Porcine satellite cells are restricted to a phenotype resembling their muscle origin

H. Zhu,* S. Park,* J. M. Scheffler,* S. Kuang,† A. L. Grant,* and D. E. Gerrard*

*Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg 24060; and
†Department of Animal Sciences, Purdue University, West Lafayette, IN 47907

ABSTRACT: Muscles in most domestic animals differ in function and growth potential based largely on muscle fiber type composition. Though much is known about satellite cells (SC), information is limited regarding how populations of SC differ with muscle fiber type, especially in pigs. Therefore, the objective of this study was to isolate and culture SC from red (RST) and white (WST) portions of the semitendinosus muscle of neonatal and adult pigs and determine their capacity to proliferate, differentiate, and express various myosin heavy chain (MyHC) isoforms in vitro. Porcine satellite cells were isolated from RST and WST muscles of 6-wk-old and adult (>6-mo-old) pigs and cultured under standard conditions. Muscle from neonatal pigs yielded nearly 10 times more (P < 0.001) presumptive satellite cells as those from adult pigs, with fusion percentages close to 60% for the former. The RST yielded more (P < 0.001) SC per gram muscle compared to WST, 8.1 ± 0.2 × 10^4 cells versus 6.7 ± 0.1 × 10^4 cells/gram muscle in young pigs, and 9.7 ± 0.4 × 10^3 cells versus 5.5 ± 0.4 × 10^3 cells/gram muscle in adult pigs, respectively. Likewise, satellite cells from RST proliferated faster (P < 0.001) than those from WST across both ages, as indicated by a shorter cell doubling time, 18.6 ± 0.8 h versus 21.3 ± 0.9 h in young pigs, and 23.2 ± 0.7 h versus 26.7 ± 0.9 h in adult pigs, respectively. As a result of shorter times to confluence, satellite cells from RST also formed myotubes earlier than those SC originating from WST. Once induced, however, SC from WST differentiated and fused faster (P < 0.05) as evidenced by fusion percentage within the first 24 h, 41.6% versus 34.3%, respectively; but reached similar ultimate fusion percentages similar to WST by 48 h. Over 90% of MyHC expressed in maximally fused SC cultures from both RST and WST was restricted to the embryonic isoform. Type IIX MyHC mRNA was not detected in any culture. Myotube cultures from RST expressed more (P < 0.01) Type I MyHC isoform mRNA than those from WST, whereas those cultures from WST expressed more (P < 0.05) Type II (including Types IIA and IIB) MyHC transcripts. These data show SC cultures from porcine fast and slow muscles express MyHC profiles largely reflective of their muscle of origin and suggest satellite cells are partially restricted to a particular muscle phenotype in which they are juxtapositioned. Understanding the molecular nature of these intrinsic control mechanisms may lead to improved strategies for augmenting meat animal growth or muscle regeneration.

Key words: porcine, satellite cell, myosin heavy chain

INTRODUCTION

Skeletal muscle growth and regeneration in postnatal animals largely depends on activation of muscle stem cells termed satellite cells (SC). Of particular interest to animal agriculture, SC become the source of nuclei contributed to growing muscle fibers and are requisite for muscle hypertrophy that occurs during normal growth (Moss and Leblond, 1971).

Modulation of overall muscle function is mainly accomplished by changing the ability of each muscle fiber to contract or metabolize energy (Brooke and Kaiser, 1970). Muscles containing greater proportions of Type I fibers are generally considered slow-twitch and utilize more oxidative metabolism, which affords muscles the ability to resist fatigue and sustain function for long periods of time. In contrast, Type II fibers, including IIX and IIB, are faster contracting and possess more glyco-
lytic metabolism but fatigue quickly, yet facilitate explosive, powerful movements. Type IIA fibers are likewise fast-contracting tending to be more oxidative (Hoh and Hughes, 1988).

