a fibre-type shift. This was shown to reflect a decrease in insulin-stimulated phosphorylation status of AKT: that is, induced AKT deletion partially rescued the immobilisation-induced atrophy and inhibition of protein synthesis, as did induced deletion of FOXO1 and TSC[239]. Whilst these authors did not investigate the role of GSK3 as a downstream mediator of AKT signalling, they argued that as far as insulin–AKT signalling as a mediator of normal growth and development, their finding that deletion of FOXO1 and TSC1 were sufficient to restore protein synthesis, muscle oxidative capacity and muscle function in the absence of AKT *in vivo*, suggested that GSK3 was not a necessary factor for AKT-mediated muscle growth. This is in line with other studies of the role of GSK3[250].

These findings suggest that the insulin/IGF-1 signalling network in rodent muscle responsible for muscle proteostasis appeared to be more complex than a simple AKT–mTORC1-dependent anabolic versus AKT–FOXO-dependent catabolic mechanism. This is consistent with our early findings that MPS rather than MPB is the main target for muscle growth regulation[15]. Furthermore other work suggests that the interactions between FOXO, mTOR and Akt include FOXO having direct inhibitory effects on mTORC1 and protein synthesis[251]. In cultured fibroblast cells, activated FOXO induces the expression of sestrin 3, which activates AMPK to phosphorylate TSC and inhibit mTORC1[252]. Sestrins are multifunctional inhibitors of mTORC1[253] also acting as amino acid sensors, which is important because muscle growth requires both insulin and amino acids[35,44,141]. Leucine specifically activates mTORC1 by binding with sestrin. This prevents the

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

inhibition by sestrin of mTORC1 by promoting its dissociation from GATOR2[207,254]. Relative sestrin concentrations vary between tissues, but in rat skeletal muscle sestrin 1 acts as the main leucine sensor, via the GATOR2 complex[46]. If FOXO1 is involved in regulating sestrin 1 in muscle, which at low leucine levels would inhibit mTORC1 via GATOR2, it may be that if FOXO is not fully inhibited by AKT, it could inhibit mTORC1 when leucine levels are not high enough to bind sestrin1 and relieve its inhibitory influence on mTORC1. Hence, FOXO may act as a rheostat in terms of its modulatory influence between insulin–AKT–mTORC1 and amino-acid signalling as suggested by Hay[251].

Finally, the insulin–AKT–mTORC1 signalling described here as revealed by the transgenic work involved the pathways presumed to occur within the myofibre. However, the insulin stimulation of MYOD1/myogenin-expressing myoblasts derived from satellite cells isolated from biopsies of adult human muscle[248] may well illustrate the pathway activated in SCs *in vivo* by IGF-1. This means that insulin/IGF-1–AKT–mTORC1 signalling can be considered as a key driver of protein synthesis and other events during both myogenesis and myofibre proteostasis.

MAPK signalling. Mitogen-activated protein kinases (MAPKs) comprise a complex, multi-branched signalling system of protein Ser/Thr kinases that convert extracellular stimuli including mechanical, mitogens and insulin/IGF-1 (as shown in Fig. 5) into a wide range of cellular responses[218,255–257]. In the present context, MAPK-mediated cell proliferation, differentiation and migration are particularly relevant to SC activation and myogenesis[120,258]. Signalling commences through three sequentially acting kinases: a MAPKK kinase (MAPKKK, or MAP3K), which acts on a MAPK kinase (MAPKK, MAP2K or MEK) to activate the MAPK. Fourteen mammalian MAPKs have been characterised, but the most extensively studied are the extracellular signal-related kinases, ERK(1/2), Jun amino-terminal kinases JNK(1/2/3) and p38 isoforms, p38(α–δ). Mechanical stimuli can activate MAPK signalling, which is not only required for the maintenance of myofibre size but also plays a role in the induction of skeletal muscle hypertrophy[259–261]. In the present context, MAPK signalling is important in both the regulation of protein synthesis and in SC activation and myogenesis as discussed above.

Both ERK, which can be activated by insulin/IGF-1 (Fig. 5), and p38 MAPKs, which are turned on by other distinct stimuli such as cytokines (not shown in Fig. 5), participate in the regulation of protein synthesis (see Proud[142]). ERK activates transcription and translation by at least two pathways as shown in Fig. 5. It phosphorylates TSC1/2, activating mTORC1 and translation. It can also activate both transcription and translation initiation independently from mTORC1 via MAPK-activated protein kinases (MAPKAPK1) and MNK1 & 2 (Mitogen-Activated Protein Kinase–Interacting Kinase 1/2) which activates one or more of the eIF4 initiation factors. p38 MAPKα/β can also activate MNK1 & 2 and translation initiation.

As for the regulation of myogenesis, p38α MAPK has been shown to be a global regulator of skeletal muscle differentiation[262]. This was indicated by chromatin-wide and transcriptome profiling integration in satellite cells derived from mice with muscle-specific deletion of p38α, and in the C2C12 murine myoblast cell line cultured in the absence or presence of the p38α/β inhibitor. p38α was shown to bind to a large set of active promoters during the transition of myoblasts from proliferation to differentiation stages.

Satellite cell signalling. The initial activation of SCs during post-natal growth at the molecular level remains incompletely understood, although Martino *et al.* has recently reviewed cellular mechanotransduction in general terms[263]. Much of the muscle-focused literature deals with responses to muscle damage[264]. However direct cell-to-cell interactions with their microenvironment offer potential mechanisms by which they can respond to mechanical force, so that the progression shown in Fig. 4 is assumed to be mainly mediated by mechano-transduction. SCs are anchored to the sarcolemma of the myofiber by cadherins, and on their apical side to the basal lamina of the endomysium via glycocalyx, integrins, syndecans, dystroglycans and sarcoglycans[265,266]. Mechanical overload by exercise or stretching could directly exert both shear and tensile stress and activate SCs in their niche[267]. The latter group have recently employed an *ex vivo* muscle myofibre bundle experimental model to show that stretching by pulsating fluid shear stress caused compression as well as considerable tensile and shear deformation of SCs in their native niche[268]. Isolated SCs responded to pulsating fluid shear stress with increased nitric oxide production, known to release hepatocyte growth factor from the ECM and to mediate SC activation, proliferation and fusion[269]. There was enhanced gene expression of c-Fos and Cdk4, known to promote cell proliferation. Also there was upregulation of IL-6 production, reported to be essential for muscle regeneration and SC proliferation[270], and for ERK 1/2 and p38 MAPK signalling.

The fibroblast growth factor (FGF) family have been implicated in skeletal muscle regeneration and satellite cell activity[271], with mRNAs of FGF1, FGF2, FGF4 and FGF6 detected in satellite cells which also express high levels of two of the FGF receptors (1 and 4). FGF2 and FGF6 particularly have been shown to regulate satellite cell function *in vivo*. FGF2 is present in the extracellular matrix and basal lamina of skeletal muscles, and is produced by fibroblasts, myofibres and SCs. FGF6 can be detected in both embryonic and adult skeletal muscle tissues, and isolated myofibres[272]. In adult mice, FGF6 is secreted by fast-twitch fibres, and its expression is increased after skeletal muscle injury.

Receptor-mediated FGF signalling involves activation of four major intracellular signalling pathways, RAS–MAPK, PI3K–AKT, phosphoinositide-specific phospholipase C (PLCγ) and signal transducer and activator of transcription (STAT)[273,274]. The complexity of FGF signalling in satellite cells reflects the capability of FGFs to control multiple cell functions that include self-renewal, expansion and terminal differentiation.

One direct downstream target of FGF signalling in satellite cells is MYOD. Upon satellite cell activation, MYOD mRNA is stabilised, permitting commitment of the satellite cell to a myoblast fate and promoting cell cycle entry. In the absence of pro-proliferative signals, MYOD drives and is necessary for satellite cell differentiation. FGF2 can also activate MYOD in SCs

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

by increasing calcium influx through the transient receptor potential canonical (TRPC1) calcium channel[275]. This is accompanied by nuclear translocation of the NFAT transcription factors NFATc2 and NFATc3, which induces MYOD expression. Thus, depending on the context, FGF signalling can promote satellite cell activation and expansion as well as promote cell cycle exit to generate either a self-renewed quiescent stem cell or a terminally differentiated myonucleus. Specific mechanisms by which these mitogens are made available to SCs in their niche have not been reported.

Phosphoproteomics of maximal-intensity contractions. The growth model shown in Fig. 1 includes mechanotransduction deriving from both stretching from bone length growth and work-induced force generation against gravity as discussed in 'Mechanotransduction during post-natal growth as indicated by animal models'. New evidence has emerged about how force development within muscle can signal to SCs. This involves work with a maximal-intensity contractions (MICs) mouse muscle model by Hornberger's' group. They report phosphoproteomic studies, identifying specific MICs-regulated phosphorylation sites[245] including those which were rapamycin sensitive[74]. They also were able to predict which kinases were involved and which were phosphorylated themselves by the MICS.

The pathways identified[245] included the regulation of mTOR, MAPK signalling, insulin signalling and ubiquitin-mediated protein degradation. These were mirrored by strong signatures for alterations in kinase activity, ubiquitin–protein transferase activity and the regulation of transcription, translation and proteolysis. The most frequent kinases identified were MAPK1 (ERK2), MAPK3 (ERK1) and cyclic AMP kinase (cAMK2α). Also identified were protein kinase C (PKCα/β), GSK3β, p38γ&δ (MAPK12/13), protein kinase A (PKAα,γ) and PKCδ. The largest group of phosphorylated kinases were members of the cAMP family.

Likely mechanotransduction signalling centres were highly phosphorylated sites on the actin cytoskeleton and especially within the contractile apparatus at the level of the Z-disc. They argued that this is consistent with the role of the Z-disc as facilitator of both longitudinal force transmission between sarcomeres and lateral transmissions via costameres. Furthermore they identified the giant protein titin and two of the cAMP mechanically sensitive kinases, obscurin and SPEG (striated muscle-specific serine/threonine protein kinase) as mediators of Z-disc phosphorylations. These two kinases are structurally similar to titin, which is known to act like a molecular spring, so all three proteins have the potential to act as mechanically sensitive kinases and mediate force transmission from the Z-disk to beyond the plasmalemma. The interaction between obscurin and titin in the sarcomere is illustrated by Mukund and Subramaniam[276].

In a subsequent study involving pre-treatment with rapamycin, the inhibitor of a subset of the mTOR signalling events, Hornberger's group showed that only a minority of the MIC-activated phosphorylation sites were mediated through a rapamycin-sensitive (RSmTOR) mechanism[74]. The phosphorylation patterns suggested the activation by MICS of various

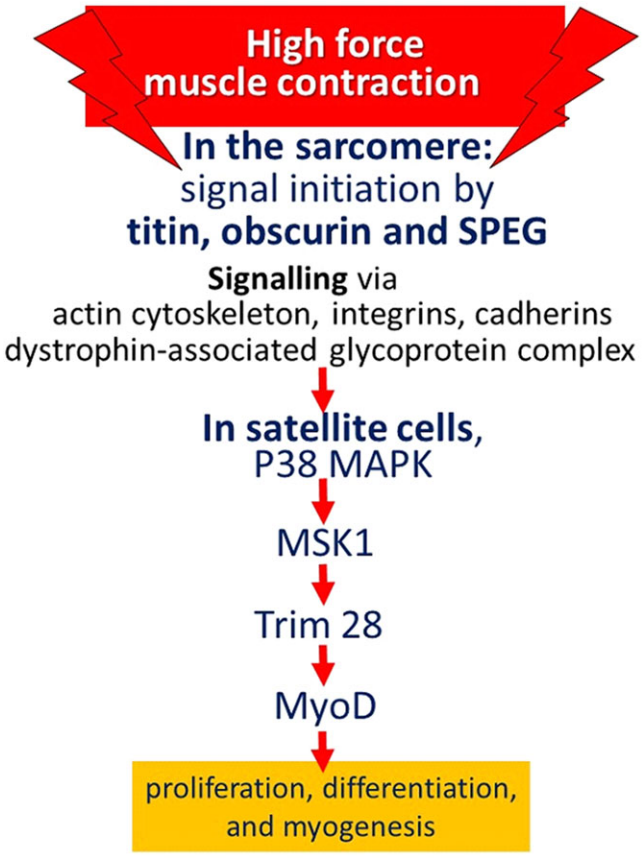

Fig. 6. Activation of satellite cells and myogenesis by maximal-intensity muscle contractions.
Phosphoproteomic data from mouse muscle, subject to electrically imposed maximal intensity contractions, identified the three giant spring-like proteins titin, obscurin and the striated preferentially expressed protein kinase (SPEG), and the transcription intermediary factor TRIM 28, as highly phosphorylated. Titin links the Z disk to each end of the sarcomere, obscurin is another giant sarcomeric signalling protein and SPEG interacts with proteins of the sarcoplasmic reticulum and the Z-band protein, desmin. It is highly likely that these proteins initiate force transmission from the sarcomere out of the myofibre activating satellite cells in their niche. The pathway is likely to involve the actin cytoskeleton, integrin-based focal adhesions, the dystrophin-associated glycoprotein complex and transmembrane calcium-dependent cadherin adhesion proteins (see Millward, 2021[5]). In satellite cells, p38 MAPK signalling to MSK1 phosphorylates TRIM28, (also known as KAP1) a transcription intermediary factor which mediates MYOD activation, enabling proliferation, differentiation, and myogenesis. Scheme based on data published by Potts *et al.*, 2017[245], Steinert *et al.*, 2021[74], Lin *et al.*, 2022[278], and Singh *et al.*, 2015[277].

isoforms of the MEK, ERK, JNK (MAPK), p38 and RSK (ribosomal s6 kinase), kinases upstream or parallel to RSmTOR. Kinases such as the 70-kDa ribosomal protein S6 kinase (p70s6K) are activated downstream of RSmTOR.

