It's not just about protein turnover: the role of ribosomal biogenesis and satellite cells in the regulation of skeletal muscle hypertrophy

Matthew Stewart Brook, Daniel James Wilkinson, Ken Smith & Philip James Atherton

To cite this article: Matthew Stewart Brook, Daniel James Wilkinson, Ken Smith & Philip James Atherton (2019): It's not just about protein turnover: the role of ribosomal biogenesis and satellite cells in the regulation of skeletal muscle hypertrophy, European Journal of Sport Science, DOI: 10.1080/17461391.2019.1569726

To link to this article: https://doi.org/10.1080/17461391.2019.1569726

Published online: 09 Feb 2019.
It’s not just about protein turnover: the role of ribosomal biogenesis and satellite cells in the regulation of skeletal muscle hypertrophy

MATTHEW STEWART BROOK1,2, DANIEL JAMES WILKINSON1,2, KEN SMITH1,2, & PHILIP JAMES ATHERTON1,2

1MRC-ARUK Centre for Musculoskeletal Ageing Research, University of Nottingham, Derby, UK & 2National Institute for Health Research (NIHR), Nottingham Biomedical Research Centre (BRC), Clinical, Metabolic and Molecular Physiology, University of Nottingham, Derby, UK

Abstract
Skeletal muscle has indispensable roles in regulating whole body health (e.g. glycemic control, energy consumption) and, in being central in locomotion, is crucial in maintaining quality-of-life. Therefore, understanding the regulation of muscle mass is of significant importance. Resistance exercise training (RET) combined with supportive nutrition is an effective strategy to achieve muscle hypertrophy by driving chronic elevations in muscle protein synthesis (MPS). The regulation of muscle protein synthesis is a coordinated process, in requiring ribosomes to translate mRNA and sufficient myonuclei density to provide the platform for ribosome and mRNA transcription; as such MPS is determined by both translational efficiency (ribosome activity) and translational capacity (ribosome number). Moreover, as the muscle protein pool expands during hypertrophy, translation capacity (i.e. ribosomes and myonuclei content) could theoretically become rate-limiting such that an inability to expand these pools through ribosomal biogenesis and satellite cell (SC) mediated myonuclear addition could limit growth potential. Simple measures of RNA (ribosome content) and DNA (SC/Myonuclei number) concentrations reveal that these pools do increase with hypertrophy; yet whether these adaptations are a pre-requisite or a limiting factor for hypertrophy is unresolved and highly debated. This is primarily due to methodological limitations and many assumptions being made on static measures or correlative associations. However recent advances within the field using stable isotope tracers shows promise in resolving these questions in muscle adaptation.

Keywords: Muscle, hypertrophy, ribosomal biogenesis, satellite cell, myonuclei

Introduction
Skeletal muscle is essential for movement, enabling the completion of everyday tasks that underpin independence. Furthermore, muscle hypertrophy is desirable among athletes and in the general population due to the rising interest in muscle health and wellbeing. Skeletal muscle is important in whole body metabolism, having significant roles in the storage and metabolism of proteins, lipids and glucose (Brook et al., 2016b; Wolfe, 2006). These roles are critical in maintaining whole-body metabolic and functional health; this is typified by the notion that progressive

Correspondence: Matthew Stewart Brook, Division of Metabolic and Molecular Physiology, Postgraduate Entry Medical School, Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3DT UK. E-mail: mszmsb@nottingham.ac.uk

© 2019 European College of Sport Science
declines in muscle mass in disease and ageing propagate frailty, metabolic comorbidities and mortality (Brook, Wilkinson, & Atherton, 2017a). As such there has been considerable research efforts from our lab (Atherton & Smith, 2012) and others’ (Phillips, Tipton, Ferrando, & Wolfe, 1999) aimed at unraveling the regulation of muscle mass. However, many aspects of hypertrophy remain unclear and debated, with a confusing array of optimal nutritive/exercise practices available (Aragon & Schoenfeld, 2013; Hulmi, Lockwood, & Stout, 2010). Understanding the mechanisms and most effective strategies to induce skeletal muscle hypertrophy would offer great resource in combating muscle wasting and add clarity to optimal exercise practices.

