REVIEW / SYNTHÈSE

Satellite cell proliferation and skeletal muscle hypertrophy

Gregory R. Adams

Abstract: Satellite cells are small, mononuclear cells found in close association with striated skeletal muscles cells (myofibers). These cells appear to function as reserve myoblasts. A critical role for these cells in the process of muscle regeneration following injury has been clearly established. In that role, satellite cells have been shown to proliferate extensively. Some of the progeny of these cells then fuse with each other to form replacement myofibers, whereas others return to quiescence, thereby maintaining this reserve population. In response to injury, activated satellite cells can also fuse with damaged but viable myofibers to promote repair and regeneration. It has also been observed that satellite cells are activated during periods of significantly increased muscle loading and that some of these cells fuse with apparently undamaged myofibers as part of the hypertrophy process. The observation that the inactivation of satellite cell proliferation prevents most of the hypertrophy response to chronic increases in loading has lead to the hypothesis that a limitation to the expansion of myofiber size is imposed by the number of myonuclei present. Recent evidence suggests that a potential limitation to muscle hypertrophy, in the absence of a reserve supply of myonuclei, may be the inability to sustain increases in ribosomes, thereby limiting translational capacity.

Key words: myonuclei, stem cells, translational capacity.

Résumé : Les cellules satellites sont de petites cellules mononucléées observées en association intime avec des cellules musculaires striées (myofibres). Les cellules satellites semblent jouer le rôle de myoblastes de réserve. D'après des études solides, elles jouent un rôle critique dans le processus de régénération musculaire à la suite d'une blessure. Au cours de ce processus, on a établi que leur nombre augmentait beaucoup. Parmi les nouvelles cellules, quelques-unes se réunissent pour former des myofibres de remplacement pendant que les autres se désactivent et retournent dans la population de réserve. En réaction à une blessure, les cellules satellites activées peuvent s'unir à des myofibres lésées mais encore viables pour en faciliter la réparation et la régénération. Selon des études, les cellules satellites sont activées au cours des périodes d'entraînement musculaire intense et quelques-unes d'entre elles s'unissent à des myofibres apparemment saines dans le processus de l'hypertrophie. Comme l'inactivation de la prolifération des cellules satellites empêche pratiquement toute la manifestation de l'hypertrophie en réponse à la surcharge musculaire, on a évoqué l'hypothèse suivante : l'accroissement des dimensions de la fibre musculaire est limité par le nombre de noyaux de fibres musculaires en place. D'après des études récentes sur la limitation potentielle de l'hypertrophie musculaire, les ribosomes n'augmentent pas leur activité en l'absence d'une réserve de noyaux de fibres musculaires disponibles, ce qui limite leur capacité de traduction.

Mots clés : noyaux de fibres musculaires, cellules souches, capacité de traduction.

[Traduit par la Rédaction]

Introduction

The constitutive cell type of skeletal muscle tissue is the myofiber. Mature mammalian myofibers are multinucleated cells formed via the fusion of individual myoblast cells during development (Cossu and Biressi 2005). To date, evidence suggests that, in vivo, these multinucleated myofibers are permanently differentiated and therefore incapable of mitotic activity (e.g., cell division) (Chambers and Mcdermott 1996; Hughes and Schiaffino 1999; Stockdale and Holtzer 1961). In addition to a complement of non-myogenic cells (fibroblasts, immune cells, etc.), myofibers are accompanied by satellite cells. Satellite cells were originally named based on their anatomical relationship to the myofiber, i.e., in close association with the myofibers. Satellite cells are undifferentiated myogenic cells present within the basal lamina of myofibers. These cells appear to be a population distinct from the myoblasts that fuse during myofiber development (Cossu and Biressi 2005). A convincing case can be made that these cells represent a muscle stem cell population (Collins and Partridge 2005). The nature of

Received 28 March 2006. Accepted 9 May 2006. Published on the NRC Research Press Web site at http://apnm.nrc.ca on 8 December 2006.

G.R. Adams. Department of Physiology and Biophysics, Medical Science I D335, University of California, Irvine, CA 92697, USA (e-mail: gradams@uci.edu).

 doi:10.1139/H06-053

satellite cells and, in particular, the degree of heterogeneity within this population of cells, is an area of intensive study (Wagers and Conboy 2005). For the purposes of this review, the umbrella term "satellite cell" will be used to denote the population of cells present within the muscle contributing to myofiber regeneration.

Satellite cells and muscle regeneration

Numerous lines of evidence indicate that, in response to muscle injury, myofiber regeneration results from the activities of satellite cells. In response to injury, satellite cells can fuse with damaged but viable myofibers to promote repair (Robertson et al. 1990, 1992*b*, 1993). Satellite cells can also initiate the de novo formation of myofibers within the basal lamina of cells that have actually been destroyed by the injury (Robertson et al. 1990; Sabourin and Rudnicki 2000; Schultz 1989; Schultz and McCormick 1994; Zammit et al. 2002).

The critical, obligatory, nature of satellite cell contributions to muscle regeneration was clearly established with selective irradiation (e.g., Robertson et al. 1992*a*). The results from a number of studies using muscle injury models indicate that relatively modest doses of radiation, below the threshold of what is generally required to induce overt cellular injury in vivo, will interfere with the regeneration of skeletal muscle (e.g., Gulati 1987; Lewis 1954; Pagel and Partridge 1999; Rathbone et al. 2003). Since there is an absence of overt cellular damage accompanying such exposures to irradiation, it has been postulated that the failure of myofibers to regenerate was a result of damage to DNA, which would prevent satellite cell proliferation (Robertson et al. 1992*a*). It would follow then that mature, permanently differentiated, mammalian myofibers would not appear to be the locus of the radiation-induced mitotic failure (Chambers and Mcdermott 1996; Stockdale and Holtzer 1961).

In light of the random nature of radiation-induced damage, the inhibitory effects of radiation on muscle regeneration are proposed to be a result of the incapacitation of satellite cell mitotic activity via the prevention of DNA replication as opposed to the inactivation of specific genes. In support of this theory, Roth and Oron (1985) were also able to prevent muscle regeneration via the pharmacological inhibition of mitosis using Vinblastine. More recently, Yan and colleaugues reported that the irradiation of mouse muscles prevented satellite cell proliferation and identified the failure to induce expression of E2f transcription factors, which are critical for expression of proteins necessary for DNA replication, as a key factor in this failure (Yan et al. 2003).

A number of studies have pointed to the critical requirement for a supply of satellite cells endogenous to the muscle for regeneration (Alameddine et al. 1989, 1994; Robertson et al. 1992*a*). As an example, studies by Alameddine and coworkers (1989, 1994) have demonstrated that the provision of autologous satellite cells to muscles that had been damaged and irradiated was able to rescue much of the muscle regeneration with regard to both morphology and function. Similarly, studies have found that, after extended periods of recovery, muscles that were damaged and irradiated showed only the deposition of a fibrotic component and did not develop any force in response to stimulation (Irintchev et al. 1997; Wernig et al. 2000). However, these authors found that the provision of cultured myoblasts resulted in significant muscle regeneration, including the ability to generate force. Interestingly, in those same studies, undamaged irradiated muscles demonstrated a small but significant deficit in function over time that was also rescued by the injection of cultured myoblasts.