Importantly, they showed that one of the most robust MIC-regulated rapamycin-insensitive phosphorylation sites was a residue on Tripartite Motif-Containing 28 (TRIM28). TRIM28 proved to be particularly important because skeletal muscle-specific TRIM28 KO mice exhibited an attenuated hypertrophic response to myotenectomy. In addition, overexpression of phosphorylated (S473) TRIM28 induced hypertrophy. This supports the concept that MICS-induced TRIM28

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

phosphorylation is a key mediator of signalling events that promote mechanical load-induced hypertrophy. The importance of TRIM28 had been shown in previous work[277]. This protein, also known as KAP1 ((Krüppel-like associated box)-associated protein 1) is part of a dynamic transcriptional regulatory system acting with transcription factors MYOD and Mef2 in a signal-dependent fashion to modulate the efficient initiation of the myogenic gene expression program in SCs. This was under the control of the kinase MSK1, downstream of p38 MAPK signalling[277]. Steinert *et al.*[74] showed that TRIM28 was required for the accretion of myonuclei during hypertrophy of the plantaris after partial removal of the gastrocnemius muscle. Most recently, this group showed with satellite-cell-specific and tamoxifen-inducible TRIM28 knockout mice that satellite-cell-derived TRIM28 was required for the normal myonuclear accretion following mechanical loading and for myofibre regeneration following injury[278]. Knockdown of TRIM28 resulted in a fusion defect in primary myoblasts studied *in vitro*. A scheme outlining the signal transduction pathway indicated by these studies is shown in Fig. 6.

Regulation of the capacity for protein synthesis. Our earliest dietary studies of MPS in muscle identified ribosomal RNA as an important locus for regulation of proteostasis and growth of muscle[24] as discussed in 'Proteostasis and turnover during skeletal muscle growth: the evidence base for the growth model'. The capacity for protein synthesis, that is, the translational apparatus, comprises ribosomes, tRNA and a number of translation regulatory proteins. Of the total cellular RNA, most is ribosomal RNA (>80%) and tRNA (10–15%). The transcription of ribosomal RNA from rDNA repeats, together with the transcription of the ~80 ribosomal proteins (rPs) and associated regulatory factors by three classes of RNA polymerases (POL I, II and III), occurs in the nucleolus. It is the most intense, complex and energy-intensive transcriptional process in the cell[279–282]. Mammalian cells contain several hundred copies of near-identical rDNA repeats distributed across multiple chromosomes grouped within the nucleolus. Transcription involves RNA Polymerase I (Pol I) resulting in the 47S precursor rRNA, which is processed to mature 18S, 5.8S, 28S rRNAs and packaged with ~80 rPs and 5S rRNA (transcribed by POL III), to form pre-ribosomal particles. These are exported into the cytoplasm where, after further processing and quality control, the mature functional 40S and 60S ribosomal particles are formed[280]. The nucleotide sequence of rRNA allows various loop structures to form and interact with the rPs and with tRNA molecules to form the functioning small and large ribosomal particles. The ~80 rPs required are synthesised in the cytoplasm from a specific class of mRNA molecules and must be transported from the cytoplasm into the nucleolus for the assembly process together with up to 200 biogenesis factors. As for tRNA, although in the genetic code, the 21 proteinogenic amino acids are encoded by 61 nucleotide triplets, in fact >500 human tRNA genes have been identified[283] and these are transcribed by Pol III[284].

The transcription of the rRNA genes by POL I is the major rate-limiting step in ribosome biogenesis[279], and is clearly an important target for regulation shown in the growth model (Fig. 1). This starts with the formation of a complex with upstream binding transcription factor (UBTF), selectivity factor 1 (SL1; also known as TIF-IB in mouse) and transcription initiation factor IA (TIF-IA, also known as RRN3), with other factors involved in translation. Overall regulation involves multiple signalling pathways[279,281,285–289]. These include ERK, AMPK, mTORC1 and P70S6K1, which allows for control by hormones and mitogens (Fig. 5), nutrients, including amino acids[290], and contractile activity. In addition, the Wnt/b-catenin/c-MYC mechanically sensitive signalling pathway involved in regulating cell growth is involved in virtually all aspects of ribosome formation and is highly expressed during skeletal muscle hypertrophy[288,291]. Insulin may well be one important control factor. It has been shown to activate POL I in fibroblasts, hepatoma cells and adipocytes[285,292]. Also insulin can regulate ribosome content in primary cultures of rat hepatocytes by accelerating the rate of transcription of rDNA, and by slowing the rate of ribosome degradation which occurs after polyribosomal disaggregation, when mRNA translation is inhibited[293]. Insulin action may be part of the mechanism of the postprandial increase in muscle ribosomes observed in our very early rat feeding studies[31]

Recent work has focused on control of the production of rPs. For these proteins control is exerted mainly at the translational stage through a common *cis*-regulatory element, the 5′ terminal oligo-pyrimidine (5′TOP) motif. These TOP mRNAs are transcribed by POL II, and comprise all 80 ribosomal proteins and a number of translation regulatory factors, and other proteins indirectly connected to the translational machinery accounting to >200 in total. Crucially, their translation is controlled by mTORC1[294–296]. Stoichiometric considerations require rapid transport of ribosomal proteins into the nucleus during POL I–III-mediated ribosome biogenesis[280], and this seems to be achieved by maintaining a cytoplasmic store of stabilised 5′TOP mRNAs. One likely mechanism involves the multimeric La-related protein 1, (LARP1)[297]. This binds through two regions of the protein to each end of the 5′TOPmRNA molecule regulating their stability and initially inhibiting their translation. Subsequent activation of their translation is a primary role of mTORC1[207,295,297–299], and a likely model is shown in Fig. 7 (see Jia *et al.*, 2021[297]). This model predicts that LARP1 acts to inhibit translation and maintain an accessible store of 5′TOPmRNAs when mTORC1 is inactive. It also acts to enable efficient translation when required after mTORC1 activation by maintaining the mRNA in a circular conformation.

5′TOP mRNAs are a small fraction (e.g. 4%[295]) of the many thousand mRNA transcripts in the cell and during cell growth the entire cellular transcriptome must be translated to enable expansion of the cellular proteome. All nuclear-encoded eukaryotic mRNAs contain a 7-methyl guanosine cap and their translation involves the binding of eIF4E to the cap as shown in Fig. 7 regardless of the subsequent oligonucleotide sequence[300,301]. Whilst it is usually stated that translation of 5′-TOP mRNAs is more sensitive to mTORC1-directed activation than Cap-dependent translation[207], the extent of any difference, if any, during active growth is not known. Furthermore, other non-mTOR kinases are known to activate cap-dependent translation such as cyclin-dependent kinase 1 (CDK1)[302], which is essential for myoblast proliferation, muscle

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

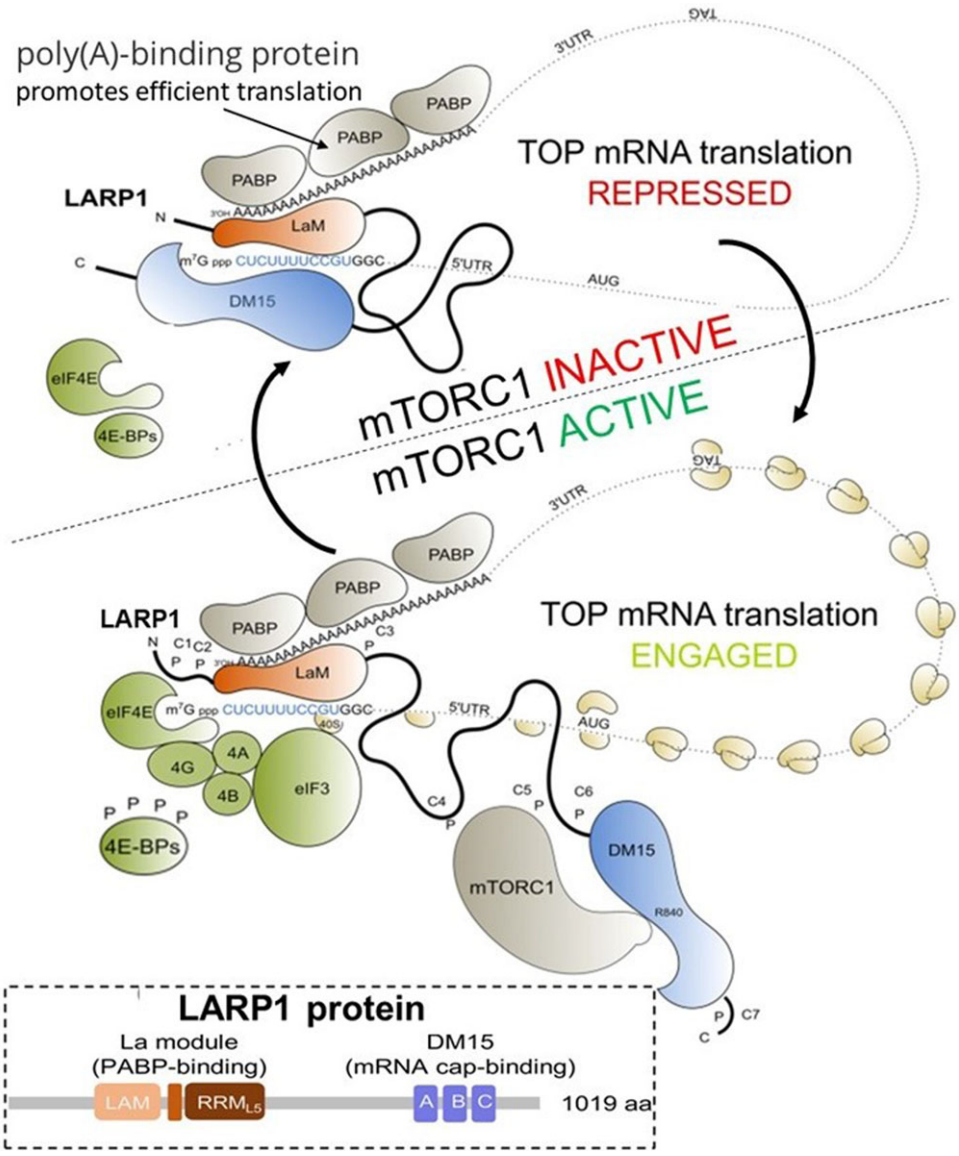

Fig. 7. Model for LARP1-mediated regulation of 5′TOP mRNA stabilisation.
As shown, LARP1 is a multimeric protein of which the carboxy-terminal DM15 domain and the mid-domain La-module play important roles in the stabilisation of 5′TOP mRNAs and inhibition of their translation. Such stabilisation is important to enable rapid production of ribosomal proteins and biogenesis factors enabling ribosome assembly when transcription of rDNA is activated during a growth stimulation which activates mTORC1. According to this model when mTORC1 is inactive and the eIF-4E initiation factor is bound to 4E-BPs, DM15 engages with the m^{7}Gppp cap and 5′TOP motif, acting like a 'pendular hook'. This blocks the 7-methyl-GTP (m7GTP) cap initiation site, blocking initiation of translation. At the same time the La-module binds with polyA-binding protein (PABP) at the polyA tail of the mRNA thereby stabilising it[344]. Activated mTORC1 phosphorylates multiple serine and threonine residues on 4E-BPs releasing eIF4E (Fig. 5) and also on DM15 enabling its release from the m7Gppp cap. This allows for the assembly of the eIF4F initiation complex involving the association of eIF4E with eIF4G which together with eIF4A and eIF3 recruit the 40S subunit of the ribosome for translation initiation. The translation initiation complex is then recruited stimulating mRNA translation, including the unwinding of mRNA secondary structure via the eIF4A helicase, and engagement with the 40S ribosome subunit after phosphorylation of its S6 protein component. Phosphorylation of the La-module does not interfere with its binding with PABP at the polyA tail. Proximity of the mRNA ends in the closed-loop structure is considered to facilitate the re-initiation during translation, that is, ribosomes are more easily engaged in the next round of initiation after termination. Copied and modified from Jia *et al.* (2021)[297] under the terms of the Creative Commons Attribution License BY-NC-ND.

regeneration and muscle fibre hypertrophy[303]. Detailed accounts of the regulation of translation have been described for muscle by Kimball and Lang[304] and in general by Proud[142].

Another post-translational regulatory mechanism important for muscle growth regulation involves modification of the polyA mRNA tail by Cytoplasmic Polyadenylation Element Binding protein 1 (CPEB1). This factor binds to the 3′ untranslated regions (UTRs) at the end of mRNA transcripts and recruits cytoplasmic polyA polymerase GLD2 to elongate the polyA tail to maintain mRNA stability. The stability of mRNAs is positively correlated with translational output and CPEB1 can consequently regulate cellular function by post-transcriptionally controlling the translation of its targeted transcripts. Zeng *et al.*[303] have shown in muscle stem cells that phosphorylation-dependent CPEB1 promoted MYOD1 protein synthesis by binding to the 3′ UTR of MYOD1mRNA, increasing its translation

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

and thereby increasing SC activation and proliferation. According to Zeng *et al.*, CPEB1 in muscle stem cells acts as a key regulator to reprogram their translational landscape, directing SC activation and subsequent proliferation[303].