Placing skeletal muscle under regular mechanical overload known as resistance exercise training (RET), is a well-known stimulator of muscle hypertrophy and its regulation is impacted based upon intensity (Kumar et al., 2009; Mitchell et al., 2012), volume (Burd et al., 2010; Kumar et al., 2012), frequency (Schoenfeld, Ogborn, & Krieger, 2016) and contraction mode (i.e. eccentric vs concentric [Franchi et al., 2014]). Intriguingly, muscle adaptation has shown to be most active during the early phases of a RET, with increases of 5–10% in muscle mass/size achieved by the first 3–6 weeks, at which point continued increases are considerably slower (Brook et al., 2015, 2016c; DeFreitas, Beck, Stock, Dillon, & Kasishke, 2011). Moreover, hypertrophic responses are markedly heterogeneous, with some individuals showing negligible changes in muscle volume in response to regular progressive RET, and others may increase up to 10% or more (Phillips et al., 2013; Stec et al., 2016). With skeletal muscle being composed of many long, multinucleated, post mitotic fibres, overall changes in muscle size occur through an increase in muscle fibre area, rather than fibre number (hyperplasia). Irrespective of loading combinations and RET paradigms employed, the fundamental mechanism driving this fibre hypertrophy is an increase in protein content, which principally must occur by muscle protein synthesis (MPS) exceeding muscle protein breakdown (MPB).

Protein synthesis essentially relies on the eukaryotic central dogma, which in its simplest form sees the flow of genetic information from DNA > RNA > Protein. However, as a muscle fibre continues to grow and the protein pool expands, the foundations that support protein synthesis (i.e. DNA > RNA) will become diluted and presumably the so called “capacity” for protein synthesis could become strained. An increase in cellular RNA is regulated by ribosomal biogenesis (translational capacity), whereas an increase in DNA represents satellite cell (SC) division and myonuclear addition, that in turn provides the platform for ribosome production and mRNA availability. Yet despite considerable progress in understanding MPS responses, those of RNA and DNA have somewhat lagged, especially in humans. As such, fundamental aspects of muscle physiology are unresolved, with one of the most rudimentary questions being can muscle hypertrophy occur in the absence of ribosomal biogenesis and myonuclear addition, or are they a pre-requisite for adaptation? In this review, we will discuss evidence of the roles of ribosomal biogenesis and SC mediated myonuclear addition in hypertrophy and highlight new methods which can further knowledge in this area.

### The regulation of skeletal muscle protein turnover

Protein synthesis is the process by which ribosomes create polypeptide chains through linking amino acids together in a specific order according to mRNA. As such, rates of protein synthesis can be modulated by the rate of mRNA translation, known as “translational efficiency”. A primary control point regulating translational efficiency and therefore protein synthesis in the majority of eukaryotic cells is by cap dependent translation. This involves the assembly of many eukaryotic initiation factors (eIF’s) to form a preinitiation complex (PIC) that interacts with the 5’ end of an mRNA to instigate protein synthesis (for more detail readers are directed to [Jackson, Hellen, & Pestova, 2010]). However, with protein synthesis being an energy demanding processes (e.g. through peptide bonding) it is unsurprising that there is myriad of regulating signaling cascades, many of which culminate on the mammalian target of rapamycin (mTOR), that integrates signals such as exercise, AA availability and energy status to coordinate cellular metabolism (Goodman et al., 2011). Some of the best understood targets of mTOR are those directly involved in cap-dependent translation, including P70S6K1, 4E-BP1, and RPS6 that can enhance translation initiation and efficiency in the absence of ribosomal biogenesis (Chesley, MacDougall, Tarnopolsky, Atkinson, & Smith, 1992).

Two of the most well documented stimulators of MPS are the protein components of a meal (particularly EAA) and RE. In the absence of nutrition, MPB exceeds MPS, such that AA’s from muscle are released to support protein synthesis and energy needs in other organs (Pozefsky, Tancredi, Moxley, Dupre, & Tobin, 1976). Upon consumption of a meal, the protein components drive a robust (>100%) yet transient (60–90 mins) increase in MPS to replenish muscle protein lost during
nutritional absence (Atherton et al., 2010). Once this is achieved the muscle becomes “full” and any additional AA’s are excreted such that excess protein consumption in healthy individuals would not result in muscle growth (Witard et al., 2014). A bout of RE in the absence of AA’s results in a similar potentiation of MPS (Kumar et al., 2009), yet due to greater increases in MPB the overall net effect is negative (Phillips et al., 1999). However, upon consumption of dietary protein in proximity to RE, MPS is enhanced further than RE or feeding alone, achieving overall positive balance and extending the muscle full point that when repeated over extended periods results in muscle hypertrophy (Atherton & Smith, 2012).