Using avian-derived SC cultures, DiMario et al. (1993) suggested that intrinsic SC differences vary with muscle type. Others, using SC MyHC expression as an indicator of fiber type programming, reported that SC from slow muscles generate both Types I and II MyHC, whereas SC from fast muscle generate exclusively Type II MyHC (Feldman and Stockdale, 1991). Similar MyHC expression patterns were later documented in rodent SC cultures (Dusterhoft and Pette, 1993; Rosenblatt et al., 1996). Taken together, these data suggest great heterogeneity exists within SC pools, but some of these differences may be preprogrammed according to a particular fiber phenotype. To date, little is known about the relationship between porcine SC and their muscle origin. Therefore, the objective of this study was to isolate SC from red (RST) and white (WST) portions of adult and neonatal pig semitendinosus muscle (ST), and determine their capacity to proliferate, differentiate, and express various MyHC isoforms in vitro.

**MATERIALS AND METHODS**

**Porcine Muscle Collection, Satellite Cell Isolation, and Cell Culture**

All experimental procedures were approved by the Virginia Tech Institutional Animal Care and use Committee. Satellite cells were isolated from ST muscles as previously described (Doumit and Merkel, 1992). Briefly, 12 neonatal (5 to 6 wks) and 6 adult (6 to 7 mo) Yorkshire, Landrace, and Duroc crossbreed pigs of mixed sex were euthanized and the ST muscle was excised, trimmed of visible connective tissue, and 10 mg each of RST and WST muscle sample were weighed before enzymatic digestion. After the addition of differentiation media, three random-ly selected plates were rinsed with PBS and fixed with 100% methanol every 12 h over a 4-d period. Cultures were stained with giemsa (Gibco, Grand Island, NY) to visualize cell structure. Ten randomly selected fields were counted with a hemocytometer. Cell yield was calculated by comparing the total number of cells with the weight of the muscle sample.

**Cell Proliferation and Myogenic Differentiation**

Satellite cell proliferation was evaluated every 24 h over a 4-d culture period using the Promega (Madison, WI) nonradioactive cell proliferation assay kit according to the manufacturer’s protocol. Briefly, after preplatting cell suspensions for 24 h, RST and WST satellite cells were collected using trypsin digestion and seeded on 96-well matrigel-coated plates at a density of 1 × 10^4 cells/cm^2. After a 24-h incubation period, an aliquot (15 μL) of dye solution was added and remained for 4 h before the addition of 100 μL of stop or solubilization solution. The optical density (OD) value of each well was measured at 570 nm after an overnight incubation.

Because satellite cells from young pigs possessed reduced doubling times, satellite cells were cultured at greater densities (2.5 × 10^4 cells/cm^2) to adjust for these differences. Once attached to the plate (~80% confluence), cultures were induced to differentiate by changing to differentiation medium containing 2% horse serum. After the addition of differentiation media, three randomly selected plates were rinsed with PBS and fixed with 100% methanol every 12 h over a 4-d period. Cultures were stained with giemsa (Gibco, Grand Island, NY) to visualize cell structure. Ten randomly selected fields were enumerated for total and fused nuclei. Fused nuclei were defined as any two or more nuclei within a continuous membrane-defined myotube. Fusion percentage was calculated by dividing the total number of fused nuclei with myotubes by the number of total nuclei within a given region of interest.

**Population Doubling Time**

Similar to that outlined above, cultures were initially preplanted, then RST- and WST-derived satellite cells were seeded on matrigel-coated, 24-well culture plates at 1 × 10^4 cells per cm^2. After a 24-h incubation to en-
sure attachment, media were changed, and this time was considered 0 h for the proliferation experiment. Cultures were grown for a 2-d period, and at 0, 12, 24, 36, and 48 h, three randomly-selected wells were harvested using classical trypsin digestions. Cell number was enumerated using an aliquot of cell suspensions and a hemocytometer. Population doubling time (PDT) was then calculated using a PDT calculating software (Roth, 2006).

**Immunohistochemistry**

Satellite cell isolation efficiency was evaluated based on paired box protein 7 (Pax7) immunostaining. In brief, cultured cells were fixed in 4% paraformaldehyde for 10 min followed by permeabilization with PBS containing 0.25% Triton X-100 (Sigma-Aldrich, St. Louis, MO) for 10 min. Cells were then blocked in PBS containing 2% BSA, 5% goat serum, and 0.2% Triton X-100 for 30 min. Primary antibody Pax7 (Developmental Studies Hybridoma Bank, Iowa city, IA) diluted in the same blocking buffer was applied on cells at 4°C overnight. Finally, cells were incubated with secondary antibody (DyLight 488, goat antimouse IgG, Fisher Scientific, Pittsburgh, PA) in 1% BSA at room temperature for 1 hour in dark. All nuclei were stained with DAPI and isolation efficiency was indicated by the percentage of Pax7 positive cells.