Finally, recent careful examination by Hornbergers group of the absolute dependency of work-induced hypertrophy on mTORC1 through its regulation of protein synthesis, has raised some unexpected findings[213]. They created skeletal muscle-specific and inducible raptor knockout mice, to eliminate signalling by mTORC1 (the raptor containing mTOR as distinct from the rictor-containing mTORC2 complex). These were unable to undergo any overload-induced hypertrophy as observed in control mice. It might have been assumed that this meant an inability of mTORC1 to direct the overload-induced increase in protein synthesis (e.g. as shown in Fig. 5). However, the raptor KO mice exhibited no reduction in the overload-induced increased protein synthesis rate observed in control mice (measured as the amount of puromycin-labelled peptides 30 min after the *in vivo* injection of puromycin). Clearly, the complete inhibition of overload-induced muscle growth implies no expansion of the proteome so that the observed increase in protein synthesis was quite inconsistent and difficult to explain apart from either incomplete translation/processing or a matching increase in autophagy due to the lack of mTORC1 signalling.

Evidence for limitation of muscle growth through a 'bag full' signal. The concept that, during muscle growth, its volume and consequent mass was limited by the connective tissue ECM in terms of the epi-, peri- and endomysial sheaths was a speculative idea at the core of a 'bag' theory of muscle growth[5,6]. It allowed muscle growth to be conveniently described in terms of 'bag' enlargement, through ECM remodelling, and 'bag' filling in terms of myofibre protein deposition. It provided a potential mechanism for the stability of muscle mass throughout the life cycle and prior to the onset of sarcopenia. Specifically during the diurnal cycle of post-absorptive losses and postprandial gains of body protein[6], losses and gains of muscle could be identified as 'bag' emptying and refilling until the 'bag-full' signal would terminate myofibre protein deposition.

The demonstration of the 'bag-full' phenomenon with studies of postprandial MPS in adults[305–308] confirmed a regulatory signal which limited protein deposition in the myofibre. However, its mechanism in terms of the signalling examined in those studies was by no means resolved. In fact, MPS fell whilst intracellular leucine concentration remained elevated and mTORC1 signalling was still active[306,308], at least those signals associated with the initiation of protein synthesis (p70S6K1, and 4EBP1). However, eEF2 phosphorylation does seem to increase coincident with the fall in MPS[300], and this indicates that the elongation phase of protein synthesis was inhibited, an effect observed in meal-fed rats in which the increased MPS in response to a meal was transient returning to baseline coincident with an increase in eEF2 phosphorylation[309]. Classically, eEF2 phosphorylation increases in response to AMPK signalling associated with energy stress, such as muscle contraction, to protect ATP and PCr levels since it is the translational phase of MPS which is so energy dependent[142]. In meal-fed rats, post-meal supplementation with either leucine or carbohydrate prevented eEF2 phosphorylation and extended the increase in MPS[310]. However, in human muscle the 'bag-full' return to baseline of MPS occurs with no indication of inhibitory AMPK signalling or energy stress in terms of muscle ATP or phosphorylcreatine concentrations[308]. This means that the triggering of the response was unexplained. Nevertheless, it represents the only observed response which could be involved in mediating the 'muscle-full' inhibition of MPS.

It was originally assumed that anabolic/catabolic signals associated with changes in muscle cell volume would be important for the 'bag-filling-bag-full' signalling concept[311]. As extensively discussed elsewhere[5], it is highly likely that myofibre volume changes occur during the feeding–fasting cycle, with evidence for linkage between cell volume, mTOR and two different anion channels associated with cell swelling[312] and shrinkage[313]. However, after reviewing the available evidence a satisfactory mechanism linking volume change to the inhibition of MPS was not immediately obvious.

There is a current discussion about the nature of volume changes within muscle during the increases in fibre cross-sectional area consequent to some types of resistance exercise in adult human muscle, although this relates to the phenomenon of sarcoplasmic hypertrophy[314]. This is a disproportionate expansion of the sarcoplasm relative to myofibril protein accretion observed in some studies of muscle growth during resistance training[315]. Potential explanations of this phenomenon offered by this group include that it is a transient phenomenon which results in cell swelling which triggers SC activation and fusion allowing subsequent myofibrillar accretion in the new MND restoring the usual balance between sarcoplasmic and myofibrillar volumes. While such data add detail to what may well occur during the bursts of post-natal muscle growth in children and adolescents it offers little insight into the molecular mechanism linking changes in overall myofibre volume to proteostasis control.

Taken together, the above indicates that, while the 'bag-full' termination of postprandial protein deposition in muscle has clearly been shown to occur, its mechanism remains to be identified. As for bag enlargement, this is an inevitable accompaniment to muscle growth. We know that increased muscle collagen synthesis is an early response to stretch-overload-induced muscle growth in fowl[78], and is observed in adult muscle stimulated by various intense exercise regimes which induce muscle growth[48,137]. As shown in Fig. 4, ECM remodelling is part of satellite cell activation and myogenesis, and can be expected to accompany the muscle hypertrophy in response to maximal muscle contractions shown in Fig. 6. During the rapid developmental muscle growth in young rodents, it would be predicted that there is continuous remodelling of the ECM. In accord with this, in our rat muscle and bone growth studies[80,81] we observed active proteoglycan synthesis in muscle during rapid growth which was reduced as growth slowed with parallel changes in muscle IGF-1[80,81,84,85]. Much more detailed studies of muscle ECM turnover during its rapid post-natal growth are needed.

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

Conclusions

This review has examined a model for post-natal muscle growth developed in experimental animals, in which its size and strength is controlled by mechanotransduction when nutrition provision is adequate. Mechanotransduction derives from both bone length growth-induced stretching and muscle force development against gravity, which generates a growth capacity through ECM remodelling and myogenesis mediated by satellite cell activation. Nutritional adequacy involves dietary protein and other key nutrients which regulates bone length growth and initiates appropriate signalling and provides substrates for protein deposition in muscle which fills the growth capacity.

As with all work with animals as models for the human condition, they show us what can occur and the evidence for the model shown in Fig. 1 as an explanation of rodent muscle growth is currently very strong. Is such a model relevant to better understanding childhood growth? The key implication of the growth model for childhood is the concept of a growth capacity for skeletal muscle, especially for the appendicular muscles, which is set by mechanotransductive forces. These forces can derive from either an increase in stature or increased demands for muscle work, but when they are minimal, which may be quite often in preschool children, dietary stimulation of muscle growth is likely to be very limited. As recently argued, the evidence from the timing of the stages of the pubertal growth spurt is consistent with this growth model explaining the phenomenology of muscle growth as a function of height growth in childhood[5].

The inability of dietary protein alone to stimulate muscle growth in childhood in excess of the growth which accompanies either height growth or in response to excessive physical demands is one explanation of the 'Early Protein Hypothesis'[316]. This postulates that dietary protein in excess of the intake required for lean tissue growth, a demand which may be quite low after the first year of life, increases plasma insulin and IGF-1 concentrations. This response mediates adipogenic activity and associated weight gain, and the programming of obesity in later childhood. This implies that the endocrine action of IGF-1 has no influence on muscle growth during stasis periods for bone length growth.

Similarly, during adult maintenance the phenotypic muscle mass can be maintained over a wide range of dietary protein intakes, with no evidence that the phenotypic lean body mass is a function of dietary protein intake, apart from that which varies with BMI and weight gain[317]. This is consistent with the 'bag' filling and enlargement concepts referred to above.

However, obvious gaps remain in the knowledge base. One is the phenomenon of saltatory, (discontinuous with leaps), growth and stasis in human height[318,319] and animal length[320]. Although this is not accepted by all, when considered in the context of the mechanotransduction of muscle growth, saltatory growth implies that passive stretch forces associated with a saltation could be much greater than those implied by a continuous but very slow rate of human long bone growth. This raises the possibility of fibre damage occurring in response to saltations, a phenomenon which could explain the anecdotal 'growing pains' reported in young children[321] and lambs[320]. This means that those experimental 'supraphysiological' models of muscle hypertrophy such as the weighted wing[21,76,78] may be more relevant to developmental growth than often accepted.

Another gap in the mechanistic knowledge base relates to the fact that some of the animal hypertrophy work discussed here does not completely discriminate between events in the myofibre as opposed to within SCs. This is important in relation to the different requirements of the growth response: acquiring new myonuclei, expanding the myofibre proteome, and connective tissue remodelling to allow expansion of the myonuclear domain within the increasing myofibre CSA. Our early studies, (Fig. 2), showed that, in the adult phase of physiological muscle growth in the male rat, considerable expansion of the myofibre proteome occurs with only a modest amount of SC activation and myogenesis as indicated by DNA accumulation. Nevertheless, connective tissue remodelling can be assumed to be a requirement at this time, as with all circumstances of growth when the myofibre CSA increases. Such remodelling could be achieved through activation of interstitial fibrogenic cells including fibro/adipocyte progenitors (FAPs)[170,322]. It would be of great interest to map the changes in these stromal cells to better understand their involvement in muscle growth.

Finally, in the context of proteostasis within the growth model shown in Fig. 1, this review has focused only on the anabolic components. Proteolysis, including lysosomal autophagy, the ubiquitin–proteasome system and calpains, together with other systems known to mediate proteolysis in muscle by poorly understood mechanisms, has not been discussed here, but have been recently reviewed elsewhere[8]. The 'turnover' component of muscle proteolysis was largely mysterious when the authors work commenced five decades ago, and notwithstanding attempts to provide molecular explanations[103], this remains the case today. It will be of great interest to see how this develops.

Acknowledgements

Much of the authors animal work described here was achieved by students, post-doctoral assistants and longer term collaborators identified here in their published work, of whom I want to particularly acknowledge Dr Peter Bates and Dr Zainal H. A. Yahya. I also thank Troy Hornberger for his sharing of his research findings.

Financial support

No financial support was received for the preparation of this paper.

Conflicts of interest

There are no conflicts of interest.

References

1. Orsso CE, Tibaes JRB, Oliveira CLP, *et al.* (2019) Low muscle mass and strength in pediatrics patients: Why should we care? *Clin Nutr* **38**, 2002–2015.