Beyond nutritive/mechano-sensitive pathways, muscle mass and RE induced hypertrophy are also regulated by hormones. Testosterone (T), growth hormone (GH) and Insulin-like growth factor 1 (IGF-1), are considered pro-anabolic, however only T has been definitively demonstrated to clearly promote MPS and subsequent hypertrophy (Griggs et al., 1989). With regard to GH and IGF-1 in humans (in young adult muscle), evidence is lacking for a direct role for these hormones in driving MPS, yet GH does increase collagen synthesis supporting a role in extracellular matrix remodeling (Doessing et al., 2010). However, that is not to say GH and IGF-1 do not hold important roles in skeletal muscle maintenance and hypertrophy, with in-vivo rodent studies demonstrating hypertrophy with IGF1 infusion (Adams & McCue, 1998) or overexpression (Musarò et al., 2001) and atrophy with IGF-1R knockout (Spangenburg, Le Roith, Ward, & Bodine, 2008). Whilst the importance of IGF-1/IGF1-R has been brought into question during overload induced hypertrophy (Spangenburg et al., 2008), IGF-1/IGF1-R may hold critical roles in muscle repair and maturation (Heron-Milhavet, Mamaeva, Leroith, Lamb, & Fernandez, 2010). In humans RE temporarily increases systemic hormones, yet associations between elevated individual hormones and resulting muscle mass have been questioned (West & Phillips, 2012). However, the entire endocrine response (i.e. T, GH, IGF1, Insulin, cortisol) has been related to muscle hypertrophy (Mangine et al., 2017) suggesting coordinated elevations in multiple hormones may have a complementary effect in stimulating enhance muscle growth.

Repeated bouts of RE supported by sufficient EAA consumption that enhance MPS is undoubtedly the major driver of protein accretion and hypertrophy. However, as myofibres increase in size and as the demand for increased protein content continues, the foundations that permit MPS could be constrained. Taking simple proxy measures of ribosome number and myonuclei content (i.e. RNA and DNA concentration) indicate these pools expand over time in response to RET which must be due to greater changes in the synthesis of these pools during this period of expansion. That being said whether changes in ribosomal and myonuclei content dictate the extent of muscle hypertrophy capacity is unclear and has remained a controversial and highly debated topic.

**The role of ribosomal biogenesis in the regulation of muscle mass and hypertrophy**

As ribosomes serve as the protein synthetic machinery of the cell, the rate of protein synthesis is not only determined by translational efficiency (discussed above) but also the total number of ribosomes (translational capacity) (Millward, Garlick, James, Nnanyelugo, & Ryatt, 1973). Ribosomes are themselves composed of RNA (ribosomal RNA (rRNA)), transcribed by RNA polymerase 1 (POL1) as a 47S pre-rRNA which is then cleaved into 28S, 18S, 5.8S and subsequently assembled with ribonuclear proteins to from a mature ribosome (Figure 1) (Chaillou, Kirby, & Mccarthy, 2014). POL1 therefore serves as a primary control point in ribosomal biogenesis, with the transcription factors TIF-1A, TIF-1B and UBF key in forming a PIC with POL1 at the rDNA promoter. The regulation of TIF-1A and UBF transcriptional activity is achieved through multiple signaling proteins, including ERK, AMPK, mTORc1 and P70S6K1 (reviewed in Kusnadi et al., 2015) enabling the control of ribosomal biogenesis to be influenced by multiple pathways such as hormones, nutrients and contractile activity. Another key regulator of ribosomal biogenesis is c-MYC, an oncprotein involved in regulating cell growth and virtually all aspects of ribosome formation. c-MYC directly upregulates many of the proteins involved in rDNA transcriptional control including UBF, TIF-1A, TIF-1B, Pol1 and many other proteins involved in the formation, processing and export of mature ribosomes. Further, c-MYC enhances POL1 transcription by remodeling rDNA chromatin structure and directly interacting with the SL1 complex, stabilising Pol1 recruitment at the promoter (van Riggelen, Yetil, & Felsher, 2010). Day-to-day fluctuations in MPS that maintain muscle mass in healthy individuals are predominantly achieved by transient increases in translational efficiency, without changes in RNA content (Chesley et al., 1992). RNA content and therefore ribosome number is likely to be adequately maintained to sustain the protein synthetic needs of habitual activity and nutritional intake, whilst being
continually replenished to maintain functional ribosomes. In support of this, ribosomal biogenesis pathways are entwined with those regulating translational efficiency (i.e. translational efficiency) and the number of available ribosomes (i.e. translational capacity). Ribosomal biogenesis is the process of ribosome production, beginning with the transcription of rDNA in the nucleus to form a 47s pre-RNA, that is subsequently cleaved and assembled into a mature ribosome. During habitual activity and dietary behaviours, the primary driver of MPS is that of AA, increasing translational efficiency whilst maintaining a sufficient number of ribosomes through the stimulation of ribosomal biogenesis. Resistance exercise (RE), along with sufficient AA intake enhances translational efficiency greater than RE or AA alone such that positive muscle protein balance is maintained. In addition, RE stimulates ribosomal biogenesis and satellite cell mediated myonuclear addition increasing translational capacity, that is likely to be key in supporting and or permitting hypertrophic adaptations. SC, satellite cell. AA, amino acid. ↑ increase. ↔ no change. T, testosterone. G, growth hormone. I, insulin-like growth factor 1.