**Myosin Heavy Chain Isoform Expression**

After maximal differentiation (d 3), RNA was isolated using TRIzol reagent according to manufacturer’s protocols (Invitrogen, San Diego, CA). Briefly, 1 mL TRIzol reagent was applied on each well, and total RNA was isolated and quantified. Likewise, total RNA was prepared from muscle tissues according to protocols outlined by Gunawan et al. (2006). First-strand cDNA was synthesized from 3 µg of total RNA using random hexanucleotide-primed cDNA synthesis. Quantitative Real time PCR was performed in duplicate using ABI 7500 Fast Real Time PCR System (Applied Biosystems, Foster City, CA). Each PCR reaction contained 200 ng of cDNA, 10-pmol primers, and ABI Power SYBR Master Mix in a total volume of 10 µL. Primers sequences for β-Actin and MyHC isoforms (embryonic and Types I, IIa, IIx, and IIb) used in qPCR are listed in Table 1. Quantitative PCR data were normalized to β-Actin and then expressed in arbitrary units, relative to the value of MyHC IIb level from adult pig RST muscle.

**Statistics**

The data was statistically analyzed with JMP using Student’s t test. Data was reported as LS means ± SE. Least square means were generated using the LSMEANS and Tukey’s adjustment for multiple comparisons. The level of significance was set at $P < 0.05$.

**RESULTS**

**Cell Yield of Muscle Samples**

Satellite cell content varied with animal age and muscle type. Cell yields from young pig muscle were over 10 times greater ($P < 0.001$) than those from adult pigs. Regardless of age, RST muscle samples yielded more ($P < 0.001$) SC per gram of muscle than WST (9.7 ± 0.4 × 10^3 cells versus 5.5 ± 0.4 × 10^3 cells/gram muscle in adult pigs; 8.1 ± 0.2 × 10^4 cells versus 6.7 ± 0.1 × 10^4 cells/gram muscle in young pigs, respectively; Fig. 1). The Pax7-positive cells accounted for 90% of total cells. No different of Pax7 positive percentage was found between RST- and WST-derived cells ($P > 0.05$).

**Proliferation Rate and Fusion Index**

Satellite cells from young pigs exhibited greater proliferative potential as defined by a shorter ($P < 0.001$) doubling time than those from adult pig muscle. Satellite cells isolated from RST proliferated faster ($P < 0.001$) than those from WST (Fig. 2A) in both young and adult pigs, as indicated by a shorter ($P < 0.05$) cell doubling time (23.2 ± 0.7 h versus 26.7 ± 0.9 h in adult pigs, respectively; 18.6 ± 0.8 h versus 21.3 ± 0.9 h in young pigs, respectively; Fig. 2).

For myogenic differentiation experiments, SC were seeded at a high density on 6-well plates (2.5 × 10^4 cells/cm^2) to reduce proliferation time and allow for quick induction of differentiation immediately after cells attached to plates. This was done to control for the differences in proliferation rates between RST and WST SC. Results revealed that once induced, SC from young pig WST had greater ($P < 0.05$) fusion percentages than those from RST during the first 24 h, 41.6% versus 34.3%, respectively; but reached similar ultimate fusion percentages by 48 h (Fig. 3). After 48 h, fully differentiated myotubes started to detach from plates and die soon, hence fusion percentage dropped significantly. Adult pig muscle-derived SC had very low differentiation potential and the fusion rate was usually less than 30% (Data not shown).

**Fiber Type-Specific Gene Expression**

Over 90% of MyHC expressed in porcine satellite cell cultures was identified as the embryonic myosin isofrom (data not shown), though no differences due to SC source were noted. As such, regardless of the muscle type, adult MyHC isoforms were expressed at relatively low levels. Even so, pair-wise comparisons of SC derived from RST and WST of adult and young pigs demonstrated that...
Intrinsic satellite cell contribution to fiber type

adult SC expressed relatively more \(P < 0.05\) fast (Type IIA and IIB), but less \(P < 0.01\) slow (Type I) MyHC isoforms than those cultures arising from young pig muscle. In both RST- and WST-derived cultures, MyHC Types I and IIA were expressed at greater levels \(P < 0.05\) than Type IIB transcripts. Type IIX MyHC mRNA was undetectable (data not shown). Comparing SC from fast and slow muscles, myotubes from RST cultures expressed more \(P < 0.01\) Type I MyHC isoform mRNA, whereas those cultures from WST expressed more fast (Types IIA and IIB) MyHC transcripts \(P < 0.05\;\text{Fig. 4}\).