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

2. Timpka S, Petersson IF, Zhou C, Englund M (2014) Muscle strength in adolescent men and risk of cardiovascular disease events and mortality in middle age: a prospective cohort study. *BMC Med* **12**, 1–8.
3. Lee DH, Keum NN, Hu FB, *et al.* (2018) Predicted lean body mass, fat mass, and all cause and cause specific mortality in men: prospective US cohort study. *BMJ* **362**, k2575.
4. Srikanthan P & Karlamangla AS (2011) Relative muscle mass is inversely associated with insulin resistance and prediabetes. Findings from the Third National Health and Nutrition Examination Survey. *J Clin Endocrinol Metab* **96**, 2898–2903.
5. Millward DJ (2021) Interactions between growth of muscle and stature: mechanisms involved and their nutritional sensitivity to dietary protein: the protein-stat revisited. *Nutrients* **13**, 1–65.
6. Millward DJ (1995) A protein-stat mechanism for regulation of growth and maintenance of the lean body mass. *Nutr Res Rev* **8**, 93–120.
7. Millward DJ, Garlick PJ, Stewart RJC, Nnanyelugo DO & Waterlow JC (1975) Skeletal muscle growth and protein turnover. *Biochem J* **150**, 235–243.
8. Millward DJ (2023) Proteostasis and turnover. In *Encyclopedia of Human Nutrition*, vol. 1. pp. 388–400 [Carballero B, editor]. Amsterdam: Elsevier, Academic Press.
9. Klaips CL, Jayaraj GG & Hartl FU (2018) Pathways of cellular proteostasis in aging and disease. *J Cell Biol* **217**, 51–63.
10. Hipp MS, Kasturi P & Hartl FU (2019) The proteostasis network and its decline in ageing. *Nat Rev Mol Cell Biol* **20**, 421–435.
11. Pey AL (2020) Protein homeostasis and disease. In *Protein Homeostasis Diseases Mechanisms and Novel Therapies*, pp. 23–37 [Pey AL editor]. Amsterdam: Elsevier Inc.
12. Waterlow JC, Garlick PJ & Millward DJ (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*. Amsterdam: North-Holland Publishing Co.
13. Millward DJ (1970) Protein turnover in skeletal muscle. I. The measurement of rates of synthesis and catabolism of skeletal muscle protein using $(^{14}C)Na_2CO_3$ to label protein. *Clin Sci* **39**, 577–590.
14. Millward DJ (1970) Protein turnover in skeletal muscle. II. The effect of starvation and a protein-free diet on the synthesis and catabolism of skeletal muscle proteins in comparison to liver. *Clin Sci* **39**, 591–603.
15. Millward DJ, Garlick PJ, Nnanyelugo D & Waterlow JC (1976) The relative importance of muscle protein synthesis and breakdown in the regulation of muscle mass. *Biochem J* **156**, 185–188.
16. Tamaki T, Akatsuka A, Yoshimura S, Roy RR & Edgerton VR (2002) New fiber formation in the interstitial spaces of rat skeletal muscle during postnatal growth. *J Histochem Cytochem* **50**, 1097–1111.
17. Winchester PK & Gonyea WJ (1992) A quantitative study of satellite cells and myonuclei in stretched avian slow tonic muscle. *Anat Rec* **232**, 369–377.
18. Antonio J & Gonyea WJ (1993) Progressive stretch overload of skeletal muscle results in hypertrophy before hyperplasia. *J Appl Physiol* **75**, 1263–1271.
19. Winchester PK, Davis ME, Alway SE & Gonyea WJ (1991) Satellite cell activation in the stretch-enlarged anterior latissimus dorsi muscle of the adult quail. *Am J Physiol Cell Physiol* **260**(2 Pt 1), C206–12.
20. Laurent GJ & Sparrow MP (1977) Changes in RNA, DNA and protein content and the rates of protein synthesis and degradation during hypertrophy of the anterior latissimus dorsi muscle of the adult fowl (*Gallus domesticus*). *Growth* **41**, 249–262.
21. Laurent G & Millward D (1980) Protein turnover during skeletal muscle hypertrophy. *Fed Proc* **39**, 42–47.
22. Henshaw EC, Hirsch CA, Morton BE & Hiatt HH (1971) Control of protein synthesis in mammalian tissues through changes in ribosome activity. *J Biol Chem* **246**, 436–446. doi: 10.1016/S0021-9258(18)62509-8
23. Jefferson LS, Koehler JO & Morgan HE (1972) Effect of insulin on protein synthesis in skeletal muscle of an isolated perfused preparation of rat hemicorpus. *Proc Natl Acad Sci U S A* **69**, 816–820.
24. Millward DJ, Garlick PJ, James WPT, Nnanyelugo DO & Ryatt JS (1973) Relationship between protein synthesis and RNA content in skeletal muscle. *Nature* **241**, 204–245.
25. Cox MD, Dalal SS, Heard CRC & Millward DJ (1984) Metabolic rate and thyroid status in rats fed diets of different protein-energy value: the importance of free T3. *J Nutr* **114**, 1609–1616.
26. Jepson MM, Bates PC & Millward DJ (1988) The role of insulin and thyroid hormones in the regulation of muscle growth and protein turnover in response to dietary protein in the rat. *Br J Nutr* **59**, 397–415.
27. Raubenheimer D & Simpson SJ (2019) Protein leverage: theoretical foundations and ten points of clarification. *Obesity* **27**, 1225–1238.
28. Giugliano R & Millward DJ (1987) The effects of severe zinc deficiency on protein turnover in muscle and thymus. *Br J Nutr* **57**, 139–155.
29. Giugliano R & Millward DJ (1984) Growth and zinc homeostasis in the severely Zn-deficient rat. *Br J Nutr* **52**, 545–560.
30. Millward DJ (2017) Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children. *Nutr Res Rev* **30**, 50–72.
31. Millward DJ, Nnanyelugo DO, James WPT & Garlick PJ (1974) Protein metabolism in skeletal muscle: the effect of feeding and fasting on muscle RNA, free amino acids and plasma insulin concentrations. *Br J Nutr* **32**, 127–142.
32. Rudar M, Fiorotto ML & Davis TA (2019) Regulation of muscle growth in early postnatal life in a swine model. *Annu Rev Anim Biosci* **7**, 309–335.
33. Wool I & Cavicchi P (1966) Insulin regulation of protein synthesis by muscle ribosomes: effect of the hormone on translation. *Proc Natl Acad Sci U S A* **56**, 991–998.
34. Jefferson L, Li JB & Rannels SR (1977) Regulation by insulin of amino acid release and protein turnover in the perfused rat hemicorpus. *J Biol Chem* **252**, 1476–1483.
35. Anthony JC, Lang CH, Crozier SJ, *et al.* (2002) Contribution of insulin to the translational control of protein synthesis in skeletal muscle by leucine. *Am J Physiol Endocrinol Metab* **282**, E1092–E1101.
36. Odedra BR, Dalal SS & Millward DJ (1982) Muscle protein synthesis in the streptozotocin-diabetic rat. A possible role for corticosterone in the insensitivity to insulin infusion in vivo. *Biochem J* **202**, 363–368.
37. Millward DJ, Odedra B & Bates PC (1983) The role of insulin, corticosterone and other factors in the acute recovery of muscle protein synthesis on refeeding food-deprived rats. *Biochem J* **216**, 583–587.
38. Millward DJ (1989) The nutritional regulation of muscle growth and protein turnover. *Aquaculture* **79**, 1–28.
39. Millward DJ (1990) The hormonal control of protein turnover. *Clin Nutr* **9**, 115–126.
40. Anthony JC, Reiter AK, Anthony TG, *et al.* (2002) Orally administered leucine enhances protein synthesis in skeletal

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

muscle of diabetic rats in the absence of increases in 4E-BP1 or S6K1 phosphorylation. *Diabetes* **51**, 928–936.
41. Davis TA, Fiorotto ML, Burrin DG, *et al.* (2002) Stimulation of protein synthesis by both insulin and amino acids is unique to skeletal muscle in neonatal pigs. *Am J Physiol Endocrinol Metab* **282**, 880–890.
42. O'Connor PMJ, Kimball SR, Suryawan A, *et al.* (2003) Regulation of translation initiation by insulin and amino acids in skeletal muscle of neonatal pigs. *Am J Physiol Endocrinol Metab* **285**, 40–53.
43. Adegoke OAJ, Beatty BE, Kimball SR & Wing SS (2019) Interactions of the super complexes: when mTORC1 meets the proteasome. *Int J Biochem Cell Biol* **117**, 105638.
44. Kimball SR (2014) Integration of signals generated by nutrients, hormones, and exercise in skeletal muscle. *Am J Clin Nutr* **99**, 237–242.
45. Kim J & Guan K-L (2019) mTOR as a central hub of nutrient signalling and cell growth. *Nat Cell Biol* **21**, 63–71.
46. Xu D, Shimkus KL, Lacko HA, Kutzler L, Jefferson LS & Kimball SR (2019) Evidence for a role for sestrin1 in mediating leucine-induced activation of mtorc1 in skeletal muscle. *Am J Physiol Endocrinol Metab* **316**, E817–E828.
47. Atherton PJ, Wilkinson DJ & Smith K (2016) Feeding modulation of amino acid utilization: role of insulin and amino acids in skeletal muscle. In *The Molecular Nutrition of Amino Acids and Proteins* [Internet], pp. 109–124 [D Dardevet, editor]: Elsevier Inc. doi: 10.1016/B978-0-12-802167-5.00009-8
48. Millward DJ & Smith K (2019) The application of stable-isotope tracers to study human musculoskeletal protein turnover: a tale of bag filling and bag enlargement. *J Physiol* **597**, 1235–1249.
49. Abdulla H, Smith K, Atherton PJ & Idris I (2016) Role of insulin in the regulation of human skeletal muscle protein synthesis and breakdown: a systematic review and meta-analysis. *Diabetologia* **59**, 44–55.
50. Greenhaff PL, Karagounis LG, Peirce N, *et al.* (2008) Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *Am J Physiol Endocrinol Metab* **295**, 595–604.
51. Wilkes EA, Selby AL, Atherton PJ, *et al.* (2009) Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia. *Am J Clin Nutr* **90**, 1343–1350.
52. Prod'homme M, Balage M, Debras E, *et al.* (2005) Differential effects of insulin and dietary amino acids on muscle protein synthesis in adult and old rats. *J Physiol* **563**, 235–248.
53. Flaim KE, Li JB & Jefferson LS (1978) Effects of thyroxine on protein turnover in rat skeletal muscle. *Am J Physiol Endocrinol Metab Gastrointest Physiol* **4**, E231–E237.
54. Brown JG, Bates PC, Holliday MA & Millward DJ (1981) Thyroid hormones and muscle protein turnover. The effect of thyroid-hormone deficiency and replacement in thryoidectomized and hypophysectomized rats. *Biochem J* **194**, 771–782.
55. Brown JG, Van Bueren J & Millward DJ (1983) The effect of tri-iodothyronine administration on protein synthesis in the diabetic rat. *Biochem J* **214**, 637–640.
56. Brown JG & Millward DJ (1983) Dose response of protein turnover in rat skeletal muscle to triiodothyronine treatment. *Biochim Biophys Acta* **757**, 182–190.
57. Salvatore D, Simonides WS, Dentice M, Zavacki AM & Larsen PR (2014) Thyroid hormones and skeletal muscle—new insights and potential implications. *Nat Rev Endocrinol* **10**, 206–214.
58. Gavin LA & Moeller M (1983) The mechanism of recovery of hepatic T4-5′-deiodinase during glucose-refeeding: role of glucagon and insulin. *Metabolism* **32**, 543–551.
59. Pascual A & Aranda A (2013) Thyroid hormone receptors, cell growth and differentiation. *Biochim Biophys Acta* **1830**, 3908–3916.
60. Odedra BR & Millward DJ (1982) Effect of corticosterone treatment on muscle protein turnover in adrenalectomized rats and diabetic rats maintained on insulin. *Biochem J* **204**, 663–672.
61. Odedra BR, Bates PC & Millward DJ (1983) Time course of the effect of catabolic doses of corticosterone on protein turnover in rat skeletal muscle and liver. *Biochem J* **214**, 617–627.
62. Jepson MM, Pell JM, Bates PC & Millward DJ (1986) The effects of endotoxaemia on protein metabolism in skeletal muscle and liver of fed and fasted rats. *Biochem J* **235**, 329–336.
63. Braun TP & Marks DL (2015) The regulation of muscle mass by endogenous glucocorticoids. *Front Physiol* **6**, 1–12.
64. Elkina Y, von Haehling S, Anker SD & Springer J (2011) The role of myostatin in muscle wasting: an overview. *J Cachexia Sarcopenia Muscle* **2**, 143–151.
65. Sartori R, Gregorevic P & Sandri M (2014) TGFβ and BMP signaling in skeletal muscle: potential significance for muscle-related disease. *Trends Endocrinol Metab* **25**, 464–471.
66. Lee JH & Jun HS (2019) Role of myokines in regulating skeletal muscle mass and function. *Front Physiol* **10**, 1–9.
67. Rodgers BD & Ward CW (2021) Myostatin/activin receptor ligands in muscle and the development status of attenuating drugs. *Endocr Rev* **XX**, 1–37.
68. Cornish SM, Bugera EM, Duhamel TA, Peeler JD & Anderson JE (2020) A focused review of myokines as a potential contributor to muscle hypertrophy from resistance-based exercise. *Eur J Appl Physiol* **120**, 941–959.
69. Lee SJ (2021) Targeting the myostatin signaling pathway to treat muscle loss and metabolic dysfunction. *J Clin Invest* **131**, 1–10.
70. Jia S & Meng A (2021) TGFβ family signaling and development. *Development (Cambridge)* **148**, 1–7.
71. Leuchtmann AB, Adak V, Dilbaz S & Handschin C (2021) The role of the skeletal muscle secretome in mediating endurance and resistance training adaptations. *Front Physiol* **12**, 1–22.
72. Severinsen MCK & Pedersen BK (2020) Muscle–organ crosstalk: the emerging roles of myokines. *Endocr Rev* **41**, 594–609.
73. Lessard SJ, MacDonald TL, Pathak P, *et al.* (2018) JNK regulates muscle remodeling via myostatin/SMAD inhibition. *Nat Commun* **9**, 1–14.
74. Steinert ND, Potts GK, Wilson GM, *et al.* (2021) Mapping of the contraction-induced phosphoproteome identifies TRIM28 as a significant regulator of skeletal muscle size and function. *Cell Rep* **34,** 108796.
75. Attwaters M & Hughes SM (2022) Cellular and molecular pathways controlling muscle size in response to exercise. *FEBS J* **289**, 1428–1456.
76. Laurent G, Sparrow M & Millward D (1978) Turnover of muscle protein in the fowl: changes in rates of protein synthesis and breakdown during hypertrophy of the anterior and posterior latissimus dorsi muscles. *Biochem J* **176**, 407–417.
77. McCormick KM & Schultz E (1994) Role of satellite cells in altering myosin expression during avian skeletal muscle hypertrophy. *Develop Dyn* **199**, 52–63.
78. Laurent G, Sparrow M, Bates P & Millward D (1978) Turnover of muscle protein in the fowl. Collagen content and turnover in cardiac and skeletal muscles of the adult fowl and the changes during stretch-induced growth. *Biochem J* **176**, 419–427.