In aggregate, the muscle regeneration literature indicates that satellite cell populations endogenous to the damaged muscle are responsible for the repair and replacement of damaged myofibers. The critical nature of this role for satellite cells has been established, in part, using irradiation as an experimental tool. To date, results from the literature suggest that intact, mytotically competent, stem cell populations outside of damaged muscles do not appear to contribute to substantial levels of regeneration in the absence of experimental interventions to promote this process, i.e., this does not appear to be a naturally occurring process (Washabaugh et al. 2004). However, in the context of experimental and (or) clinical settings, the potential for myoblast transplantation has made great strides and holds the promise of future treatments for diseases such as muscular dystrophy (Skuk and Tremblay 2003).

Satellite cells and muscle hypertrophy

There have been a number of studies that have demonstrated that the muscle hypertrophy process appears to involve the addition of nuclei to existing myofibers (e.g., Salleo et al. 1983; Schiaffino et al. 1972, 1976; Snow 1990). In light of the historical findings indicating that irradiation prevented muscle regeneration, a number of investigators were stimulated to study the impact of this treatment on the phenomenon of muscle hypertrophy. The results of such studies have uniformly indicated that previous irradiation can prevent some or all of the hypertrophy normally induced by increased muscle loading (Adams et al. 2002; Phelan and Gonyea 1997; Robertson et al. 1992*a*; Rosenblatt and Parry 1992, 1993; Rosenblatt et al. 1994). For example, a series of papers published by Rosenblatt and colleagues have shown that, in response to functional overload (surgical removal of synergist muscles), irradiated myofibers do not hypertrophy or increase their myonuclear number, but do alter their myosin heavy chain (MHC) isoform profile from a faster to a slower phenotype (Rosenblatt and Parry 1992, 1993; Rosenblatt et al. 1994). We recently reported that the cells of irradiated muscles respond to functional overload with a number of cellular and molecular changes that indicate that signaling pathways and intracellular processes associated with muscle hypertrophy appear to be intact and functioning appropriately (Adams et al. 2002). In spite of what appeared to be appropriate initial cellular and molecular responses, these irradiated muscles failed to add myonuclei or to hypertrophy. As part of that study we found that the ability of irradiated muscles to increase oxidative capacity is intact, indicating that this was not a limiting factor for hypertrophy. Similarly, Li et al. (2006) recently reported that muscle irradiation treatment does not appear to prevent angiogenesis.

A number of studies involving resistance exercise training

have observed increases in satellite cell activity and (or) the addition of myonuclei in both animals and humans (e.g., Cabric et al. 1987; Kadi et al. 1999; Kadi and Thornell 2000). Similarly, the results from studies using hormonal interventions, such as testosterone treatment, which induce hypertrophy also indicate that satellite cells participate via the provision of additional myonuclei (Herbst and Bhasin 2004). However, the majority of the intervention studies conducted specifically to assess the obligatory nature of the contribution of satellite cells to the hypertrophy process have used the synergist ablation model. This method of inducing compensatory hypertrophy is most likely chosen because it is the animal resistance exercise model that produces the most robust levels of mechanical stress and therefore extensive hypertrophic responses. However, it is generally acknowledged that this model involves some inflammation in the early stages. This suggests the possibility that the obligatory nature of satellite cell contributions to hypertrophy may be unique to this model. However, in contravention to this caveat, Li et al. (2006) have reported that an endurance-running protocol could induce skeletal muscle hypertrophy in mice, and that previous irradiation prevented the hypertrophy response. In addition, Barton-Davis et al. (1999) have reported results demonstrating that satellite cells contribute significantly to muscle hypertrophy induced by the overexpression of insulin-like growth factor I (IGF-I). In that study, the authors reported that irradiation prevented a significant portion of the hypertrophy response, but that it had no impact on specific force of mouse muscles, providing an additional indication that irradiation does not directly impact myofiber function.

Taken together the results from a large body of literature indicate that irradiated myofibers adapt in a manner similar to non-irradiated myofibers with regard to most processes intrinsic to the myofiber, such as the qualitative expression of contractile protein isoforms, but that they are unable to generate more than a modest increase in the quantity of protein accumulated in the myofibers. The common finding from studies of muscle hypertrophy following irradiation has provided a consistent observation of decreased or absent cell proliferation, presumably due to a failure to add myonuclei from the satellite cell pool. In contrast to findings in mammals, in avian muscles, irradiation appears to prevent stretch-induced cellular proliferation, but prevents only a relatively small proportion of the hypertrophy induced by stretching the muscles (Lowe and Alway 1999).

It is important to note that there appears to be some threshold level of muscle hypertrophy that is sensitive to the requirement for the addition of myonuclei. It has been observed in human studies that moderate levels of muscle hypertrophy can occur in the absence of significant levels of myonuclear incorporation (e.g., Kadi et al. 2004). It seems quite logical that the relationship between myofiber size and myonuclear number would have a fairly wide range (e.g., Barton-Davis et al. 1999). There would be an appreciable metabolic and resource expense associated with the constant shedding of nuclei or activation of satellite cell proliferation in response to moderate fluctuations in muscle loading. It also seems reasonable to expect that, after a period of rapid satellite cell or myoblast cell line activity (i.e., proliferation, differentiation, and fusion) there would be a period of protein synthesis to reestablish the myonuclear–myofiber size ratio in the absence of further cell replication events (Nader et al. 2005; Rommel et al. 2001).

Along these same lines there is evidence that some degree of hypertrophy can be observed in previously irradiated muscles as well. In a study involving long-term overloading and irradiation of skeletal muscles in rats we found that, in the initial period, there was a small but significant increase in the mass and myofibrillar protein content of irradiated muscles (Adams et al. 2002). During the first 15 days of increased loading, the DNA content of the irradiated muscles also increased by a small but significant amount. This suggests the possibility that a small number of satellite cells within the irradiated muscles may have been able to complete mitosis. These cells may have been either undamaged by the irradiation treatment or were able to affect repair of their DNA (Mozdziak et al. 1996). Alternatively, this increase in muscle DNA may have represented an influx of cells from outside of the irradiated muscles. There were also indications that the small degree of hypertrophy seen in the irradiated muscles may have been supported by a population of satellite cells that was quiescent but committed to differentiation without the need to proliferate. This source could contribute a finite supply of new myonuclei to overloaded myofibers. In support of this possibility, we have previously reported that some of the earliest molecular level changes seen in overloaded muscles are indicative of myogenic differentiation rather than proliferation (Adams et al. 1999). Myogenic cell differentiation that proceeds proliferation has also been observed in response to muscle injury (Grounds et al. 1992; Rantanen et al. 1995; Yablonka-Reuveni 1995).