DISCUSSION

Satellite cells are often referred to as adult muscle stem cells “trapped” between the basal lamina and sarcolemma. However, Bischoff and Heintz (1994) notes that during muscle fiber development, muscle progenitor cells often have great affinity to myotube surfaces in order to facilitate the genesis of nascent fibers. Satellite cells, like their embryonic relatives, reside in contact with myotubes prior to the development of the basal lamina, thus giving the teleological perspective of entrapment. Though some have argued that the origin of satellite cells may be nonsomitic (De Angelis et al., 1999; Ferrari et al., 1998), elegant tracking studies firmly support the notion that satellite cells emanate from the somite, the dermomyotome where embryonic muscle progenitors originate (Gros et al., 2005). Thus, even though satellite cells are a distinct population of myogenic progenitor cells (Cossu and Molinaro, 1987), they likely migrate with embryonic muscle progenitor cells and become enveloped in close proximity to developing muscle fibers. During prenatal skeletal muscle development in the pig, primary fibers form between 35 and 55 d of gestation and exclusively express

Table 1. Nucleotide sequences of the primers used for quantitative reverse transcription-PCR (Da Costa et al., 2003).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Direction</th>
<th>Primer sequence</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>forward</td>
<td>5'-CCA TTC AAT CGG TAG TAG CG-3’</td>
<td>U07786</td>
</tr>
<tr>
<td></td>
<td>reverse</td>
<td>5'-CTA ACC CGT TGA ACC CCA TT-3’</td>
<td></td>
</tr>
<tr>
<td>MyHC slow/I</td>
<td>forward</td>
<td>5'-GGC CCC TTC CAG CTT GA-3’</td>
<td>L10129</td>
</tr>
<tr>
<td></td>
<td>reverse</td>
<td>5'-TGG CTG CGC CTT GGT TT-3’</td>
<td></td>
</tr>
<tr>
<td>MyHC IIA</td>
<td>forward</td>
<td>5'-TTA AAA AGC TCC AAG AAC TGT TTC A-3’</td>
<td>U17772</td>
</tr>
<tr>
<td></td>
<td>reverse</td>
<td>5'-CCA TTT CCT GGT CGG AAC TC-3’</td>
<td></td>
</tr>
<tr>
<td>MyHC IIX</td>
<td>forward</td>
<td>5'-TGC TCC AAG TTC GCC ACT-3’</td>
<td>U90719</td>
</tr>
<tr>
<td></td>
<td>reverse</td>
<td>5'-GGC TGC GGG TTA TTG ATG G-3’</td>
<td></td>
</tr>
<tr>
<td>MyHC IIB</td>
<td>forward</td>
<td>5'-CAC TTT AAG TAG TTG TCT GCC TTG AG-3’</td>
<td>U90720</td>
</tr>
<tr>
<td></td>
<td>reverse</td>
<td>5'-GGC AGC AGG GCA CTA GAT GT-3’</td>
<td></td>
</tr>
<tr>
<td>MyHC embryonic</td>
<td>forward</td>
<td>5'-CCC GCC TGT GTG ATG T-3’</td>
<td>XM003131994.2</td>
</tr>
<tr>
<td></td>
<td>reverse</td>
<td>5'-GGT GTC GGC TGC GAG TCA CA-3’</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** Satellite cell yield (number) per gram muscle of RST (red semitendinosus) and WST (white semitendinosus) from adult and young pig. Means \((\pm SE)\) bearing different letters differ \((P < 0.05)\).  