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

79. Yahya ZAH & Millward DJ (1994) Dietary protein and the regulation of long-bone and muscle growth in the rat. *Clin Sci* **87**, 213–224.
80. Yahya ZAH, Tirapegui JO, Bates PC & Millward DJ (1994) Influence of dietary protein, energy and corticosteroids on protein turnover, proteoglycan sulphation and growth of long bone and skeletal muscle in the rat. *Clin Sci* **87**, 607–618.
81. Yahya ZAH, Bates PC, Tirapegui JO, Morrell D, Buchanan C & Millward DJ (1988) IGF-1 concentrations in protein deficient rat plasma and tissues in relation to protein and proteoglycan synthesis rates. *Biochem Soc Trans* **16**, 624–625.
82. Tirapegui J, Yayha ZAH, Bates PC, Millward DJ (1994) Dietary energy, glucocorticoids and the regulation of long bone and muscle growth. *Clin Sci* **87**, 599–606.
83. Parker DF, Round JM, Sacco P & Jones DA (1990) A Cross-sectional survey of upper and lower limb strength in boys and girls during childhood and adolescence. *Ann Hum Biol* **17**, 199–211.
84. Yahya ZAH (1991) *Dietary and Hormonal Regulation of Bone and Muscle Growth in the Rat [PhD]*. London: School of Hygiene and Tropical Medicine.
85. Yahya ZAH, Bates PC & Millward DJ (1990) Responses to protein deficiency of plasma and tissue insulin-like growth factor-I levels and proteoglycan synthesis rates in rat skeletal muscle and bone. *J Endocrinol* **127**, 56–79.
86. Yahya ZAH, Bates PC, Tirapegui JO & Millward DJ (1989) Influence of dietary protein, energy and corticosterone on the hormonal stimulation of muscle and bone growth. *Biochem Soc Trans* **17**, 738–739.
87. Millward DJ (1989) The Endocrine Response to Dietary Protein: the Anabolic Drive on Growth. In *Milk Proteins: Nutritional, Clinical, Functional and Technological Aspects*, pp. 49–61 [CA Barth and E Schlimme, editors]. Steinkopff; New York: Springer.
88. Borowik AK, Davidyan A, Peelor FF, *et al.* (2022) Skeletal muscle nuclei in mice are not post-mitotic. *Function* **4**, zqac059.
89. Matsumoto T, Wakefield L, Tarlow BD & Grompe M (2020) In vivo lineage tracing of polyploid hepatocytes reveals extensive proliferation during liver regeneration. *Cell Stem Cell* **26**, 34–47.e3.
90. Millward D, Bates P & Laurent G (1976) The relationship between the growth of the DNA-unit in muscle and protein turnover. *Proc Nutr Soc* **36**, 35A.
91. Bates PC & Millward DJ (1983) Myofibrillar protein turnover. *Biochem J* **214**, 587–592.
92. Laurent G, Sparrow M, Bates P & Millward D (1978) Turnover of muscle protein in the fowl (*Gallus domesticus*). Rates of protein synthesis in fast and slow skeletal, cardiac and smooth muscle of the adult fowl. *Biochem J* **176**, 393–401.
93. Giordani L, He GJ, Negroni E, *et al.* (2019) High-dimensional single-cell cartography reveals novel skeletal muscle-resident cell populations. *Mol Cell* **74**, 609–621.e6.
94. Petrany MJ, Swoboda CO, Sun C, *et al.* (2020) Single-nucleus RNA-seq identifies transcriptional heterogeneity in multinucleated skeletal myofibers. *Nat Commun* **11**, 6374, 1–12.
95. Burleigh IG (1977) Observations on the number of nuclei within the fibres of some red and white muscles. *J Cell Sci* **23**, 269–284 .
96. Qaisar R & Larsson L (2014) What determines myonuclear domain size? *Indian J Physiol Pharmacol* **58**, 1–12.
97. Liu JX, Höglund AS, Karlsson P, *et al.* (2009) Myonuclear domain size and myosin isoform expression in muscle fibres from mammals representing a 100 000-fold difference in body size. *Exp Physiol* **94**, 117–129.
98. Murach KA, Englund DA, Dupont-Versteegden EE, McCarthy JJ & Peterson CA (2018) Myonuclear domain flexibility challenges rigid assumptions on satellite cell contribution to skeletal muscle fiber hypertrophy. *Front Physiol* **9**, 1–7.
99. van der Meer SFT, Jaspers RT & Degens H (2011) Is the myonuclear domain size fixed? *J Musculoskelet Neuronal Interact* **11**, 286–297.
100. Mantilla CB, Sill RV, Aravamudan B, Zhan WZ & Sieck GC (2008) Developmental effects on myonuclear domain size of rat diaphragm fibers. *J Appl Physiol* **104**, 787–794.
101. Mccarthy JJ, Mula J, Miyazaki M, *et al.* (2011) Effective fiber hypertrophy in satellite cell-depleted skeletal muscle. *Development* **138**, 3657–3666.
102. Bates PC, Grimble GK, Sparrow MP & Millward DJ (1983) Myofibrillar protein turnover. Synthesis of protein-bound 3-methylhistidine, actin, myosin heavy chain and aldolase in rat skeletal muscle in the fed and starved states. *Biochem J* **214**, 593–605.
103. Millward DJ (1980) Protein degradation in muscle and liver. In *Comprehensive Biochemistry*, vol. **19B**, pp. 153–232 [M Florkin, A Neuberger and M Van Deenan, editors]. Amsterdam: Elsevier/North Holland.
104. Cass JA, Williams CD, Irving TC, *et al.* (2021) A mechanism for sarcomere breathing: volume change and advective flow within the myofilament lattice. *Biophys J* **120**, 4079–4090.
105. Willingham TB, Kim Y, Lindberg E, Bleck CKE & Glancy B (2020) The unified myofibrillar matrix for force generation in muscle. *Nat Commun* **11**, 1–10.
106. Schultz E (1996) Satellite cell proliferative compartments in growing skeletal muscles. *Dev Biol* **175**, 84–94.
107. McCarthy JJ & Esser KA (2007) Counterpoint: satellite cell addition is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol* **103**, 1100–1102.
108. Connor RSO & Pavlath GK (1985) Point: satellite cell addition is obligatory for skeletal muscle hypertrophy. *J Appl Physiol* **103**, 1099–100.
109. Rehfeldt C, Mantilla CB, Sieck GC, *et al.* (2007) Point: counterpoint "Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy" comments. *J Appl Physiol* **103**, 1104–1106.
110. Murach KA, Fry CS, Kirby TJ, *et al.* (2018) Starring or supporting role? Satellite cells and skeletal muscle fiber size regulation. *Physiology* **33**, 26–38.
111. Murach KA, Fry CS, Dupont-Versteegden EE, McCarthy JJ & Peterson CA (2021) Fusion and beyond: Satellite cell contributions to loading-induced skeletal muscle adaptation. *FASEB J* **35**, 1–15.
112. Prasad V & Millay DP (2021) Skeletal muscle fibers count on nuclear numbers for growth. *Semin Cell Dev Biol* **119**, 3–10.
113. Englund DA, Figueiredo VC, Dungan CM, *et al.* (2020) Satellite cell depletion disrupts transcriptional coordination and muscle adaptation to exercise. *Function* **2**, 1–18.
114. Bachman JF & Chakkalakal JV (2022) Insights into muscle stem cell dynamics during postnatal development. *FEBS J* **289**, 2710–2722.
115. Moss FP & Leblond CP (1971) Satellite cells as the source of nuclei in muscles of growing rats. *Anat Rec* **170**, 421–435.
116. White RB, Biérinx AS, Gnocchi VF & Zammit PS (2010) Dynamics of muscle fibre growth during postnatal mouse development. *BMC Dev Biol* **10**, 21, 1–11.
117. Zammit PS (2017) Function of the myogenic regulatory factors Myf5, MyoD, Myogenin and MRF4 in skeletal muscle, satellite cells and regenerative myogenesis. *Semin Cell Dev Biol* **72**, 19–32.
118. Gattazzo F, Laurent B, Relaix F, Rouard H & Didier N (2020) Distinct phases of postnatal skeletal muscle growth govern the

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

progressive establishment of muscle stem cell quiescence. *Stem Cell Rep* **15**, 597–611.
119. Segalés J, Perdiguero E & Muñoz-Cánoves P (2014) Epigenetic control of adult skeletal muscle stem cell functions. *FEBS J* **282**, 1571–1588.
120. Segalés J, Perdiguero E & Muñoz-Cánoves P (2016) Regulation of muscle stem cell functions: a focus on the p38 MAPK signaling pathway. *Front Cell Dev Biol* **4**, 1–15.
121. Rugowska A, Starosta A & Konieczny P (2021) Epigenetic modifications in muscle regeneration and progression of Duchenne muscular dystrophy. *Clin Epigenet* **13**, 1–25.
122. Verma M, Asakura Y, Murakonda BSR, *et al.* (2018) Muscle satellite cell cross-talk with a vascular Niche maintains quiescence via VEGF and Notch signaling. *Cell Stem Cell* **23**, 530–543.e9.
123. Zhang L, Noguchi YT, Nakayama H, *et al.* (2019) The CalcR-PKA-Yap1 axis is critical for maintaining quiescence in muscle stem cells. *Cell Rep* **29**, 2154–2163.
124. Eliazer S, Muncie JM, Christensen J, *et al.* (2019) Wnt4 from the Niche controls the Mechano-properties and quiescent state of muscle stem cells. *Cell Stem Cell* **25**, 654–665.e4.
125. Chen W, Datzkiw D & Rudnicki MA (2020) Satellite cells in ageing: use it or lose it. *Open Biol* **10**, 1–11.
126. Ancel S, Stuelsatz P & Feige JN (2021) Muscle stem cell quiescence: controlling stemness by staying asleep. *Trends Cell Biol* **31**, 556–568.
127. Abuammah A, Maimari N, Towhidi L, *et al.* (2018) New developments in mechanotransduction: cross talk of the Wnt, TGF-β and Notch signalling pathways in reaction to shear stress. *Curr Opin Biomed Eng* **5**, 96–104.
128. Totaro A, Castellan M, Di Biagio D & Piccolo S (2018) Crosstalk between YAP/TAZ and Notch signaling. *Trends Cell Biol* **28**, 560–573.
129. Kim JH, Han GC, Seo JY, *et al.* (2016) Sex hormones establish a reserve pool of adult muscle stem cells. *Nat Cell Biol* **18**, 930–940.
130. Tajbakhsh S & Mourikis P (2018) Reciprocal signalling by Notch–Collagen V–CALCR retains muscle stem cells in their niche. *Nature* **557**, 714–718.
131. Purslow PP (2020) The structure and role of intramuscular connective tissue in muscle function. *Front Physiol* **11**, 1–15.
132. Jorgenson KW, Phillips SM & Hornberger TA (2020) Identifying the structural adaptations that drive the mechanical load-induced growth of skeletal muscle: a scoping review. *Cells* **9**, 1–32.
133. Kjaer M, Jørgensen NR, Heinemeier K & Magnusson SP (2015) Exercise and regulation of bone and collagen tissue biology. *Prog Mol Biol Transl Sci* **135**, 259–291.
134. Rayagiri SS, Ranaldi D, Raven A, *et al.* (2018) Basal lamina remodeling at the skeletal muscle stem cell niche mediates stem cell self-renewal. *Nat Commun* **9**, 1–12.
135. Fry CS, Kirby TJ, Kosmac K, McCarthy JJ & Peterson CA (2017) Myogenic progenitor cells control extracellular matrix production by fibroblasts during skeletal muscle hypertrophy. *Cell Stem Cell* **20**, 56–69.
136. Murach KA, Vechetti IJ, Van Pelt DW, *et al.* (2020) Fusion-independent satellite cell communication to muscle fibers during load-induced hypertrophy. *Function* **1**, 1–15.
137. Brightwell CR, Latham CM, Thomas NT, Keeble AR, Murach KA & Fry CS (2022) A glitch in the matrix: the pivotal role for extracellular matrix remodeling during muscle hypertrophy. *Am J Physiol. Cell Physiol NLM* **323**, C763–C771.
138. Kineman RD, del Rio-Moreno M & Sarmento-Cabral A (2018) 40 years of IGF1: understanding the tissue-specific roles of IGF1/IGF1R in regulating metabolism using the Cre/loxP system. *J Mol Endocrinol.* BioScientifica Ltd. **61**, T187–T198.
139. White MF & Kahn CR (2021) Insulin action at a molecular level – 100 years of progress. *Mol Metab* **52**, 1–20.
140. Boucher J, Kleinridders A & Kahn CR (2014) Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb Perspect Biol* **6**, a009191.
141. Dennis MD, Baum JI, Kimball SR & Jefferson LS (2011) Mechanisms involved in the coordinate regulation of mTORC1 by insulin and amino acids. *J Biol Chem* **286**, 8287–8296.
142. Proud CG (2019) Phosphorylation and signal transduction pathways in translational control. *Cold Spring Harb Perspect Biol* **11**, 1–22.
143. Proud CG (2006) Regulation of protein synthesis by insulin. *Biochem Soc Trans* **34**, 213–236.
144. Yakar S, Werner H & Rosen CJ (2018) 40 years of IGF1: insulin-like growth factors: actions on the skeleton. *J Mol Endocrinol.* BioScientifica Ltd. **61**, T115–T137.
145. LeRoith D, Holly JMP & Forbes BE (2021) Insulin-like growth factors: Ligands, binding proteins, and receptors. *Mol Metab* **52**, 1–16.
146. Vassilakos G, Lei H, Yang Y, *et al.* (2019) Deletion of muscle IGF-I transiently impairs growth and progressively disrupts glucose homeostasis in male mice. *FASEB J* **33**, 181–194.
147. Mavalli MD, DiGirolamo DJ, Fan Y, *et al.* (2010) Distinct growth hormone receptor signaling modes regulate skeletal muscle development and insulin sensitivity in mice. *J Clin Invest* **120**, 4007–4020.
148. Baker J, Liu JP, Robertson EJ & Efstratiadis A (1993) Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* **75**, 73–82.
149. Poreba E & Durzynska J (2020) Nuclear localization and actions of the insulin-like growth factor 1 (IGF-1) system components: transcriptional regulation and DNA damage response. *Mutat Res – Rev Mutat Res.* Elsevier B.V. **784**, 10837, 1–19.
150. Philippou A, Maridaki M, Pneumaticos S & Koutsilieris M (2014) The complexity of the IGF1 gene splicing, posttranslational modification and bioactivity. *Mol Med.* University of Michigan **20**, 202–214.
151. Siddle K (2012) Molecular basis of signaling specificity of insulin and IGF receptors: neglected corners and recent advances. *Front Endocrinol* **3**, 34, 1–24.
152. Belfiore A, Malaguarnera R, Vella V, *et al.* (2017) Insulin receptor isoforms in physiology and disease: an updated view. *Endocr Rev* **38**, 379–431.
153. Vassilakos G & Barton ER (2019) Insulin-like growth factor i regulation and its actions in skeletal muscle. *Compr Physiol* **9**, 413–438.
154. Wallis M (2009) New insulin-like growth factor (IGF)-precursor sequences from mammalian genomes: the molecular evolution of IGFs and associated peptides in primates. *Growth Horm IGF Res* **19**, 12–23.
155. Wallis M (2019) Characterization of a novel alternatively-spliced 5′ exon in the human insulin-like growth factor I (IGF-I) gene, expressed in liver and some cancers. *Growth Horm IGF Res* **46–47**, 36–43.
156. Bikle DD, Tahimic C, Chang W, Wang Y, Philippou A & Barton ER (2015) Role of IGF-I signaling in muscle bone interactions. *Bone* **80**, 79–88.
157. Annibalini G, Bielli P, De Santi M, *et al.* (2016) MIR retroposon exonization promotes evolutionary variability and generates species-specific expression of IGF-1 splice variants. *Biochim Biophys Acta Gene Regul Mech* **1859**, 757–768.
158. Durzynska J, Philippou A, Brisson BK, Nguyen-McCarty M & Barton ER (2013) The pro-forms of insulin-like growth factor I (IGF-I) are predominant in skeletal muscle and alter IGF-I receptor activation. *Endocrinology* **154**, 1215–1224.