Figure 1. Overview in the signaling of muscle translational efficiency and translational capacity. Muscle protein synthesis (MPS) is determined by both the rate of mRNA translation by ribosomes (i.e. translational efficiency) and the number of available ribosomes (i.e. translational capacity). Ribosomal biogenesis is the process of ribosome production, beginning with the transcription of rDNA in the nucleus to form a 47s pre-RNA, that is subsequently cleaved and assembled into a mature ribosome. During habitual activity and dietary behaviours, the primary driver of MPS is that of AA, increasing translational efficiency whilst maintaining a sufficient number of ribosomes through the stimulation of ribosomal biogenesis. Resistance exercise (RE), along with sufficient AA intake enhances translational efficiency greater than RE or AA alone such that positive muscle protein balance is maintained. In addition, RE stimulates ribosomal biogenesis and satellite cell mediated myonuclear addition increasing translational capacity, that is likely to be key in supporting and or permitting hypertrophic adaptations. SC, satellite cell. AA, amino acid. ↑ increase. ↔ no change. T, testosterone. G, growth hormone. I, insulin-like growth factor 1.

Continually replenished to maintain functional ribosomes. In support of this, ribosomal biogenesis pathways are entwined with those regulating translational efficiency (i.e. mTORc1) (Mayer & Grummt, 2006) (Figure 1) and in response to chronic muscle loading or nutritional modulation, bio-markers of increased ribosomal biogenesis are observed (i.e. Increased c-MYC / 47s pre-RNA) (Stec, Mayhew, Ostlund Farrants, & Nader, 2012).

With rRNA comprising 85% of the total RNA pool, increases or decreases in the size of this pool are indicative proxies of changes in the rates of ribosomal biogenesis. Early models of overload induced hypertrophy in animals demonstrated rapid and substantial increases in RNA, with attempts to prevent RNA synthesis in vivo inhibiting muscle growth (Goldberg & Goodman, 1969). However, the specificity of these compounds to inhibit RNA synthesis and not DNA synthesis is a confounding factor. More recent preclinical models to approach this subject have utilised synergist ablation (SA) techniques combined with regular sampling to create a time-course of molecular events. At the onset of SA, there is an early increase in RNA concentration, 45S-pre-rRNA and a coordinated expression in regulators of POL1 transcription, indicative of ribosomal biogenesis and increased translational capacity at the onset of hypertrophy (von Walden, Casagrande, Ostlund Farrants, & Nader, 2012). Early increases in RNA content with SA have been demonstrated previously (Miyazaki, Mccarthy, Fedele, & Esser, 2011) however, whether this precedes or limits
muscle hypertrophy is unclear as muscle mass increases similarly at this time. Moreover, early increases in muscle weight may be influenced by water (oedema), such that increased ribosome content may occur before increases in muscle mass (Marino et al., 2008). Supporting this notion, the use of electrical stimulation to replicate RE in vivo showed that 12 h after RE there was a significant increase pre-rRNA and a trend for greater total RNA—again supporting early active ribosomal biogenesis prior to hypertrophy (West et al., 2016). Furthermore, using EU labelled RNA, Kirby showed that early increases in ribosomal biogenesis were due to an increase in translational output per myonuclei, not an increase in active myonuclei, suggesting that myonuclei already possess a capacity to maintain greater levels of transcription in the absence myonuclei addition and meet the transcriptional demands for hypertrophy (Kirby et al., 2016).