**Figure 2.** (A) Proliferation rates of RST- (red semitendinosus) and WST- (white semitendinosus) derived satellite cells. Optical density (OD) were collected at 0, 24, 48, 72, and 96 h. (B) Cell doubling time of RST- and WST-derived satellite cells. Results are means \(\pm SE\). Means \((\pm SE)\) bearing different letters differ \((P < 0.05)\).
slow MyHC (Lefaucheur and Vigneron, 1986; Suzuki and Cassens, 1980). Secondary fibers, which form between 55 to 95 d of gestation, usually include an internal layer of Type IIA fibers and an external layer of Type IIB fibers expressing the corresponding MyHCs (Lefaucheur and Vigneron, 1986). In contrast to primary fibers, slow MyHC (Type I) is not expressed in any secondary fibers until late gestation (Lefaucheur et al., 1995). This could indeed explain why the majority of MyHC transcripts detected from our SC cultures are predominately embryonic forms of the contractile-specific gene; essentially, they are assuming a very immature muscle program and therefore, when isolated and cultured, express predominately an embryonic program. The differences between in vivo and in vitro muscle development are likely a result of innervation and endocrine signaling differences that are difficult to recapitulate in a tissue culture plate. Clearly, several have shown that extrinsic signals are capable of altering muscle fiber type. For example, acute denervation of muscle induces a muscle fiber type transition from a slow to a fast phenotype (DiMario and Stockdale, 1997; Viguie et al., 1997). Muscle cross-innervations studies have likewise shown the importance of innervations in driving muscle fiber type composition (Gauthier et al., 1983). Moreover, slow frequency electrical stimulation in vivo increases MyHC Type I expression differently in denervated fast or slow muscles; while slow and fast fre-
frequency of electrical stimulation on the same muscle induces muscle fiber type transitions differently (Ausoni et al., 1990; Kalhovde et al., 2005). Even though these studies demonstrated conclusively that neither innervation nor electrical stimulation is capable of changing muscle fiber type completely, intrinsic mechanisms exist within the muscle that may limit the plasticity of the muscle to a particular insult. This restricted flexibility, whatever the mechanism, may also reside in those satellite cells held in close proximity to fibers differing greatly in their ability to metabolize energy and contract.

In this study, we isolated SC from RST and WST of young and adult pigs in order to ascertain whether intrinsic differences occur in SC originating from vastly different muscles as those reported for other species, and whether these differences were altered with age. Indeed, regardless of muscle type, more satellite cells were liberated from neonatal pig muscle consistent with the findings of others (Allbrook et al., 1971; Cardasis and Cooper, 1975; Feldman and Stockdale, 1991). Though it is possible that protocols used in our laboratory preferentially favor isolation of a greater proportion of the total population available from this more immature, less-extensively developed muscle tissue (Grimby, 1995; Larsson et al., 1978; Lexell et al., 1988), it is intuitively clear that significant muscle cell hypertrophy rate is incomplete at earlier ages, and therefore greater numbers of muscle fibers are collected in a given sample. As such, greater satellite cells would be liberated from such tissue. This is especially for pigs at 5 to 6 wk postnatal, where the diameter of muscle fibers, though not measured in these pigs, is roughly one-quarter that found in adult muscle (Rehfeldt et al., 1999). These data support the notion that satellite cells are an exhaustible reservoir of presumptive myoblasts that, though they actively contribute to their DNA to support the muscle-fiber-based hypertrophy that normally occurs with growth, they seemed to be depleted in adult muscle.

However, using our protocols, significantly more SC were isolated from the RST than the WST, regardless of age. These results are consistent with those of Kelly (1978), and Gibson and Schultz (1982), and though not completely understood, this increase in satellite cell number may be related to the heightened amount of protein turnover normally occurring in redder, more oxidative, fatigue-resistant muscle (Dadoune et al., 1978) that must constantly function to repair muscles that support the postural and ambulatory function of the animal. Given the greater protein turnover rates and the fact that red muscles tend to contain more mRNA for major contractile genes (Collins et al., 2005; Hawke and Garry, 2001), it seems plausible that these muscles need more satellite cells to maintain a high level of transcript level, not necessarily to support hypertrophy but also to repair myofibrillar structures that are damaged by continual use. The ability of SC from red muscle to proliferate faster would also contribute to a greater need for satellite cells in a tissue that needs to replace structural proteins more rapidly and more often. Regardless, these data show that satellite cell abundance is greater in slower contracting muscle of young piglets and argues a functional necessity in both regards.