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

159. Annibalini G, Contarelli S, De Santi M, *et al.* (2018) The intrinsically disordered E-domains regulate the IGF-1 pro-hormones stability, subcellular localisation and secretion. *Sci Rep* **8**, 8919, 1–13.
160. Matheny RW, Nindl BC & Adamo ML (2010) Minireview: mechano-growth factor: a putative product of IGF-I gene expression involved in tissue repair and regeneration. *Endocrinology* **151**, 865–875.
161. Siegfried JM, Kasprzyk PG, Treston AM, Mulshine JL, Quinn KA & Cuttitta F (1992) A mitogenic peptide amide encoded within the E peptide domain of the insulin-like growth factor IB prohormone. *Proc Natl Acad Sci U S A* **89**, 8107–8111.
162. Fornaro M, Hinken AC, Needle S, *et al.* (2014) Mechano-growth factor peptide, the COOH terminus of unprocessed insulin-like growth factor 1, has no apparent effect on myoblasts or primary muscle stem cells. *Am J Physiol Endocrinol Metab* **306**, 150–156.
163. Rotwein P (2014) Editorial: the fall of mechanogrowth factor? *Mol Endocrinol* **28**, 155–156.
164. Brisson BK, Spinazzola J, Park SH & Barton ER (2014) Viral expression of insulin-like growth factor I E-peptides increases skeletal muscle mass but at the expense of strength. *Am J Physiol Endocrinol Metab* **306**, 965–974.
165. Ascenzi F, Barberi L, Dobrowolny G, *et al.* (2019) Effects of IGF-1 isoforms on muscle growth and sarcopenia. *Aging Cell* **18**, e12954, 1–11.
166. Dehkhoda F, Lee CMM, Medina J & Brooks AJ (2018) The growth hormone receptor: mechanism of receptor activation, cell signaling, and physiological aspects. *Front Endocrinol.* Frontiers Media S.A. **9**, 35, 1–23.
167. Alfieri CM, Evans-Anderson HJ & Yutzey KE (2007) Developmental regulation of the mouse IGF-I exon 1 promoter region by calcineurin activation of NFAT in skeletal muscle. *Am J Physiol Cell Physiol* [Internet] **292**, C1887–C1894. www.ajpcell.org
168. Wynes MW & Riches DWH (2003) Induction of macrophage insulin-like growth factor-I expression by the Th2 cytokines IL-4 and IL-13 1. *J Immunol* [Internet] **171**, 3550–3559. http://journals.aai.org/jimmunol/article-pdf/171/7/3550/1174133/3550.pdf
169. Iresjö BM, Diep L & Lundholm K (2022) Initiation of muscle protein synthesis was unrelated to simultaneously upregulated local production of IGF-1 by amino acids in non-proliferating L6 muscle cells. *PLoS One* **17**, e027092, 1–11.
170. Wosczyna MN & Rando TA (2018) A muscle stem cell support group: coordinated cellular responses in muscle regeneration. *Dev Cell* [Internet] **46**, 135–143. doi: 10.1016/j.devcel.2018.06.018
171. Christov C, Chrétien F, Abou-Khalil R, *et al.* (2007) Muscle satellite cells and endothelial cells: CloseNeighbors and privileged partners. *Mol Biol Cell* **18**, 1397–1409.
172. Allard JB & Duan C (2018) IGF-binding proteins: why do they exist and why are there so many? *Front Endocrinol (Lausanne)* **9**, 1–12.
173. Bang P (2019) Pediatric implications of normal insulin-GH-IGF-Axis physiology. In Feingold K, Anawalt B, Boyce A, Al E, editors. *Endotext – NCBI Bookshelf.* MDText.com., 1–20.
174. Leung KC, Doyle N, Ballesteros M, Waters MJ & Ho KKY (2000) Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. *J Clin Endocrinol Metab* **85**, 4712–4720.
175. Oxvig C (2015) The role of PAPP-A in the IGF system: location, location, location. *J Cell Commun Signal* **9**, 177–187.
176. Belfiore A, Frasca F, Pandini G, Sciacca L & Vigneri R (2009) Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. *Endocr Rev* **30**, 586–623.
177. Bailyes EM, Nave BT, Soos MA, Orr SR, Hayward AC & Siddle K (1997) Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues : quantification of individual receptor species by selective immunoprecipitation and immunoblotting [Internet]. *Biochem J* **327**. http://portlandpress.com/biochemj/article-pdf/327/1/209/624474/bj3270209.pdf
178. Slaaby R (2015) Specific insulin/IGF1 hybrid receptor activation assay reveals IGF1 as a more potent ligand than insulin. *Sci Rep* **5**, 1–5.
179. Kelly GM, Buckley DA, Kiely PA, Adams DR & O'Connor R (2012) Serine phosphorylation of the insulin-like growth factor I (IGF-1) receptor C-terminal tail restrains kinase activity and cell growth. *J Biol Chem* **287**, 28180–28194.
180. O'Neill BT, Lee KY, Klaus K, *et al.* (2016) Insulin and IGF-1 receptors regulate FoxO-mediated signaling in muscle proteostasis. *J Clin Invest* **126**, 3433–3446.
181. Kitamura T, Kitamura Y, Nakae J, *et al.* (2004) Mosaic analysis of insulin receptor function. *J Clin Invest* **113**, 209–219.
182. Liu JP, Baker J, Perkins AS, Roberteon EJ & Efetratiadie A (1993) Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf.1) and type 1 IGF receptor (Igflr). *Cell* **75**, 59–72.
183. Mavalli MD, DiGirolamo DJ, Fan Y, *et al.* (2010) Distinct growth hormone receptor signaling modes regulate skeletal muscle development and insulin sensitivity in mice. *J Clin Invest* **120**, 4007–4020.
184. O'Neill BT, Lauritzen HPMM, Hirshman MF, Smyth G, Goodyear LJ & Kahn CR (2015) Differential role of insulin/IGF-1 receptor signaling in muscle growth and glucose homeostasis. *Cell Rep* [Internet] **11**, 1220–1235. doi: 10.1016/j.celrep.2015.04.037
185. Spangenburg EE, Le Roith D, Ward CW & Bodine SC (2008) A functional insulin-like growth factor receptor is not necessary for load-induced skeletal muscle hypertrophy. *J Physiol* **586**, 283–291.
186. Jacob R, Xiaoyue HU, Niederstock D, *et al.* (1996) IGF-I stimulation of muscle protein synthesis in the awake rat: Permissive role of insulin and amino acids. *Am J Physiol Endocrinol Metab* **270**, E60–E66.
187. Bark TH, McNurlan MA, Lang CH & Garlick PJ (1998) Increased protein synthesis after acute IGF-I or insulin infusion is localized to muscle in mice. *Am J Physiol Endocrinol Metab* **275**, 118–123.
188. Rommel C, Bodine SC, Clarke BA, *et al.* (2001) Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Alt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nat Cell Biol* **3**, 1009–1113.
189. Stewart CE & Pell JM (2010) Point: IGF is the major physiological regulator of muscle mass. *J Appl Physiol* **108**, 1820–1821.
190. Flueck M & Goldspink G (2010) Counterpoint: IGF is not the major physiological regulator of muscle mass. *J Appl Physiol* **108**, 1821–1823.
191. Spangenburg E, Phillips S, Yang S, *et al.* (2010) Comments on point: counterpoint: IGF is/is not the major physiological regulator of muscle mass. *J Appl Phsiol* **108**, 1825–1831.
192. Flueck M & Goldspink G (2010) Last word on point: counterpoint: IGF is/is not the major physiological regulator of muscle mass. *J Appl Physiol* **108**, 1833.
193. McKay BR, O'Reilly CE, Phillips SM, Tarnopolsky MA & Parise G (2008) Co-expression of IGF-1 family members with myogenic regulatory factors following acute damaging

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

muscle-lengthening contractions in humans. *J Physiol* **586**, 5549–5560.
194. Grubb A, Joanisse S, Moore DR, *et al.* (2014) IGF-1 colocalizes with muscle satellite cells following acute exercise in humans. *Appl Physiol Nutr Metab* **39**, 514–518.
195. Kirby TJ, Patel RM, McClintock TS, Dupont-Versteegden EE, Peterson CA & McCarthy JJ (2016) Myonuclear transcription is responsive to mechanical load and DNA content but uncoupled from cell size during hypertrophy. *Mol Biol Cell* **27**, 788–798.
196. Czerwinski SM, Martin JM & Bechtel PJ (1994) Modulation of IGF mRNA abundance during stretch-induced skeletal muscle hypertrophy and regression. *J Appl Physiol* **76**, 2026–2030.
197. McKoy G, Ashley W, Mander J, *et al.* (1999) Expression of insulin growth factor-1 splice variants and structural genes in rabbit skeletal muscle induced by stretch and stimulation. *J Physiol* **516**, 583–592.
198. Goldspink DF, Cox VM, Smith SK, *et al.* (1995) Muscle growth in response to mechanical stimuli. *Am J Physiol Endocrinol Metab* **268**, 7532362.
199. Perrone CE, Fenwick-Smith D & Vandenburgh HH (1995) Collagen and stretch modulate autocrine secretion of insulin-like growth factor-1 and insulin-like growth factor binding proteins differentiated skeletal muscle cells. *J Biol Chem* **270**, 2099–2106.
200. Tonkin J, Temmerman L, Sampson RD, *et al.* (2015) Monocyte/macrophage-derived IGF-1 orchestrates murine skeletal muscle regeneration and modulates autocrine polarization. *Mol Ther* **23**, 1189–1200.
201. Goldberg AL, Etlinger JD, Goldspink DF & Jablecki C (1975) Mechanism of work-induced hypertrophy of skeletal muscle. *Med Sci Sports* **7**, 185–198.
202. Baker SA & Rutter J (2023) Metabolites as signalling molecules. *Nat Rev Mol Cell Biol* Nature Research. **24**, 355–374.
203. Zhu J & Thompson CB (2019) Metabolic regulation of cell growth and proliferation. *Nat Rev Mol Cell Biol.* Nature Publishing Group **20**, 436–450.
204. Liu GY & Sabatini DM (2020) mTOR at the nexus of nutrition, growth, ageing and disease. *Nat Rev Mol Cell Biol.* Nature Research **21**, 183–203.
205. Yoon MS (2017) mTOR as a key regulator in maintaining skeletal muscle mass. *Front Physiol.* Frontiers Media S.A **8**, 788, 1–9.
206. McCarthy JJ & Murach KA (2018) Anabolic and catabolic signaling pathways that regulate skeletal muscle mass. In *Nutrition and Enhanced Sports Performance: Muscle Building, Endurance, and Strength*, pp. 275–290 [Debasis Bagchi, Sreejayan Nair and Chandan K. Sen, editors]. Amsterdam: Elsevier, Academic Press.
207. Valvezan AJ & Manning BD (2019) Molecular logic of mTORC1 signalling as a metabolic rheostat. *Nat Metab* [Internet] **1**, 321–333. doi: 10.1038/s42255-019-0038-7
208. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S & Sabatini DM (2010) Ragulator-rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* **141**, 290–303.
209. Betz C & Hall MN (2013) Where is mTOR and what is it doing there? *J Cell Biol* **203**, 563–574.
210. Song Z, Moore DR, Hodson N, *et al.* (2017) Resistance exercise initiates mechanistic target of rapamycin (mTOR) translocation and protein complex co-localisation in human skeletal muscle. *Sci Rep* **7**, 5028, 1–14.
211. Tinline-Goodfellow CT, Lees MJ & Hodson N (2022) The skeletal muscle fiber periphery: a nexus of mTOR-related anabolism. *Sports Med Health Sci* **5**, 10–19.
212. Goodman CA (2014) The role of mTORC1 in regulating protein synthesis and skeletal muscle mass in response to various mechanical stimuli. *Rev Physiol Biochem Pharmacol* **166**, 43–95.
213. You JS, Mcnally RM, Jacobs BL, *et al.* (2019) The role of raptor in the mechanical load-induced regulation of mTOR signaling, protein synthesis, and skeletal muscle hypertrophy. *FASEB J* **33**, 4021–4034.
214. Lin KH, Wilson GM, Blanco R, *et al.* (2021) A deep analysis of the proteomic and phosphoproteomic alterations that occur in skeletal muscle after the onset of immobilization. *J Physiol* **599**, 2887–2906.
215. Yoshida T & Delafontaine P (2020) Mechanisms of IGF-1-mediated regulation of skeletal muscle hypertrophy and atrophy. *Cells* **9**, 1–25.
216. Hoxhaj G & Manning BD (2020) The PI3K–AKT network at the interface of oncogenic signalling and cancer metabolism. *Nat Rev Cancer* **20**, 74–88.
217. Manning BD & Toker A (2017) AKT/PKB signaling: navigating the network. *Cell* **169**, 381–405.
218. Cargnello M & Roux PP (2011) Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. *Microbiol Mol Biol Rev* **75**, 50–83.
219. Han J, Wu J & Silke J (2020) An overview of mammalian p38 mitogen-activated protein kinases, central regulators of cell stress and receptor signaling. [version 1; peer review: 2 approved] *F1000Research*, 9(F1000 Faculty Rev), 653, 1–20.
220. Goh Q, Song T, Petrany MJ, *et al.* (2019) Myonuclear accretion is a determinant of exercise-induced remodeling in skeletal muscle. *eLife* **8**, 1–19.
221. Fukada SI, Akimoto T & Sotiropoulos A (2020) Role of damage and management in muscle hypertrophy: Different behaviors of muscle stem cells in regeneration and hypertrophy. *Biochim Biophys Acta Mol Cell Res* **1867**, 118742.
222. Addinsall AB, Cacciani N, Akkad H, *et al.* (2021) JAK/STAT inhibition augments soleus muscle function in a rat model of critical illness myopathy via regulation of complement C3/3R. *J Physiol* **599**, 2869–2886.
223. Wen Y, Englund DA, Peck BD, Murach KA, McCarthy JJ & Peterson CA (2021) Myonuclear transcriptional dynamics in response to exercise following satellite cell depletion. *iScience* **24**, 102838, 1–17.
224. Kaczmarek A, Kaczmarek M, Ciałowicz M, *et al.* (2021) The role of satellite cells in skeletal muscle regeneration—the effect of exercise and age. *Biology (Basel)* **10**, 1–18.
225. Jabre S, Hleihel W & Coirault C (2021) Nuclear mechano-transduction in skeletal muscle. *Cells* **10**, 1–18.
226. Zhou S, Han L, Weng M, *et al.* (2021) Paxbp1 controls a key checkpoint for cell growth and survival during early activation of quiescent muscle satellite cells. *Proc Natl Acad Sci U S A* **118**, 1–12.
227. Murach KA, Dungan CM, von Walden F & Wen Y (2022) Epigenetic evidence for distinct contributions of resident and acquired myonuclei during long-term exercise adaptation using timed in vivo myonuclear labeling. *Am J Physiol-Cell Physiol* **322**, C86–C93.
228. Dayanidhi S & Lieber RL (2014) Skeletal muscle satellite cells: mediators of muscle growth during development and implications for developmental disorders. *Muscle Nerve* **50**, 723–732.
229. Oertel G (1988) Morphometric analysis of normal skeletal muscles in infancy, childhood and adolescence. An autopsy study. *J Neurol Sci* **88**, 303–313.
230. Kawano F, Takeno Y, Nakai N, *et al.* (2008) Essential role of satellite cells in the growth of rat soleus muscle fibers. *Am J Physiol Cell Physiol* **295**, 458–467.