Satellite cells and sarcopenia

The literature regarding a potential role for satellite cells in aging-related muscle atrophy, i.e., sarcopenia, has been less clear than the relationship of satellite cells to muscle regeneration or hypertrophy. Studies have indicated that aging does not appear to depress the inherent ability of satellite cells to activate in response to various perturbations (Chakravarthy et al. 2000; Conboy et al. 2005; Dedkov et al. 2003; Putman et al. 2001). We recently reported that, in the muscles of both young and old rats, cyclin D1 mRNA levels (potentially indicating increased cell-cycle activity) increased following acute resistance exercise (Haddad and Adams 2006). However, there was a significant delay in the cyclin D1 response of the older muscles. This observation appears to be in accord with in vitro studies demonstrating a lag in the proliferative responses of satellite cells from aged muscles (Schultz and Lipton 1982).

Some of the confusion with regard to the impact of aging on satellite cell function, particularly the maintenance of myonuclear number, may partly stem from the lack of a clear understanding of how the myonuclear domain changes with age. This, in turn, could be a result of the diversity of methods used to quantitate the myonuclear domain (see Brack et al. 2005). Recently, Brack et al. (2005) provided a comprehensive analysis of changes in both myonuclear domain and satellite cell abundance in the muscles of mice. They found that there were fiber-type-specific declines in

satellite cell number that appeared to be related to reduced numbers of myonuclei per unit length and hence to increase in nuclear domain size. Early in the aging process, they found that decreases in myonuclei per unit muscle length preceded sarcopenia in larger muscle fibers. This finding lead these authors to speculate that the age-related decrease in myofiber size may actually be a compensatory response to an inadequate capacity for myonuclear replacement to maintain nuclear domain size. This concept contrasts with observations of muscle inactivity induced by the silencing of neural signaling in which muscle atrophy precedes the decline in myonuclear number (Zhong et al. 2005).

Satellite cell identification

It should be noted that there are a relatively large number of cell surface molecules that have been proposed as markers of satellite cells (myogenic precursor cells, side population cells, etc.) (see Cossu and Biressi 2005). Most likely, the identification of satellite cells using various markers is in some way conditional, relative to the state of the tissue, thereby rendering the interpretation of the literature on satellite cell behavior and regulation difficult to interpret at times.

Adding myonuclei and compensatory hypertrophy

Accepting the premise that there is a threshold level of compensatory muscle hypertrophy, above which the addition of myonuclei becomes necessary, the next question becomes "Why?". In response to a chronic increase in loading, the task confronting the affected myofibers is simply to increase the compliment of various cellular (and extracellular) components that the cells routinely synthesize to maintain cellular homeostasis. This contrasts with the case of muscle regeneration in which the loss of myofibers, or portions thereof, renders the requirement for cellular proliferation intuitively obvious.

In an attempt to shed light on this question we conducted a long-term (3 month) study in rats using bilateral leg muscle overloading (soleus and gastrocnemius ablation) in conjunction with unilateral leg irradiation (Adams et al. 2002). In that study, we assessed cellular- and molecular-level changes induced by overloading in an attempt to identify loading-sensitive processes altered by the irradiation treatment. Since the control was the contralateral non-irradiated muscle from the same animal, a differential response would clearly be a function of the irradiation treatment.

As noted above, in the irradiated muscles, we observed a small increase in myofibrillar protein in the early stages of the treatment. However, beyond this minor early adaptation, the hypertrophy response was essentially abrogated by the irradiation treatment. The results of that study also indicated that the inhibition of the hypertrophy response was not related to the ability to produce mRNA in general or muscle-specific mRNAs since, for example, the conversion from fast to slow MHC expression was actually accentuated in the irradiated muscles.

During the initial period of increased loading (e.g., 3–7 d) we observed that the various cellular- and molecular-level responses of irradiated and contralateral muscles were not notably different. This suggests that the insult imposed by the radiation treatment did not affect the ability of the myofibers and their myonuclei to initiate adaptive responses. However, as the period of loading progressed there were obvious changes in intracellular signaling that were negatively affected by irradiation. In particular, components of intracellular signaling pathways associated with the regulation of protein translation returned to baseline in the irradiated muscles, but they remained elevated in the contralateral muscles. For example, in irradiated muscles, the activating phosphorylation of the p70 ribosomal S6 kinase (S6K1) was initially increased, but declined to resting levels after 3 d (Adams et al. 2002). S6K1 phosphorylation is known to be initiated by interventions that induce muscle hypertrophy, such as resistance exercise or growth-factor stimulation, (Fig. 1) (Adams et al. 2002; Baar and Esser 1999; Haddad and Adams 2002, 2004, 2006).

S6K1 is a critical component of signaling that induces an increase in the translational apparatus, i.e., ribosomal RNA (rRNA), proteins, and elongation factors (Fig. 1) (Dufner and Thomas 1999; Ruggero and Sonenberg 2005). Current thinking is that S6K1 increases the transcription of rRNA via the activation of an unknown kinase which, in turn, activates the rRNA transcription factor UBF (upstream binding factor) (Hannan et al. 2003). Hannan et al. demonstrated that effects of S6K1 activity are important for the hypertrophy of non-proliferating cardiomyocytes, indicating that the importance of S6K1 for hypertrophy is separate from proliferation related processes. Similarly, the phenotype of S6K-deficient mice indicates that S6K1 is important for the regulation of myofiber size, but does not appear to be involved with cell proliferation (Ohanna et al. 2005).

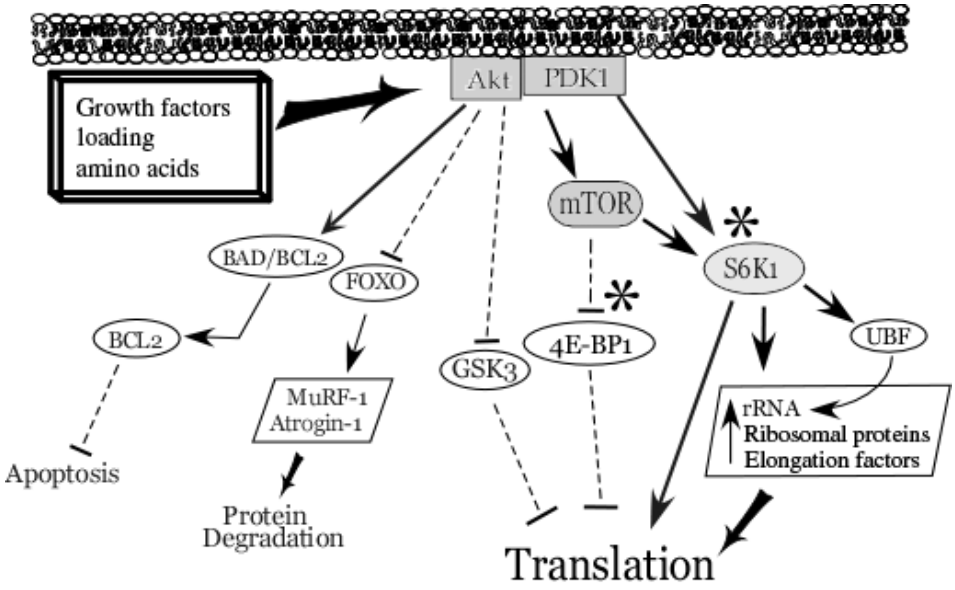
In addition to S6K1, another regulatory step related to translation was also inhibited as a result of irradiation. Irradiation resulted in a failure to maintain hyperphosphoryalation of the eukaryotic initiation factor 4E binding protein (4E-BP1) (Adams et al. 2002). The hyperphosphorylation of 4E-BP1 is critical for increased translation of mRNAs with 5′ cap structuring (Fig. 1) (Ruggero and Sonenberg 2005).