In agreement with the rapid onset of ribosomal biogenesis in animal models, contractile stimulation in humans also causes a rapid expression in markers of rDNA transcription, including increased expression of 45S-pre-rRNA 24 h hours after RE (Stec et al., 2016). Further RNA content increased after two bouts of RE (Bickel et al., 2005), a response which is sustained at 3 and 6-weeks of continued RET (Brook et al., 2015). Some of the strongest evidence supporting a requirement of ribosome biogenesis in muscle hypertrophy has been demonstrated using k-means cluster analysis. After 4-weeks of RET, 42 older adults (60–75y) were categorised into three responder groups based on the extent of hypertrophy of type II fibres, showing either no change (-7%), a modest increase (22%) or extreme increase (83%). At the onset of training all groups had the same RNA content, whilst after training there was no increase in RNA concentration in the “no change group”, a trend for an increase in the “modest group” (+9%) and a significant increase in the “extreme group” (+26) (Stec et al., 2016); a finding recently reproduced in young individuals when clustered by change in vastus lateralis thickness (Mobley et al., 2018). Similarly we have shown that age-related deficits in long term MPS and resulting hypertrophy were allied with inferior ribosomal biogenesis and a lack of c-MYC induction (Brook et al., 2016a). Together, these studies have demonstrated that increases in translational capacity are often present in those with the greatest hypertrophy, yet this does not necessarily mean it is a prerequisite or limiting factor (Figure 2). However, taking a large group (n=66) of mixed gender and age individuals, only extreme hypertrophic responders increased total RNA content after the first exercise bout, seemingly positioning those that can rapidly expand ribosomal capacity at a hypertrophic advantage (Table I). That being said, after 16-weeks, both

![Figure 2. Mechanisms of increased translational capacity and fibre CSA. Skeletal muscle hypertrophy occurs through an increase in protein synthesis, protein content and therefore overall fibre CSA. Presumably this would lead to a dilution in the foundations of protein synthesis (i.e. translational capacity) in both Ribosomal content (RNA) and myonuclei number (DNA). However, whether fibre hypertrophy can occur without an increase in translational capacity via ribosomal biogenesis or satellite cell mediated myonuclear addition remains a contentious issue. Further, as shown above, an expansion in translation capacity can result from varied adaptations. 1 – Lack of fibre hypertrophy due to an absence of increased in translational capacity, 2 – Fibre hypertrophy in the absence of an increase in translational capacity, 3 – Fibre hypertrophy driven by an increase in translational capacity from existing myonuclei, 4 – Fibre hypertrophy driven by an increase in translational capacity from satellite cell mediated myonuclear addition, 5 – Fibre hypertrophy driven by an increase in translational capacity from both existing new myonuclei. CSA, cross sectional area. SC, satellite cell. ↑ increase. ↔ no change.](image-url)

It’s not just about protein turnover 5
moderate and non-responders showed equal increases in RNA content despite diminished hypertrophy, highlighting muscle growth is a coordinate process and cannot rely on increases in translational capacity alone (Kim, Petrella, Cross, & Bamman, 2007).

Role of satellite cells (SC) in the regulation of skeletal muscle mass and hypertrophy

Skeletal muscles cells are multinucleated but terminally differentiated cells (Heron & Richmond, 1993). However, skeletal muscle also possess a residual pool of resident muscle stem cells – SC (Mauro, 1961). Unlike their sub-sarcolemma Myonuclei counterparts, SC are located in the extracellular matrix between the sarcolemma and the basal lamina and in being a type of stem cell (unlike myonuclei) (Bintliff & Walker, 1960), SC possess mitotic potential. The physiological role of SC is thought to be the provision of “new” nuclei to existing myofibers, supporting transcriptional capacity, while at the same time ensuring through division, sustenance of an extracellular SC population. Upon activation, SC exist quiescence and enter the cell cycle where they can proliferate as myoblasts and potentially terminally differentiate. A population of these cells will undergo asymmetric cell division where by one daughter cell remains quiescent maintaining SC number and a continued source of myonuclei (Moss & Leblond, 1971; Troy et al., 2012). The contended role of SC in the regulation of hypertrophy was derived from the concept that each nuclei of a multi-nucleated myofibre appears to synthesise protein for a close vicinity domain (Figure 2) (Gundersen, Sanes, & Merlie, 1993; Hall & Ralston, 1989) and that this karyoplasmatic ratio is held constant (Allen, Roy, & Edgerton, 1999). Obviously, this means that as a muscle hypertrophies, the fixed nuclei content of terminally differentiated muscle cells essentially becomes “diluted” to a point that a new source of nuclei is needed to support the transcriptional requirements of supporting a larger myofiber volume. Therefore, the potential role for SC in regulating and/or limiting muscle hypertrophy has many foundations that warrant detailed consideration.