Several studies in rodents have compared the myogenic differentiation potential of SC from both fast and slow muscle types. Some suggest that the expression of MyHC differ (Wehrle et al., 1994), while others do not (Barjot et al., 1998; Dusterhoft et al., 1990). Our present data showed significant difference in Types I, IIA, and IIB MyHC expression between RST- and WST-derived SC cultures. In prenatal muscle development, primary and secondary muscle fibers are sequentially formed and the dominant MyHC isoforms expressed are embryonic, perinatal, and MyHC Type I (Lyons et al., 1990). Adult fast MyHC mRNA is detected as early as 35 d gestation in pigs and occurs in the order of IIA, IIX, and IIB, respectively, which curiously coincides with their position in the skeletal MyHC cluster (Da Costa et al., 2003). In our data, the majority of MyHC expression in both RST and WST SC cultures appeared to be restricted to the embryonic isoform (over 90%), while the second most abundant MyHC isoforms expressed was Type I MyHC. Type IIA isoforms were expressed at similar, but lower levels to Type I MyHC, whereas Type IIB expression was the least prominent adult contractile protein expressed. This unique in vitro MyHC expression pattern may be predetermined, or restricted, by cell lineage that reflects the early prenatal muscle fiber development. In both young and adult pig RST- and WST-derived SC cultures, a greater expression of MyHC Type I suggest the potential possibility that a portion of cells arise from primary muscle fibers, which are intrinsically capable of formation without innervation (Miller and Stockdale, 1986). Also, when comparing cultures from adult and young pigs, we observed a tendency for those derived from adult pig muscle to express more fast MyHC and less slow Type I MyHC than those emanating from more juvenile pigs. Furthermore, myotube cultures derived from RST of both adult and young pig expressed more Type I, while WST-derived SC expressed more Type IIB indicating the existence of preprogrammed SC lineages from different muscle origins. Interestingly, unlike rodent and avian cultures, our pig muscle SC cultures from RST and WST not only co-expressed Types I and II, but also expressed predominantly embryonic MyHC isoform. The exact reason for this is not entirely known, but is most likely related to limitations of an in vitro system. First and foremost, as mentioned above, these cultures lack neuronal stimulation. Though cultured myotubes contract, they do so spontaneously and not as a result of direction innervations. Clearly, innervations could directly modify gene regulation (Crow and Stockdale, 1986; Spangenburg and
Booth, 2003) or by indirectly affecting them through signal transductions involving calcium (Jordan et al., 2004, 2005; Marks, 1997). We have shown halothane-positive pigs possessing a mutated RYR1 (calcium release channel) have altered MyHC profiles, both expression and production of the isoforms. Second, in vitro, traditional coated plates clearly fail to mimic in vivo environments, where cells are forced to develop and mature in a two-dimensional environment. Finally, pig muscle cell cultures in our studies, as well as others from other species, were only capable of surviving a very short period of time after induction of differentiation (3 days). This issue alone may be the single most important factor limiting expression of adult contractile proteins. The process of myofibrillogenesis involves the coordinated organization of the entire myofibril plus its position in the muscle cells and attachment to the cell membrane. At the same time, myonuclei migrate or are forced to the periphery of the maturing muscle fiber. This does not occur in cultured myotubes and may simply be a function of the limited time these cultures remain viable under very artificial conditions. Even so, however, the fact that cultures indeed express any adult isoform, regardless of the type, clearly argues against the aforementioned and suggests other mechanisms, intrinsic or otherwise, may be restricting myotube maturation events. Of course, we assume that a subpopulation of cells, or myotubes, are responsible for expressing what little adult MyHC detected, but we have not proven this is indeed true. It is equally possible that a small fraction of all transcripts from every myotube is expressing the adult phenotype. Additional studies, most likely involving in situ hybridizations or immunocytochemistry protocols would be required address this issue, even though transcript stability and epitope homology among the isoforms, respectively, would make such studies difficult. Regardless, our data suggest that porcine SC are preprogrammed in their muscle of origin to express genes associated with a specific muscle fiber type, which supports the hypothesis that satellite cells can make an intrinsic contribution to fiber type determination. The question of whether fast and slow muscle-derived SC can grow and differentiate into fast and slow muscle fibers, or vice versa, may largely depend on how these embryonic myotubes grow and fuse into an adult phenotype.

REFERENCES


Intrinsic satellite cell contribution to fiber type