Hypertrophy and translational capacity

Interestingly, in overloaded irradiated muscles, another key point of divergence involved an increase in total RNA present in muscles (Adams et al. 2002). Since the bulk of RNA present in skeletal muscle is ribosomal (>85%), large changes in this measure are generally accepted as being indicative of alterations in the translational capacity of the tissue (Hannan et al. 1998). In non-irradiated contralateral muscles, a continuing overloading stimulus resulted in an extended anabolic state evidenced by the sustained increase in RNA. However, in irradiated muscles, subject to the same loading, the elevation in total RNA was not sustained. The time course of S6K1 phosphorylation and RNA content suggests that changes in regulation, including signaling via S6K1, were altered by irradiation (Fig. 2). Interestingly, the changes in the expression of various loading-sensitive mRNAs did not demonstrate this pattern of abrupt divergence (Adams et al. 2002).

An additional mechanism for the regulation of rRNA production involves changes in the phosphorylation of retino-

Fig. 1. In skeletal muscle, signaling via the Akt–mTOR pathway is sensitive to growth factors (e.g., IGF-I) and mechanical loading. Akt–mTOR signaling can promote increased protein synthesis in a number of ways, including an increase in the initiation of translation (decreased 4E-BP1 inhibition via hyperphosphorylation) and increasing translational capacity (increased S6K1 activity). This pathway can also promote anti-catabolic processes via decreased ubiquitin-mediated protein degradation (inhibition of the FOXO transcription factor) and possibly via decreased apoptosis. In studies using irradiation to inhibit loading-induced muscle hypertrophy, the phosphorylation of S6K1 and the hyperphosphorylation of 4E-BP1 were both negatively effected by irradiation (Adams et al. 2002). This suggests that the requirement for additional myonuclei may involve the ability to chronically up-regulate translational capacity. Akt, protein kinase B or Rac-1; PDK1, 3-phosphoinositide-dependent protein kinase-1; mTOR, mammalian target of rapamycin (also called RAFT-1, FRAP, RAPT-1); 4E-BP1, eukaryotic initiation factor 4 binding protein-1 (also called PHAS-1); S6K1, p70 S6 kinase; GSK3, glycogen synthase kinase-3; FOXO, member of the forkhead transcription factor family; Atrogen-1, a ubiquitin E3 ligase; MuRF-1, muscle ring finger 1 (ubiquitin E3 ligase); BAD, regulator of programmed cell death, pro-apoptotic; Bcl2, regulator of programmed cell death, anti-apoptotic.



blastoma (Rb) gene products (Hannan et al. 1998). Nader et al. (2005) recently presented convincing evidence that this mechanism may be in operation during in vitro myotube growth. In that study, increases in cyclin D expression paralleled the phosphorylation and inactivation of Rb during serum-induced increases in myotube size in the absence of continuing nuclear addition. These authors found that the inhibition of mTOR via rapamycin prevented myotube growth and the increase in cyclin D expression.

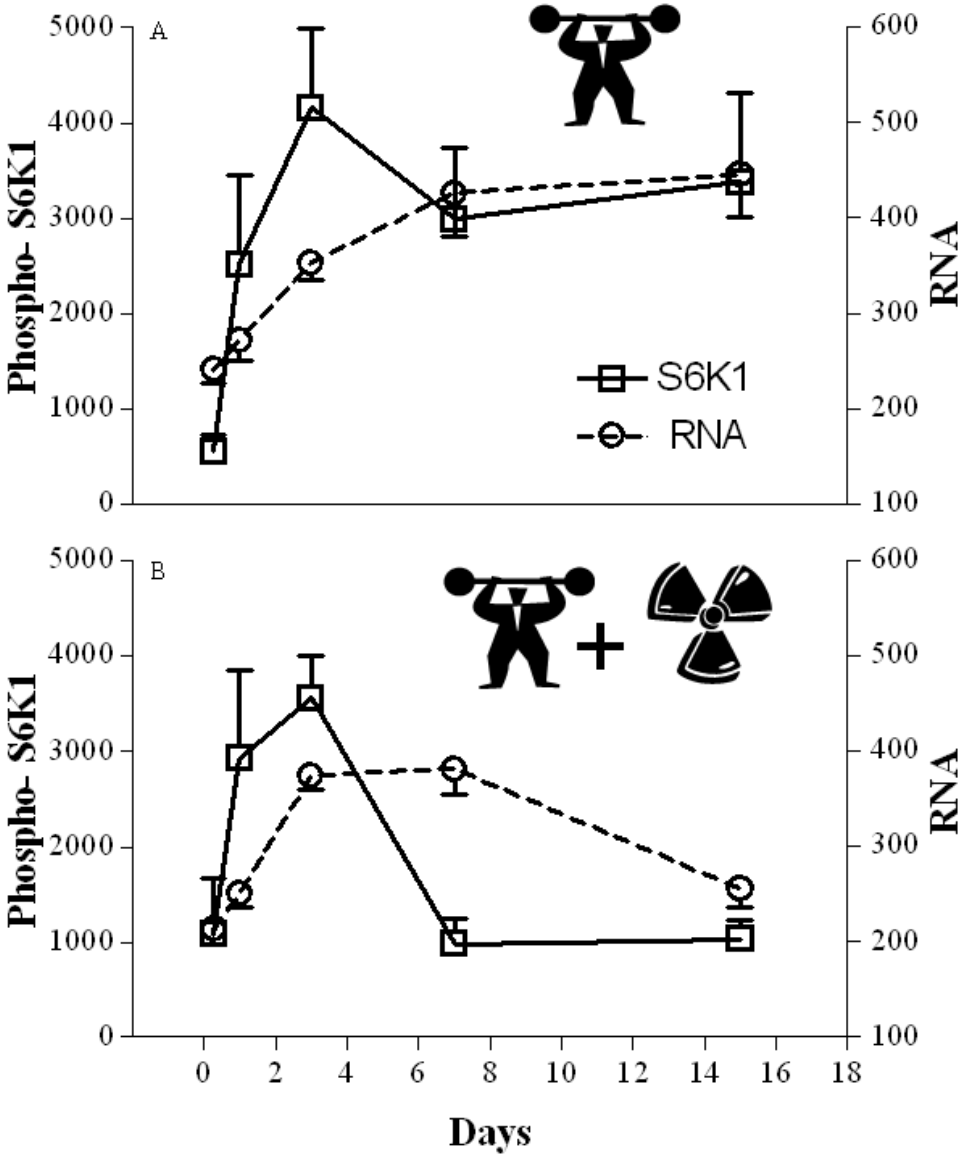
It is interesting to note that cyclin D1 expression often parallels the increase in total RNA present in skeletal muscles during increased loading. For example, in a recent study, we found that just two bouts of resistance exercise resulted in an increase in RNA and that there was a significant correlation between total RNA and cyclin D1 mRNA expression in the muscles from both young (r^2 = 0.43, p = 0.0001) and old (r^2 = 0.24, p = 0.009) rats (Haddad and Adams 2006). However, in preparation for this review, we conducted analysis of the RNA – cyclin D1 relationship from our previous paper involving irradiation (Adams et al. 2002). We found that there was a significant correlation between RNA and cyclin D1 in the non-irradiated muscles (r^2 = 0.35, p = 0.0001), but not in the irradiated overloaded muscles (r^2 = 0.07, p = 0.12). There was, however, a very robust increase in cyclin D1 expression in the irradiated overloaded muscles. Cyclin D1 mRNA expression in the irradiated muscles was increased 3- to 7-fold at time points when total RNA levels were at baseline values. This appears to suggest that, in the absence of increased signaling in the mTOR pathway (e.g., S6K1 and 4E-BP1 phosphorylation), elevated cyclin D1 expression was not sufficient to promote increases in rRNA. Along these same lines, Hannan et al. (2003) reported that rapamycin inhibited increases in rRNA in cells that harbored functionally inactivated Rb . These authors concluded that increased S6K1 activity was required to produce an increase in rRNA (Fig. 1).