Numerous pre-clinical studies have been carried out with the intention to resolve the “mechanistic” role of SC as determinants of muscle hypertrophy, using genetic, chemical or irradiation techniques (Rosenblatt, Yong, & Parry, 1994). Non-genetic based animal studies aimed at delineating the role of SC in post-natal muscle growth tended to focus on DNA synthesis inhibition by gamma-irradiation or by way of chemical DNA synthesis inhibitors. Such techniques led to early reports that SC were indispensable for muscle hypertrophy (Rosenblatt et al., 1994). However, such approaches were blighted by lack of specificity in targeting SC activity; instead targeting all cells displaying mitotic activity (e.g. fibroblasts, endothelial cells). Since skeletal muscle hypertrophy is the summation of complex and coordinated tissue remodeling involving multiple cell types, it could be argued that impaired muscle hypertrophy in these studies was not a result of ablating SC activity per se, but rather of suppressing mitotic activity in multiple cell types involved in hypertrophy. This thesis is supported by genetic approaches, creation of mouse strains in which conditional ablation of SC (i.e. PAX7 expressing cells) could achieve SC depletion (~90%) in adult mice. To determine the role of SC in hypertrophy, SC depleted animals and wild-type counterparts were exposed to SA. Intriguingly, it was shown that >90% depletion of SC did not limit muscle hypertrophy despite an absence of myonuclei accretion (McCarthy et al., 2011). Instead, there was an expansion of myonuclear domains (lack of BrdU (DNA) positive myonuclei) in SC-depleted muscle, a finding arguing against there being a critical upper limit for myonuclear domain. In a second study, using the same model, it was reported that SC-depletion also did not impact on skeletal muscle re-

### Table I. Analysis of human subjects after undergoing 16 weeks of progressive resistance exercise training clustered by vastus lateralis fibre cross sectional area (Bamman, Petrella, Kim, Mayhew, & Cross, 2007) with their respective increases in muscle satellite cell/myonuclear (Petrella, Kim, Mayhew, Cross, & Bamman, 2008) and RNA content (Kim et al., 2007).

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Baseline fibre area</th>
<th>Δ Fibre area</th>
<th>Baseline SC</th>
<th>Δ SC</th>
<th>Baseline myonuclei</th>
<th>Δ Myonuclei</th>
<th>Myonuclear domain</th>
<th>RNA 24 h</th>
<th>RNA 16 wk.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>↑</td>
</tr>
<tr>
<td>Mod</td>
<td>–</td>
<td>↑</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>↑</td>
<td>–</td>
<td>–</td>
<td>↑</td>
</tr>
<tr>
<td>Xtr</td>
<td>–</td>
<td>↑↑</td>
<td>↑↑</td>
<td>–</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Ref (Bamman et al., 2007)</td>
<td>(Petrella et al., 2008)</td>
<td>(Kim et al., 2007)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
growth following hind limb suspension-induced atrophy (Jackson et al., 2012). While this also points to a non-obligatory requirement for SC in muscle growth, this was in direct contradiction to the early Rosenblatt studies and, moreover, recent inducible PAX7 depletion studies contesting a non-obligatory role of SC e.g. due to methodological concerns (mis-quantification of myofibre hypertrophy and inclusion of regenerating fibres in the data analyses [Egner, Bruusgaard, & Gundersen, 2016]). As such, there remains uncertainty around the role of SC in muscle hypertrophy, despite significant advances in animal models. However, PAX7 depletion studies have demonstrated SC to be indispensable in muscle regeneration (Lepper, Partridge, & Fan, 2011), with SC also showing key roles in regulating the muscle fibre micro-environment during hypertrophy and ageing (Fry et al., 2014, 2015), highlighting the important roles SC play in processes other than hypertrophy.

Consistent with the notion of constant myonuclear domains and a role for SC in muscle hypertrophy, recruitment of new nuclei from SC fusing with the pre-existing muscle fibre syncitia has been noted as a feature of muscle hypertrophy in humans (Allen et al., 1999). Moreover, altered regulation in numerous myogenic regulatory factors (MRF’s), as proxies for SC activation, proliferation and differentiation have been shown to be up-regulated in the hours following a bout of RE (McKay et al., 2008), with expansion of the SC pool and Myonuclei addition accompanying muscle fibre hypertrophy in the early stages of RET (Snijders et al., 2016). However, an important and contentious question remains to be fully resolved: during “normal” skeletal muscle hypertrophy- is myonuclear addition a physiological pre-requisite for muscle hypertrophy or adaptation? While there are few studies in humans to address this, Petrella et al. (2008) applied a cluster analysis to examine relationships between inter-individual variance in hypertrophic responsiveness to resistance exercise and that of SC activity. In this study, it was demonstrated that so-called “high-responders” for RET-induced hypertrophy displayed an increased SC number at baseline, and also increased myonuclei number in response to resistance exercise (vs. less responsive individuals) (Table 1). Since they observed that high-responders expanded their myonuclear domains (i.e. cytoplasm-to-nucleus-ratio), the authors suggested this was the driving force behind demand for myonuclear addition from SC sources to support myofibre hypertrophy in successful growth-adaptors. Which factors communicate this need for increased “transcriptional capacity” is unknown. Nonetheless, it could also be that a poor “intrinsic” capacity for hypertrophy, is the driving force behind the lack of increase in myofibre nuclear number such that one’s ability to sustain positive increases in net protein balance rather than to stimulate SC activity, is a rate limiting physiological step for human skeletal muscle hypertrophy.