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

231. Song H, Cho S, Lee HY, Lee H & Song W (2018) The effects of progressive resistance exercise on recovery rate of bone and muscle in a rodent model of hindlimb suspension. *Front Physiol* **9**, 1–10.
232. Desplanches D, Mayet MH, Sempore B & Flandrois R (1987) Structural and functional responses to prolonged hindlimb suspension in rat muscle. *J Appl Physiol* **63**, 558–563.
233. Zhang S, Ueno D, Ohira T, *et al.* (2021) Depression of bone density at the weight-bearing joints in Wistar Hannover rats by a simulated mechanical stress associated with partial gravity environment. *Front Cell Dev Biol* **9**, 1–10.
234. Zhu WG, Hibbert JE, Lin KH, *et al.* (2021) Weight pulling: a novel mouse model of human progressive resistance exercise. *Cells* **10**, 1–21.
235. Eftestøl E, Egner IM, Lunde IG, *et al.* (2016) Increased hypertrophic response with increased mechanical load in skeletal muscles receiving identical activity patterns. *Am J Physiol Cell Physiol* **311**, C616–C629.
236. Bamman MM, Roberts BM & Adams GR (2018) Molecular regulation of exercise-induced muscle fiber hypertrophy. *Cold Spring Harb Perspect Med* **8**, a029751, 1–16.
237. Lim C, Nunes EA, Currier BS, McLeod JC, Thomas ACQ & Phillips SM (2022) An evidence-based narrative review of mechanisms of resistance exercise-induced human skeletal muscle hypertrophy. *Med Sci Sports Exerc* **54**, 1546–1559.
238. Sawan SA, Hodson N, Babits P, Malowany JM, Kumbhare D & Moore DR (2021) Satellite cell and myonuclear accretion is related to training-induced skeletal muscle fiber hypertrophy in young males and females. *J Appl Physiol* **131**, 871–880.
239. Jaiswal N, Gavin M, Loro E, *et al.* (2021) AKT controls protein synthesis and oxidative metabolism via combined mTORC1 and FOXO1 signalling to govern muscle physiology. *J Cachexia Sarcopenia Muscle* **13**, 495–514.
240. Ochoa D, Jarnuczak AF, Viéitez C, *et al.* (2020) The functional landscape of the human phosphoproteome. *Nat Biotechnol* **38**, 365–373.
241. Franciosa G, Martinez-Val A & Olsen JV () Deciphering the human phosphoproteome. *Nat Biotechnol* **38**, 285–286.
242. Needham EJ, Hingst JR, Parker BL, *et al.* (2022) Personalized phosphoproteomics identifies functional signaling. *Nat Biotechnol* **40**, 576–584.
243. Sharma K, D'Souza RCJ, Tyanova S, *et al.* (2014) Ultradeep human phosphoproteome reveals a distinct regulatory nature of Tyr and Ser/Thr-based signaling. *Cell Rep* **8**, 1583–1594.
244. Reimann L, Schwäble AN, Fricke AL, *et al.* (2020) Phosphoproteomics identifies dual-site phosphorylation in an extended basophilic motif regulating FILIP1-mediated degradation of filamin-C. *Commun Biol* **3**, 1–19.
245. Potts GK, McNally RM, Blanco R, *et al.* (2017) A map of the phosphoproteomic alterations that occur after a bout of maximal-intensity contractions. *J Physiol* **595**, 5209–5226.
246. Martinez-Val A, Bekker-Jensen DB, Steigerwald S, *et al.* (2021) Spatial-proteomics reveals phospho-signaling dynamics at subcellular resolution. *Nat Commun* **12**, 7113, 1–17.
247. Hakuno F & Takahashi SI (2018) 40 years of IGF1: IGF1 receptor signaling pathways. *J Mol Endocrinol* BioScientifica Ltd. **61**, T69–T86.
248. Batista TM, Jayavelu AK, Wewer Albrechtsen NJ, *et al.* (2020) A cell-autonomous signature of dysregulated protein phosphorylation underlies muscle insulin resistance in type 2 diabetes. *Cell Metab* **32**, 844–859.e5.
249. Lai KMV, Gonzalez M, Poueymirou WT, *et al.* (2004) Conditional activation of Akt in adult skeletal muscle induces rapid hypertrophy. *Mol Cell Biol* **24**, 9295–9304.
250. Theeuwes WF, Pansters NAM, Gosker HR, *et al.* (2020) Recovery of muscle mass and muscle oxidative phenotype following disuse does not require GSK-3 inactivation. *Biochim Biophys Acta Mol Basis Dis* **1866**, 165740.
251. Hay N (2011) Interplay between FOXO, TOR, and Akt. *Biochim Biophys Acta Mol Cell Res* **1813**, 1965–1970.
252. Chen CC, Jeon SM, Bhaskar PT, *et al.* (2010) FoxOs Inhibit mTORC1 and activate Akt by inducing the expression of Sestrin3 and Rictor. *Dev Cell* **18**, 592–604.
253. Ro SH, Fay J, Cyuzuzo CI, *et al.* (2020) SESTRINs: emerging dynamic stress-sensors in metabolic and environmental health. *Front Cell Dev Biol* **8**, 603421, 1–13.
254. Lawrence RE & Zoncu R (2019) The lysosome as a cellular centre for signalling, metabolism and quality control. *Nat Cell Biol* **21**, 133–142.
255. Morrison DK (2012) MAP kinase pathways. *Cold Spring Harb Perspect Biol* **4**, 1–6.
256. Ronkina N & Gaestel M (2022) MAPK-activated protein kinases: servant or partner? *Annu Rev Biochem* **91**, 505–540.
257. Li L, Zhao GD, Shi Z, Qi LL, Zhou LY & Fu ZX (2016) The Ras/Raf/MEK/ERK signaling pathway and its role in the occurrence and development of HCC (Review). *Oncol Lett* **12**, 3045–3050.
258. Brennan CM, Emerson CP, Owens J & Christoforou N (2021) p38 MAPKs – roles in skeletal muscle physiology, disease mechanisms, and as potential therapeutic targets. *JCI Insight* **6,** e149915, 1–11.
259. Haddad F & Adams GR (2004) Inhibition of MAP/ERK kinase prevents IGF-I-induced hypertrophy in rat muscles. *J Appl Physiol* **96**, 203–210.
260. Kramer HF & Goodyear LJ (2007) Exercise, MAPK, and NF-κB signaling in skeletal muscle. *J Appl Physiol* **103**, 388–395.
261. Shi H, Scheffler JM, Zeng C, *et al.* (2009) Mitogen-activated protein kinase signaling is necessary for the maintenance of skeletal muscle mass. *Am J Physiol Cell Physiol* **296**, 1040–1048.
262. Segalés J, Islam ABMMK, Kumar R, *et al.* (2016) Chromatin-wide and transcriptome profiling integration uncovers p38α MAPK as a global regulator of skeletal muscle differentiation. *Skelet Muscle* **6**, 1–15.
263. Martino F, Perestrelo AR, Vinarský V, Pagliari S & Forte G (2018) Cellular mechanotransduction: from tension to function. *Front Physiol* **9**, 1–21.
264. Anderson JE (2021) Key concepts in muscle regeneration: muscle "cellular ecology" integrates a gestalt of cellular cross-talk, motility, and activity to remodel structure and restore function. *Eur J Appl Physiol* **122**, 273–300.
265. Loreti M & Sacco A (2022) The jam session between muscle stem cells and the extracellular matrix in the tissue microenvironment. *NPJ Regen Med* **7**, 1–15.
266. Yin H, Price F & Rudnicki MA (2013) Satellite cells and the muscle stem cell niche. *Physiol Rev* **93**, 23–67.
267. Boers HE, Haroon M, Le Grand F, Bakker AD, Klein-Nulend J & Jaspers RT (2018) Mechanosensitivity of aged muscle stem cells. *J Orthop Res* **36**, 632–641.
268. Haroon M, Klein-Nulend J, Bakker AD, *et al.* (2021) Myofiber stretch induces tensile and shear deformation of muscle stem cells in their native niche. *Biophys J* **120**, 2665–2678.
269. Tatsumi R (2010) Mechano-biology of skeletal muscle hypertrophy and regeneration: Possible mechanism of stretch-induced activation of resident myogenic stem cells. *Anim Sci J* **81**, 11–20.
270. Serrano AL, Baeza-Raja B, Perdiguero E, Jardí M & Muñoz-Cánoves P (2008) Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. *Cell Metab* **7**, 33–44.
271. Pawlikowski B, Vogler TO, Gadek K & Olwin BB (2017) Regulation of skeletal muscle stem cells by fibroblast growth factors. *Develop Dyn* **246**, 359–367.