Satellite cells and translational capacity

The observation that signaling associated with the regulation of translation, as well as translational capacity itself, is down-regulated as a result of irradiation, indicates that the addition of myonuclei may play a critical role in this aspect of the cellular responses to increased loading.

Parallel changes in RNA and muscle size have been observed in both animals and humans (e.g., Adams et al. 2002; Haddad et al. 2005). The amount of RNA, and therefore ribosomal RNA, present in skeletal muscles decreases precipitously as an initial response to a decrease in muscle activation and loading (e.g., Haddad et al. 2003). In the case of acute muscle unloading, this would be expected to result in a rapid decrease in the protein synthetic capacity of the muscle most likely accounting for a portion of the observed atrophy (Haddad et al. 2003). Interestingly, we found that relative to ambulatory controls total RNA levels were depressed in the atrophied muscles of spinal cord injury patients and that just two bouts of resistance exercise could in-

Fig. 2. Increased muscle loading in the absence (A) or presence (B) of irradiation to incapacitate satellite cell proliferation. Anabolic stimuli result in an increase in activity in the Akt–mTOR pathway (see Fig. 1). In this circumstance, mTOR activates S6K1, leading to an increase in protein translational capacity as evidenced by a sustained increase in total RNA. In muscles that were not irradiated, increased loading (synergist ablation) resulted in a sustained increase in S6K1 phosphorylation, which induced a large increase in total muscle RNA content (A). However, if prior to overloading muscles were exposed to radiation to prevent satellite cell proliferation, the increase in S6K1 phosphorylation and RNA content could not be sustained, thereby preventing most of the hypertrophy response (B) (Adams et al. 2002).



itiate the normalization of this parameter (Bickel et al. 2003).

One of the primary limitations that might be imposed by the bulk amount of DNA present in a given myofiber is the ability to sustain large increases in transcription. (Montagne 2000). This limitation may not be particularly critical, since the translation of mRNA is subject to potential amplification via multiple translation events, i.e., concurrent translation via polyribosomes, allowing for the production of many proteins from one transcript. In contrast, transcripts such as ribosomal RNA (rRNA) and transfer RNAs (tRNA) are final gene products, thus their mass production requires many DNA templates (Gregory 2001; Montagne 2000). The number of copies of rRNA and tRNA genes can only be increased via the acquisition of additional copies of the template DNA. Accepting the premise that multinucleated myofibers and their nuclei are post-mitotic, an increase in the number of copies of the DNA must come from a source external to the myofiber, most likely via the progeny of satellite cells.

As reviewed by Booth et al. (1998), there is evidence that a general increase in translational efficiency occurs at the onset of muscle hypertrophy. However, sustained increases in protein production appear to require substantial increases in the translational machinery. For example, in the hypertrophying heart, early adaptations include an increase in translational efficiency and an acceleration of the synthesis of new ribosomes (Nagatomo et al. 1999; Siehl et al. 1985).

In general, under steady-state conditions, there is a relatively constant relationship between the amount of DNA

and RNA present in muscle cells across a wide range of muscle sizes (Millward et al. 1975). Obviously, this relationship can be altered during periods of intense anabolic activity. For example, the results of our long-term irradiation study indicate that there was an approximately 30% increase in the RNA–DNA ratio after 15 d of overloading in control muscles. However, the overloaded and irradiated muscles were unable to increase this ratio (Adams et al. 2002). In that study, after 90 d of overloading, the RNA content of the non-irradiated contralateral muscles was increased by ~1.8-fold; however, the RNA–DNA ratio was no longer elevated relative to control values due to the acquisition of new myonuclei as evidenced by DNA content and microscopic analysis of myonuclei.

The fact that disequilibrium in RNA–DNA ratios was observed early in the hypertrophy process indicates that the production of ribosomal RNA can be increased by a given complement of myonuclei. Therefore it is not intuitively obvious why myofibers could not just up-regulate the production of the translational apparatus until sufficient amounts of protein have been produced to allow for compensatory hypertrophy. One limiting factor could be some form of functional compartmentation resulting from limitations in the movement of gene products. For example, studies have shown that the gene products appropriate to myofibers will have a relatively limited range of distribution, i.e., in the region surrounding the originating myonucleus (Chretien et al. 2005; Pavlath et al. 1989; Ralston and Hall 1992). In contrast, non-native gene products such as green fluorescent protein (GFP) can be widely distributed along the length of a myofiber (Chretien et al. 2005).

An additional mechanism limiting the influence of a given myonucleus could reside in the targeting of ribosomes to specific locations thereby limiting their distribution. Along those lines, Horne and Hesketh (1990*a*, 1990*b*) reported that, during the development of muscle hypertrophy, there is a significant increase in the population of ribosomes colocalized with myofibrillar components. Such findings suggest that increases in ribosomes during muscle hypertrophy may be targeted to specific locations where the production of contractile proteins will take place. This could conceivably represent a mechanism by which the distribution of rRNA may be spatially limited relative to a given myonucleus.

Taken together, the above observations suggest the hypothesis that the addition of myonuclei to muscle fibers is in some way necessary for sustained increase in translational capacity in response to substantial, chronic increases in muscle loading.