**Perspectives on the roles of ribosomal biogenesis and SC in muscle hypertrophy**

Over two decades on from initial studies, this leaves one to ponder, how might these results be reconciled? Firstly, it should be pointed out that the SA model is a blunt tool of accelerated surgically-induced muscle growth in rodents, which cannot accurately represent RE-induced hypertrophy for which there exist no robust pre-clinical models. As such, whether or not ribosomal biogenesis and myonuclear addition are “required” or “obligatory” in experimental models of rapid muscle hypertrophy, is not really as critical an issue as whether they are involved in human physiological muscle hypertrophy induced by resistance exercise. At present only tentative conclusions can thus be made: (i) increased ribosomal content and myonuclear incorporation into muscle may be associated with human muscle hypertrophy, (ii) increases in muscle CSA area, ribosome content and myonuclear number may be subject to inter-individual variation in the contribution of MPS and SC in humans, and (iii) while ribosomal biogenesis and SC incorporation are a feature of hypertrophy, according to genetic models, SC activity may or may not be dispensable for muscle hypertrophy, whilst equivalent studies investigating ribosomal biogenesis are yet to be performed. Collectively, these data suggest caution in interpreting data derived from studies on this topic and highlight that the involvement of ribosomal biogenesis and SC incorporation, particularly in human physiological hypertrophy, remains somewhat of an open question; one that requires new technologies for use in vivo, in humans.

**Advances in isotopic techniques to quantify nucleic acid polymerisation: the future?**

Despite assumptions and limitations relating to so-called “obligatory requirements” for ribosomal biogenesis and myonuclear addition in muscle hypertrophy, their physiological roles remain a highly contentious issue, primarily due to methodological and experimental limitations. Our current understanding relies heavily on inference from static measures such as histological staining and correlative changes of cell size with myonuclei number or DNA/RNA content (Kadi et al., 2004; Petrella et al., 2006). Moreover, the use of unrepresentative animal models
of hypertrophy (i.e. SA) alongside irradiated or genetic models of SC ablation to determine contribution of SCs to hypertrophy has further clouded our understanding (McCarthy et al., 2011; Rosenblatt et al., 1994). Therefore, there is a need to develop novel dynamic measures of ribosomal biogenesis and myonuclear addition in vivo.

Historical measures of DNA and RNA synthesis used radiolabeled pyrimidine analogues such as 3H-thymidine or BrDU (Waldman et al., 1991). However due to their highly toxic nature and the ability to alter cell proliferation in vivo, there use has been limited (Asher et al., 1995). Stable isotope tracers offer an attractive alternative, being safe for human use and (in most cases) have the same chemical behaviour as their naturally abundant cousin with their use in in vivo metabolism is far reaching (Wilkinson et al., 2017). There are many sites for potential stable isotope incorporation during nucleotide synthesis, however ~80–90% of deoxyribose arises from extracellular glucose providing a route for continuous reliable uptake of an isotopically enriched compound (Neese et al., 2002). For instance by using labelled glucose the turnover of a range of blood cells has been made (Macallan et al., 2009); however due to the need to maintain a constant ingestion or intravenous infusion of stable isotopically labeled glucose, these techniques are only useful for fast turning over cell populations i.e. 24–48 h (Macallan et al., 2009). However, for cells with much slower proliferation rates, such as that of the SC of post-mitotic skeletal muscle cells, an alternative technique is required, and recent novel developments in the application of the “universal” stable isotope tracer deuterium oxide (D2O) has led to great step forward in this field (Brook et al., 2017b).