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

272. Xie Y, Su N, Yang J, *et al.* (2020) FGF/FGFR signaling in health and disease. *Signal Transduct Target Ther* **5**, 181, 1–38.
273. Brewer JR, Mazot P & Soriano P (2016) Genetic insights into the mechanisms of Fgf signaling. http://www.genesdev.org/cgi/doi/10.1101/gad.277137
274. Ornitz DM & Itoh N (2015) The fibroblast growth factor signaling pathway. *Wiley Interdiscip Rev Dev Biol* **4**, 215–266.
275. Liu Y & Schneider MF (2014) FGF2 activates TRPC and Ca^{2+} signaling leading to satellite cell activation. *Front Physiol* **5**, 1–10.
276. Mukund K & Subramaniam S (2020) Skeletal muscle: a review of molecular structure and function, in health and disease. *Wiley Interdiscip Rev Syst Biol Med* **12**, 1–46.
277. Singh K, Cassano M, Planet E, *et al.* (2015) A KAP1 phosphorylation switch controls MyoD function during skeletal muscle differentiation. *Genes Dev* **29**, 513–525.
278. Lin K, Lemens J, Torbey M, Steinert N & Hornberger T (2022) The role of satellite cell derived TRIM28 in mechanical loading-induced and injury-induced myogenesis. *FASEB J* **36**, S1, R2414 (abs).
279. Sharifi S & Bierhoff H (2018) Regulation of RNA polymerase I transcription in development, disease, and aging. *Annu Rev Biochem* **87**, 51–73.
280. Kressler D, Hurt E & Baßler J (2017) A puzzle of life: crafting ribosomal subunits. *Trends Biochem Sci* **42**, 640–654.
281. Piazzi M, Bavelloni A, Gallo A, Faenza I & Blalock WL (2019) Signal transduction in ribosome biogenesis: a recipe to avoid disaster. *Int J Mol Sci* **20**, 2718, 1–47.
282. Pelletier J, Thomas G & Volarevic S (2017) Ribosome biogenesis in cancer: new players and therapeutic avenues. *Nat Publ Group* **18**, 51–63.
283. Parisien M, Wang X & Pan T (2013) Diversity of human tRNA genes from the 1000-Genomes Project. *RNA Biol* **10**, 1853–1867.
284. Advani VM & Ivanov P (2019) Translational control under stress: reshaping the translatome. *BioEssays* **41**, 1–10.
285. Liu L & Pilch PF (2016) PTRF/Cavin-1 promotes efficient ribosomal RNA transcription in response to metabolic challenges. *eLife* **5**, 1–20.
286. Brook MS, Wilkinson DJ, Smith K & Atherton PJ (2019) It's not just about protein turnover: the role of ribosomal biogenesis and satellite cells in the regulation of skeletal muscle hypertrophy. *Eur J Sport Sci* **19**, 952–963.
287. Kusnadi EP, Hannan KM, Hicks RJ, Hannan RD, Pearson RB & Kang J (2015) Regulation of rDNA transcription in response to growth factors, nutrients and energy. *Gene* **556**, 27–34.
288. Chaillou T, Kirby TJ & Mccarthy JJ (2014) Ribosome biogenesis: emerging evidence for a central role in the regulation of skeletal muscle mass. *J Cell Physiol* **229**, 1584–1594.
289. Fyfe JJ, Bishop DJ, Bartlett JD, *et al.* (2018) Enhanced skeletal muscle ribosome biogenesis, yet attenuated mTORC1 and ribosome biogenesis-related signalling, following short-term concurrent versus single-mode resistance training. *Sci Rep* **8**, 1–21.
290. Proud CG (2014) Control of the translational machinery by amino acids. *Am J Clin Nutr* **99**, 231–236.
291. Figueiredo VC & McCarthy JJ (2019) Regulation of ribosome biogenesis in skeletal muscle hypertrophy. *Physiology* **34**, 30–42.
292. Hannan KM, Rothblum LI & Jefferson LS (1998) Regulation of ribosomal DNA transcription by insulin. *Am J Physiol* **275**, 130–138.
293. Antonetti DA, Kimball SR, Horetsky RL & Jefferson LS (1993) Regulation of rDNA transcription by insulin in primary cultures of rat hepatocytes. *J Biol Chem* **268**, 25277–25284.
294. Meyuhas O & Kahan T (2015) The race to decipher the top secrets of TOP mRNAs. *Biochim Biophys Acta Gene Regul Mech* **1849**, 801–811.
295. Philippe L, van den Elzen AMG, Watson MJ & Thoreen CC (2020) Global analysis of LARP1 translation targets reveals tunable and dynamic features of 5′ TOP motifs. *Proc Natl Acad Sci U S A* **117**, 5319–5328.
296. Tcherkezian J, Cargnello M, Romeo Y, *et al.* (2014) Proteomic analysis of cap-dependent translation identifies LARP1 as a key regulator of 5′TOP mRNA translation. *Genes Dev* **28**, 357–371.
297. Jia JJ, Lahr RM, Solgaard MT, *et al.* (2021) MTORC1 promotes TOP mRNA translation through site-specific phosphorylation of LARP1. *Nucleic Acids Res* **49**, 3461–3489.
298. Berman AJ, Thoreen CC, Dedeic Z, Chettle J, Roux PP & Blagden SP (2021) Controversies around the function of LARP1. *RNA Biol* **18**, 207–217.
299. Al-Ashtal HA, Rubottom CM, Leeper TC & Berman AJ (2021) The LARP1 La-module recognizes both ends of TOP mRNAs. *RNA Biol* **18**, 248–258.
300. Richter JD & Sonenberg N (2005) Regulation of cap-dependent translation by eIF4E inhibitory proteins. *Nature* **433**, 477–480.
301. Nandagopal N & Roux PP (2015) Regulation of global and specific mRNA translation by the mTOR signaling pathway. *Translation* **3**, e983402.
302. Shuda M, Velásquez C, Cheng E, *et al.* (2015) CDK1 substitutes for mTOR kinase to activate mitotic cap-dependent protein translation. *Proc Natl Acad Sci U S A* **112**, 5875–5882.
303. Zeng W, Yue L, Lam KSW, *et al.* (2022) CPEB1 directs muscle stem cell activation by reprogramming the translational landscape. *Nat Commun* **13**, 1–19.
304. Kimball SR & Lang CH (2018) Mechanisms underlying muscle protein imbalance induced by alcohol. *Annu Rev Nutr* **38**, 197–217.
305. Bohé J, Aili Low JF, Wolfe RR & Rennie MJ (2001) Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *J Physiol* **532**, 575–579.
306. Atherton PJ, Etheridge T, Watt PW, *et al.* (2010) Muscle full effect after oral protein: time-dependent concordance and discordance between human muscle protein synthesis and mTORC1 signaling. *Am J Clin Nutr* **92**, 1080–1088.
307. Mitchell WK, Phillips BE, Williams JP, *et al.* (2015) A dose – rather than delivery profile –dependent mechanism regulates the "muscle-full" effect in response to oral essential amino acid intake in young men. *J Nutr* **145**, 207–214.
308. Mitchell WK, Phillips BE, Hill I, *et al.* (2017) Human skeletal muscle is refractory to the anabolic effects of leucine during the postprandial muscle-full period in older men. *Clin Sci* **131**, 2643–2653.
309. Norton LE, Layman DK, Bunpo P, Anthony TG, Brana DV & Garlick PJ (2009) The leucine content of a complete meal directs peak activation but not duration of skeletal muscle protein synthesis and mammalian target of rapamycin signaling in rats. *J Nutr* **139**, 1103–1109.
310. Wilson GJ, Layman DK, Moulton CJ, *et al.* (2011) Leucine or carbohydrate supplementation reduces AMPK and eEF2 phosphorylation and extends postprandial muscle protein synthesis in rats. *Am J Physiol Endocrinol Metab* **301**, 1236–1242.
311. Häussinger D, Gerok W, Roth E & Lang F (1993) Cellular hydration state: an important determinant of protein catabolism in health and disease. *Lancet* **341**, 1330–1332.
312. Kumar A, Xie L, Ta CM, *et al.* (2020) SWELL1 regulates skeletal muscle cell size, intracellular signalling, adiposity and glucose metabolism. *eLife* **9**, 1–27.

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press

313. Gosmanov AR, Lindinger MI & Thomason DB (2003) Riding the tides: K^+ concentration and volume regulation by muscle Na^+-K^+-$2Cl^-$ cotransport activity. *News Physiol Sci* **18**, 196–200.
314. Roberts MD, Haun CT, Vann CG, Osburn SC & Young KC (2020) Sarcoplasmic hypertrophy in skeletal muscle: a scientific "Unicorn" or resistance training adaptation? *Front Physiol* Frontiers Media S.A. **11**, 816, 1–16.
315. Haun CT, Vann CG, Osburn SC, *et al.* (2019) Muscle fiber hypertrophy in response to 6 weeks of high-volume resistance training in trained young men is largely attributed to sarcoplasmic hypertrophy. *PLoS One* **14**, e0215267, 1–22.
316. Koletzko B, Von Kries R, Monasterolo RC, *et al.* (2009) Can infant feeding choices modulate later obesity risk? *Am J Clin Nutr* **89**, 1502–1509.
317. Millward DJ, Truby H, Fox KR, Livingstone MBE, Macdonald IA & Tothill P (2014) Sex differences in the composition of weight gain and loss in overweight and obese adults. *Br J Nutr* **111**, 933–943.
318. Lampl M & Johnson ML (1997) Identifying saltatory growth patterns in infancy: a comparison of results based on measurement protocol. *Am J Hum Biol* **9**, 343–355.
319. Lampl M & Schoen M (2017) How long bones grow children: mechanistic paths to variation in human height growth. *Am J Hum Biol* **29**, e22983.
320. Noonan KJ, Farnum CE, Leiferman EM, Lampl M, Markel MD & Wilsman NJ (2004) Growing pains: are they due to increased growth during recumbency as documented in a lamb model? *J Pediatr Orthop* **24**, 726–731.
321. Lehman PJ & Carl RL (2017) Growing pains: when to be concerned. *Sports Health* **9**, 132–138.
322. Evano B & Tajbakhsh S (2018) Skeletal muscle stem cells in comfort and stress. *NPJ Regen Med* **3**, 1–13.
323. Maes C & Kronenberg HM (2016) Bone development and remodeling. In *Endocrinology: Adult and Pediatric*, 7th ed. Vol. **1**, pp. 1038–1062.e8 [JL Jameson, LJ De Groot, editors]. Amsterdam: Elsevier.
324. Hallett SA, Ono W & Ono N (2019) Growth plate chondrocytes: skeletal development, growth and beyond. *Int J Mol Sci* **20**, 1–17.
325. Millward DJ (2017) Undernutrition, infection, and poor growth in infants and children. In *Nutrition, Immunity, and Infection*, pp. 83–109 [PC Calder, AD Kulkarni, editors]. Boca Raton, FL: CRC Press, Taylor & Francis Group.
326. Nimmanon T, Ziliotto S, Morris S, Flanagan L & Taylor KM (2017) Phosphorylation of zinc channel ZIP7 drives MAPK, PI3K and mTOR growth and proliferation signalling. *Metallomics* **9**, 471–481.
327. Sabatini DM (2017) Twenty-five years of mTOR: uncovering the link from nutrients to growth. *Proc Natl Acad Sci U S A* **114**, 11818–11825.
328. Hozain S, Hernandez A, Fuller J, Sharp G & Cottrell J (2021) Zinc chloride affects chondrogenesis via VEGF signaling. *Exp Cell Res* **399**, 112436.
329. Burgess D, Iversen T & Cottrell J (2018) Zinc chloride treatment in ATDC5 cells induces chondrocyte maturation. *Int J Regen Med: Sci Reposit* **1**, 1–11.
330. Mammoto A, Mammoto T & Ingber DE (2012) Mechanosensitive mechanisms in transcriptional regulation. *J Cell Sci* **125**, 3061–3073.
331. Hornberger TA (2011) Mechanotransduction and the regulation of mTORC1 signaling in skeletal muscle. *Int J Biochem Cell Biol* **43**, 1267–1276.
332. Jansen KA, Atherton P & Ballestrem C (2017) Mechanotransduction at the cell-matrix interface. *Semin Cell Dev Biol* **71**, 75–83.
333. Stutchbury B, Atherton P, Tsang R, Wang DY & Ballestrem C (2017) Distinct focal adhesion protein modules control different aspects of mechanotransduction. *J Cell Sci* **130**, 1612–1624.
334. Miller BF, Olesen JL, Hansen M, *et al.* (2005) Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *J Physiol* **567**, 1021–1033.
335. Moore DR, Phillips SM, Babraj JA, Smith K & Rennie MJ (2005) Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions. *Am J Physiol Endocrinol Metab* **288**, 1153–1159.
336. Figueiredo VC, Wen Y, Alkner B, *et al.* (2021) Genetic and epigenetic regulation of skeletal muscle ribosome biogenesis with exercise. *J Physiol* **599**, 3363–3384.
337. Jin J, Bakker AD, Wu G, Klein-Nulend J & Jaspers RT (2019) Physicochemical Niche conditions and mechanosensing by osteocytes and myocytes. *Curr Osteoporos Rep* **17**, 235–249.
338. Frost HM (2003) Bone's Mechanostat: a 2003 update. *Anat Rec Part A Dis Mol Cell Evol Biol* **275**, 1081–1101.
339. Zanou N & Gailly P (2013) Skeletal muscle hypertrophy and regeneration: Interplay between the myogenic regulatory factors (MRFs) and insulin-like growth factors (IGFs) pathways. *Cell Mol Life Sci* **70**, 4117–4130.
340. Cisternas P, Henriquez JP, Brandan E & Inestrosa NC (2014) Wnt signaling in skeletal muscle dynamics: Myogenesis, neuromuscular synapse and fibrosis. *Mol Neurobiol* **49**, 574–589.
341. Tu MK, Levin JB, Hamilton AM & Borodinsky LN (2016) Calcium signaling in skeletal muscle development, maintenance and regeneration. *Cell Calcium* **59**, 91–97.
342. Zhan M, Jin B, Chen SE, Reecy JM & Li YP (2007) TACE release of TNF-α mediates mechanotransduction-induced activation of p38 MAPK and myogenesis. *J Cell Sci* **120**, 692–701.
343. Eid Mutlak Y, Aweida D, Volodin A, *et al.* (2020) A signaling hub of insulin receptor, dystrophin glycoprotein complex and plakoglobin regulates muscle size. *Nat Commun* [Internet] **11**, 1–17. doi: 10.1038/s41467-020-14895-9
344. Mattijssen S, Kozlov G, Gaidamakov S, *et al.* (2021) The isolated La-module of LARP1 mediates 3′ poly(A) protection and mRNA stabilization, dependent on its intrinsic PAM2 binding to PABPC1. *RNA Biol* **18**, 275–289.

https://doi.org/10.1017/S0954422423000124 Published online by Cambridge University Press