Summary

The majority of the studies conducted in vivo suggest that a substantial increase in the size of myofibers in the muscles of mature mammals requires the availability of satellite cells that can provide additional myonuclei to support hypertrophy. The reasons for the requirement have yet to be established. Data from irradiation studies appears to indicate that, in the absence of a source for additional nuclei, myofibers down-regulate ribosomal biogenesis, thereby restraining translational capacity and blunting the anabolic response to increased loading.

Acknowledgements

Supported by the National Space Biology Research Institute (US) (NSBRI-NCC9-58) and the National Institutes of Health (US) (R01 HD-39676).

References

Adams, G.R., Haddad, F., and Baldwin, K.M. 1999. Time course of changes in markers of myogenesis in overloaded rat skeletal muscles. J. Appl. Physiol. **87**: 1705–1712. PMID:10562612.

Adams, G.R., Caiozzo, V.J., Haddad, F., and Baldwin, K.M. 2002. Cellular and molecular responses to increased skeletal muscle loading after irradiation. Am. J. Physiol. **283**: C1182–C1195.

Alameddine, H.S., Dehaupas, M., and Fardeau, M. 1989. Regeneration of skeletal muscle fibers from autologous satellite cells multiplied *in vitro*. An experimental model for testing cultured cell myogenicity. Muscle Nerve, **12**: 544–555. doi:10.1002/mus.880120705. PMID:2674704.

Alameddine, H.S., Louboutin, J.P., Dehaupas, M., Sebille, A., and Fardeau, M. 1994. Functional recovery induced by satellite cell grafts in irreversibly injured muscles. Cell Transplant. **3**: 3–14. PMID:8162290.

Baar, K., and Esser, K. 1999. Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. Am. J. Physiol. **276**: C120–C127. PMID:9886927.

Barton-Davis, E.R., Shoturma, D.I., and Sweeney, H.L. 1999. Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. Acta Physiol. Scand. **167**: 301–305. doi:10.1046/j.1365-201x.1999.00618.x. PMID:10632630.

Bickel, C.S., Slade, J.M., Haddad, F., Adams, G.R., and Dudley, G.A. 2003. Acute molecular responses of skeletal muscle to resistance exercise in able-bodied and spinal cord-injured subjects. J. Appl. Physiol. **94**: 2255–2262. PMID:12611774.

Booth, F.W., Tseng, B.S., Fluck, M., and Carson, J.A. 1998. Molecular and cellular adaptation of muscle in response to physical training. Acta Physiol. Scand. **162**: 343–350. doi:10.1046/j.1365-201X.1998.0326e.x. PMID:9578380.

Brack, A.S., Bildsoe, H., and Hughes, S.M. 2005. Evidence that satellite cell decrement contributes to preferential decline in nuclear number from large fibres during murine age-related muscle atrophy. J. Cell Sci. **118**: 4813–4821. doi:10.1242/jcs.02602. PMID:16219688.

Cabric, M., Appell, H.J., and Resic, A. 1987. Effects of electrical stimulation of different frequencies on the myonuclei and fiber size in human muscle. Int. J. Sports Med. **8**: 323–326. PMID:3679646.

Chakravarthy, M.V., Davis, B.S., and Booth, F.W. 2000. IGF-I restores satellite cell proliferative potential in immobilized old skeletal muscle. J. Appl. Physiol. **89**: 1365–1379. PMID:11007571.

Chambers, R.L., and Mcdermott, J.C. 1996. Molecular basis of skeletal muscle regeneration. Can. J. Appl. Physiol. **21**: 155–184. PMID:8792022.

Chretien, F., Dreyfus, P.A., Christov, C., Caramelle, P., Lagrange, J.L., Chazaud, B., and Gherardi, R.K. 2005. *In vivo* fusion of circulating fluorescent cells with dystrophin-deficient myofibers results in extensive sarcoplasmic fluorescence expression but limited dystrophin sarcolemmal expression. Am. J. Pathol. **166**: 1741–1748. PMID:15920159.

Collins, C.A., and Partridge, T.A. 2005. Self-renewal of the adult skeletal muscle satellite cell. Cell Cycle, **4**: 1338–1341. PMID:16177569.

Conboy, I.M., Conboy, M.J., Wagers, A.J., Girma, E.R., Weissman, I.L., and Rando, T.A. 2005. Rejuvenation of aged progenitor cells

by exposure to a young systemic environment. Nature (London), **433**: 760–764. doi:10.1038/nature03260. PMID:15716955.

Cossu, G., and Biressi, S. 2005. Satellite cells, myoblasts and other occasional myogenic progenitors: possible origin, phenotypic features and role in muscle regeneration. Semin. Cell Dev. Biol. **16**: 623–631. doi:10.1016/j.semcdb.2005.07.003. PMID:16118057.

Dedkov, E.I., Borisov, A.B., Wernig, A., and Carlson, B.M. 2003. Aging of skeletal muscle does not affect the response of satellite cells to denervation. J. Histochem. Cytochem. **51**: 853–863. PMID:12810836.

Dufner, A., and Thomas, G. 1999. Ribosomal S6 kinase signaling and the control of translation. Exp. Cell Res. **253**: 100–109. doi:10.1006/excr.1999.4683. PMID:10579915.

Gregory, T.R. 2001. Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. Biol. Rev. Camb. Philos. Soc. **76**: 65–101. doi:10.1017/S1464793100005595. PMID:11325054.

Grounds, M.D., Garrett, K.L., Lai, M.C., Wright, W.E., and Beilharz, M.W. 1992. Identification of skeletal muscle precursor cells *in vivo* by use of MyoD1 and myogenin probes. Cell Tissue Res. **267**: 99–104. doi:10.1007/BF00318695. PMID:1310442.

Gulati, A.K. 1987. The effect of X-irradiation on skeletal muscle regeneration in the adult rat. J. Neurol. Sci. **78**: 111–120. doi:10.1016/0022-510X(87)90083-9. PMID:3572447.

Haddad, F., and Adams, G.R. 2002. Acute cellular and molecular responses to resistance exercise. J. Appl. Physiol. **93**: 394–403. PMID:12070230.

Haddad, F., and Adams, G.R. 2004. Inhibition of MAP/ERK kinase prevents IGF-I induced hypertrophy in rat muscles. J. Appl. Physiol. **96**: 203–210. PMID:12959952.

Haddad, F., and Adams, G.R. 2006. Aging sensitive cellular and molecular mechanisms associated with skeletal muscle hypertrophy. J. Appl. Physiol. **100**(4): 1188–1203. PMID:16373446.

Haddad, F., Roy, R.R., Zhong, H., Edgerton, V.R., and Baldwin, K.M. 2003. Atrophy responses to muscle inactivity. I. Cellular markers of protein deficits. J. Appl. Physiol. **95**: 781–790. PMID:12716870.

Haddad, F., Baldwin, K.M., and Tesch, P.A. 2005. Pretranslational markers of contractile protein expression in human skeletal muscle: effect of limb unloading plus resistance exercise. J. Appl. Physiol. **98**: 46–52. PMID:15298986.

Hannan, K.M., Hannan, R.D., and Rothblum, L.I. 1998. Transcription by RNA polymerase I. Front. Biosci. **3**: 376–398.