D2O has some unique properties that make it ideally suited for the measurement of skeletal muscle nucleic acid pools. Administered orally, either as a single bolus or continuously (daily or weekly), D2O rapidly equilibrates with body water creating a homogenous, slowly turning over, precursor pool available for use by multiple substrates. The deuterium from the body water can then be incorporated onto different substrates at stable C–H positions through biological reductions during de novo synthesis, and the metabolic flux of these substrate pools can be calculated from the measurement of the amount of the label that is incorporated (Hellerstein, 2004). Furthermore, the homogeneous distribution among the body water pool provides an easily accessible and measurable surrogate precursor pool (Dufner & Previs, 2003), which due to the relatively slow turnover of the body water pool, allows for the measurement of metabolic turnover over periods of hours–days–weeks–months (Brook et al., 2015; Wilkinson et al., 2014). These unique properties of D2O are ideal therefore for application to studies of human skeletal muscle where relatively limited cellular proliferation occurs.

Work in animals in vivo has already highlighted the utility of these D2O methods for understanding the metabolic control of skeletal muscle, with DNA and RNA synthesis shown to be active in mature skeletal muscle of rats (Brook et al., 2017c; Drake et al., 2015). Building on this, using D2O Robinson and colleagues have been able to detect rates of DNA turnover in adult human skeletal muscle, again showing slow yet detectable rates of DNA turnover of ~0.03%/d, and a suggestion for increased rates with exercise (Robinson, Turner, Hellerstein, Hamilton, & Miller, 2011). However, few studies have investigated human muscle beyond this, in part due to lack of appropriate validation and technical development of these D2O techniques. However, we have made recent advances within the field to rectify this. Developing and validating highly sensitive GC-MS/MS techniques we were able to detect very low levels of deuterium incorporation into newly synthesised ribonucleotides, enabling us to make the first measures of RNA synthesis in humans in muscle. Further we showed that dynamic increases in RNA synthesis (i.e. ribosomal biogenesis) were active at the onset of RE, presumably playing an integral role in the prevailing hypertrophy (Brook et al., 2016a, 2017c). Further, rates of ribosomal biogenesis were strongly associated with rates of MPS, highlighting that changes in translational efficiency and translational capacity are a coordinated process during hypertrophy (Brook et al., 2017c). The enhanced sensitivity of these techniques are not limited to just RNA synthesis, if new DNA has been synthesised then deuterium will be incorporated and isolation of SC/myonuclear fractions will have the potential to show without doubt the occurrence of cellular proliferation and therefore myonuclear addition. These newly developed techniques are widely applicable and if combined with appropriately designed studies have the potential to definitively determine the likely physiological role of ribosomal biogenesis and myonuclear addition in human muscle growth.

Conclusion

Understanding how ribosomal biogenesis and SC mediated myonuclear addition influence MPS and ultimately muscle hypertrophy would make significant advances in the field of muscle physiology. However, despite many years of research and technological advances, many of these questions remain unresolved. The use of overload induced hypertrophy
in animal models has provided valuable information in clearly demonstrated that an expansion of translational capacity and an increase in myonuclei are aspects of, and correlated with muscle growth (Nakada, Ogawara, Kawada, Maekawa, & Ishii, 2016). However further progress to determine if these adaptations are a pre-requisite or dictate hypertrophy are lacking and often rely on a time course of associated measures. The development of genetic animal models to explore SC have and will continue to show great promise, yet currently contrasting results are obtained (Egner, Bruusgaard, & Gundersen, 2017), whilst equivalent models to investigate ribosomal adaptations are lacking. Still, a confounding problem with SA is the lack of physiological relevance to RE induced hypertrophy. In human studies, conclusions are often based on associations between cellular changes and the extent of hypertrophy. The rapid technical advances in recent years with regards to D2O stable isotope tracer methods based on the work our lab and others (Brook et al., 2017c; Mathis et al., 2017; Neese et al., 2002), finally provides researchers with the tools necessary to hone in on the dynamics of RNA and DNA turnover in relation to maintenance and growth of human muscle mass and in ageing and catabolic diseases.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
The authors would like to acknowledge the Dunhill Medical Trust (R364/1112), The Physiological Society, a Medical Research Council Confidence in Concept award and the MRC-Arthritis Research UK Centre for Musculoskeletal Ageing Research (MR/R502364/1 and MR/P021220/1) for supporting our work in this area. All authors contributed equally to this manuscript.

References


Egner, I. M., Bruusgaard, J. C., & Gundersen, K. (2017). An apparent lack of effect of satellite cell depletion on hypertrophy could be due to methodological limitations. Response to 'Methodological issues limit interpretation of negative effects of satellite cell depletion on adult muscle hypertrophy'. Development, 144, 1365–1367.


Wilkinson, D. J., Brook, M. S., Smith, K., & Atherton, P. J. (2017). Stable isotope tracers and exercise physiology: Past,