Hannan, K.M., Brandenburger, Y., Jenkins, A., Sharkey, K., Cavanaugh, A., Rothblum, L., et al. 2003. mTOR-dependent regulation of ribosomal gene transcription requires S6K1 and is mediated by phosphorylation of the carboxy-terminal activation domain of the nucleolar transcription factor UBF. Mol. Cell. Biol. **23**: 8862–8877. doi:10.1128/MCB.23.23.8862-8877.2003. PMID:14612424.

Herbst, K.L., and Bhasin, S. 2004. Testosterone action on skeletal muscle. Curr. Opin. Clin. Nutr. Metab. Care, **7**: 271–277. PMID:15075918.

Horne, Z., and Hesketh, J. 1990*a*. Increased association of ribosomes with myofibrils during the skeletal-muscle hypertrophy induced either by the beta-adrenoceptor agonist clenbuterol or by tenotomy. Biochem. J. **272**: 831–833. PMID:2268305.

Horne, Z., and Hesketh, J. 1990*b*. Immunological localization of ribosomes in striated rat muscle. Biochem. J. **268**: 231–236. PMID:2188648.

Hughes, S.M., and Schiaffino, S. 1999. Control of muscle fiber size: a crucial factor in ageing. Acta Physiol. Scand. **167**: 307–312. doi:10.1046/j.1365-201x.1999.00619.x. PMID:10632631.

Irintchev, A., Langer, M., Zweyer, M., Theisen, R., and Wernig, A. 1997. Functional improvement of damaged adult mouse muscle by implantation of primary myoblasts. J. Physiol. **500**: 775–785. PMID:9161990.

Kadi, F., and Thornell, L.E. 2000. Concomitant increases in myonuclear and satellite cell content in female trapezius muscle following strength training. Histochem. Cell Biol. **113**: 99–103. doi:10.1007/s004180050012. PMID:10766262.

Kadi, F., Eriksson, A., Holmner, S., Butler-Browne, G.S., and Thornell, L.E. 1999. Cellular adaptation of the trapezius muscle in strength-trained athletes. Histochem. Cell Biol. **111**: 189–195. doi:10.1007/s004180050348. PMID:10094415.

Kadi, F., Schjerling, P., Andersen, L.L., Charifi, N., Madsen, J.L., Christensen, L.R., and Andersen, J.L. 2004. The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles. J. Physiol. **558**: 1005–1012. doi:10.1113/jphysiol.2004.065904. PMID:15218062.

Lewis, R.B. 1954. Changes in striated muscle following single intense doses of X-rays. Lab. Invest. **3**: 48–55. PMID:13131820.

Li, P., Akimoto, T., Zhang, M., Williams, R.S., and Yan, Z. 2006. Resident stem cells are not required for exercise-induced fiber type-switching and angiogenesis, but required for activity-dependent muscle growth. Am. J. Physiol. **290**(6): C1461–C1468. PMID:16407413.

Lowe, D.A., and Alway, S.E. 1999. Stretch-induced myogenin, MyoD, and MRF4 expression and acute hypertrophy in quail slow-tonic muscle are not dependent upon satellite cell proliferation. Cell Tissue Res. **296**: 531–539. doi:10.1007/s004410051314. PMID:10370140.

Millward, D.J., Garlick, P.J., Stewart, R.J., Nnanyelugo, D.O., and Waterlow, J.C. 1975. Skeletal-muscle growth and protein turnover. Biochem. J. **150**: 235–243. PMID:1180916.

Montagne, J. 2000. Genetic and molecular mechanisms of cell size control. Mol. Cell Biol. Res. Commun. **4**: 195–202. PMID:11409911.

Mozdziak, P.E., Schultz, E., and Cassens, R.G. 1996. The effect of *in vivo* and *in vitro* irradiation (25 Gy) on the subsequent *in vitro* growth of satellite cells. Cell Tissue Res. **283**: 203–208. doi:10.1007/s004410050530. PMID:8593649.

Nader, G.A., McLoughlin, T.J., and Esser, K.A. 2005. mTOR function in skeletal muscle hypertrophy: Increased ribosomal RNA via cell cycle regulators. Am. J. Physiol. **289**: C1457–C1465. doi:10.1152/ajpcell.00165.2005.

Nagatomo, Y., Carabello, B.A., Hamawaki, M., Nemoto, S., Matsuo, T., and McDermott, P.J. 1999. Translational mechanisms accelerate the rate of protein synthesis during canine pressure-overload hypertrophy. Am. J. Physiol. **277**: H2176–H2184. PMID:10600835.

Ohanna, M., Sobering, A.K., Lapointe, T., Lorenzo, L., Praud, C., Petroulakis, E., et al. 2005. Atrophy of S6K1(–/–) skeletal muscle cells reveals distinct mTOR effectors for cell cycle and size control. Nat. Cell Biol. **7**: 286–294. doi:10.1038/ncb1231. PMID:15723049.

Pagel, C.N., and Partridge, T.A. 1999. Covert persistence of mdx mouse myopathy is revealed by acute and chronic effects of irradiation. J. Neurol. Sci. **164**: 103–116. doi:10.1016/S0022-510X(99)00061-1. PMID:10402020.

Pavlath, G.K., Rich, K., Webster, S.G., and Blau, H.M. 1989. Localization of muscle gene products in nuclear domains. Nature (London), **337**: 570–573. doi:10.1038/337570a0. PMID:2915707.

Phelan, J.N., and Gonyea, W.J. 1997. Effect of radiation on satel-

lite cell activity and protein expression in overloaded mammalian skeletal muscle. Anat. Rec. **247**: 179–188. doi:10.1002/(SICI)1097-0185(199702)247:2<179::AID-AR4>3.0.CO;2-T. PMID:9025997.

Putman, C.T., Sultan, K.R., Wassmer, T., Bamford, J.A., Skorjanc, D., and Pette, D. 2001. Fiber-type transitions and satellite cell activation in low-frequency-stimulated muscles of young and aging rats. J. Gerontol. A Biol. Sci. Med. Sci. **56**: B510–B519. PMID:11723143.

Ralston, E., and Hall, Z.W. 1992. Restricted distribution of mRNA produced from a single nucleus in hybrid myotubes. J. Cell Biol. **119**: 1063–1068. doi:10.1083/jcb.119.5.1063. PMID:1447288.

Rantanen, J., Hurme, T., Lukka, R., Heino, J., and Kalimo, H. 1995. Satellite cell proliferation and the expression of myogenin and desmin in regenerating skeletal muscle: evidence for two different populations of satellite cells. Lab. Invest. **72**: 341–347. PMID:7898053.

Rathbone, C.R., Wenke, J.C., Warren, G.L., and Armstrong, R.B. 2003. Importance of satellite cells in the strength recovery after eccentric contraction-induced muscle injury. Am. J. Physiol. **285**: R1490–R1495.

Robertson, T.A., Grounds, M.D., Mitchell, C.A., and Papadimitriou, J.M. 1990. Fusion between myogenic cells *in vivo*: an ultrastructural study in regenerating murine skeletal muscle. J. Struct. Biol. **105**: 170–182. doi:10.1016/1047-8477(90)90111-O. PMID:2100148.

Robertson, T.A., Grounds, M.D., and Papadimitriou, J.M. 1992*a*. Elucidation of aspects of murine skeletal muscle regeneration using local and whole body irradiation. J. Anat. **181**: 265–276. PMID:1295865.

Robertson, T.A., Papadimitriou, J.M., and Grounds, M.D. 1992*b*. Fusion between a myogenic cell in the satellite cell position and undamaged adult myofiber segments. Experientia, **48**: 394–395. doi:10.1007/BF01923439. PMID:1582497.

Robertson, T.A., Papadimitriou, J.M., and Grounds, M.D. 1993. Fusion of myogenic cells to the newly sealed region of damaged myofibers in skeletal muscle regeneration. Neuropathol. Appl. Neurobiol. **19**: 350–358. PMID:8232756.

Rommel, C., Bodine, S.C., Clarke, B.A., Rossman, R., Nunez, L., Stitt, T.N., et al. 2001. Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. Nat. Cell Biol. **3**: 1009–1013. doi:10.1038/ncb1101-1009. PMID:11715022.

Rosenblatt, J.D., and Parry, D.J. 1992. Gamma irradiation prevents compensatory hypertrophy of overloaded mouse extensor digitorum longus muscle. J. Appl. Physiol. **73**: 2538–2543. PMID:1490967.

Rosenblatt, J.D., and Parry, D.J. 1993. Adaptation of rat extensor digitorum longus muscle to gamma irradiation and overload. Pflugers Arch. **423**: 255–264. doi:10.1007/BF00374404. PMID:8321629.

Rosenblatt, J.D., Yong, D., and Parry, D.J. 1994. Satellite cell activity is required for hypertrophy of overloaded adult rat skeletal muscle. Muscle Nerve, **17**: 608–613. doi:10.1002/mus.880170607. PMID:8196703.

Roth, D., and Oron, U. 1985. Repair mechanisms involved in muscle regeneration following partial excision of the rat gastrocnemius muscle. Exp. Cell Biol. **53**: 107–114. PMID:3979655.

Ruggero, D., and Sonenberg, N. 2005. The Akt of translational control. Oncogene, **24**: 7426–7434. doi:10.1038/sj.onc.1209098. PMID:16288289.

Sabourin, L.A., and Rudnicki, M.A. 2000. The molecular regulation of myogenesis. Clin. Genet. **57**: 16–25. doi:10.1034/j.1399-0004.2000.570103.x. PMID:10733231.

Salleo, A., LaSpada, G., Falzea, G., Denaro, M.G., and Cicciarello, R. 1983. Response of satellite cells and muscle fibers to long-term compensatory hypertrophy. J. Submicrosc. Cytol. **15**: 929–940. PMID:6655769.

Schiaffino, S., Bormioli, S.P., and Aloisi, M. 1972. Cell proliferation in rat skeletal muscle during early stages of compensatory hypertrophy. Virchows Arch. B. Cell Pathol. **11**: 268–273.

Schiaffino, S., Pierobon Bormioli, S., and Aloisi, M. 1976. The fate of newly formed satellite cells during compensatory muscle hypertrophy. Virchows Arch. B. Cell Pathol. **21**: 113–118.

Schultz, E. 1989. Satellite cell behavior during skeletal muscle growth and regeneration. Med. Sci. Sports Exerc. **21**: S181–S186. PMID:2691829.

Schultz, E., and Lipton, B.H. 1982. Skeletal muscle satellite cells: changes in proliferation potential as a function of age. Mech. Ageing Dev. **20**: 377–383. doi:10.1016/0047-6374(82)90105-1. PMID:7166986.

Schultz, E., and McCormick, K.M. 1994. Skeletal muscle satellite cells. Rev. Physiol. Biochem. Pharmacol. **123**: 213–257. PMID:8209136.

Siehl, D., Chua, B.H., Lautensack-Belser, N., and Morgan, H.E. 1985. Faster protein and ribosome synthesis in thyroxine-induced hypertrophy of rat heart. Am. J. Physiol. **248**: C309–C319. PMID:3156508.

Skuk, D., and Tremblay, J.P. 2003. Myoblast transplantation: the current status of a potential therapeutic tool for myopathies. J. Muscle Res. Cell Motil. **24**: 285–300. PMID:14620742.

Snow, M.H. 1990. Satellite cell response in rat soleus muscle undergoing hypertrophy due to surgical ablation of synergists. Anat. Rec. **227**: 437–446. doi:10.1002/ar.1092270407. PMID:2393096.

Stockdale, F.E., and Holtzer, H. 1961. DNA synthesis and myogenesis. Exp. Cell Res. **24**: 508–520. doi:10.1016/0014-4827(61)90450-5. PMID:13917284.

Wagers, A.J., and Conboy, I.M. 2005. Cellular and molecular signatures of muscle regeneration: current concepts and controversies in adult myogenesis. Cell, **122**: 659–667. doi:10.1016/j.cell.2005.08.021. PMID:16143100.

Washabaugh, C.H., Ontell, M.P., and Ontell, M. 2004. Nonmuscle stem cells fail to significantly contribute to regeneration of normal muscle. Gene Ther. **11**: 1724–1728. doi:10.1038/sj.gt.3302353. PMID:15385949.

Wernig, A., Zweyer, M., and Irintchev, A. 2000. Function of skeletal muscle tissue formed after myoblast transplantation into irradiated mouse muscles. J. Physiol. **522**: 333–345. doi:10.1111/j.1469-7793.2000.t01-2-00333.x. PMID:10639108.

Yablonka-Reuveni, Z. 1995. Developmental and postnatal regulation of adult myoblasts. Microsc. Res. Tech. **30**: 366–380. doi:10.1002/jemt.1070300504. PMID:7787236.

Yan, Z., Choi, S., Liu, X., Zhang, M., Schageman, J.J., Lee, S.Y., et al. 2003. Highly coordinated gene regulation in mouse skeletal muscle regeneration. J. Biol. Chem. **278**: 8826–8836. doi:10.1074/jbc.M209879200. PMID:12477723.

Zammit, P.S., Heslop, L., Hudon, V., Rosenblatt, J.D., Tajbakhsh, S., Buckingham, M.E., Beauchamp, J.R., and Partridge, T.A. 2002. Kinetics of myoblast proliferation show that resident satellite cells are competent to fully regenerate skeletal muscle fibers. Exp. Cell Res. **281**: 39–49. doi:10.1006/excr.2002.5653. PMID:12441128.

Zhong, H., Roy, R.R., Siengthai, B., and Edgerton, V.R. 2005. Effects of inactivity on fiber size and myonuclear number in rat soleus muscle. J. Appl. Physiol. **99**: 1494–1499. doi:10.1152/japplphysiol.00394.2005. PMID:15994244.