

Satellite Cells Isolated from Sheep Skeletal Muscle are not Homogeneous

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Abstract

Clonally-derived satellite cells were used to determine if satellite cells reside in sheep skeletal muscle as a homogeneous population. Fifty-two cell clones were isolated from sheep semi-membranosus ($n = 28$) and semitendinosus ($n = 24$) muscles. Muscle type was not a factor in determining the outcome for any of the characteristics measured. The time period required for each clone to proliferate to 100 cells was determined by daily cell count determination. Forty-seven of the 52 clones (90%) proliferated to 100 cells in non-conditioned medium. The largest difference between satellite cell clones was that 4 clones required ≥ 16 d to obtain a colony size of 100. In contrast, 16 clones proliferated to 100 cells in ≤ 10 d. Eighty-seven percent of the clones that grew to 100 cells were satellite cells. Six cell strains (5 myogenic and 1 non-myogenic) were further characterized on the basis of variables of growth and differentiation. Differences in proliferation rate between satellite cell strains were evident and maintained for two subpassages. Differences in plating densities did not effect the relative proliferation of satellite cell strains. However, a marked decrease (~ 2 fold) in proliferation for 3 of the 5 satellite cell strains occurred between the fourth and sixth passage. Some satellite cell strains differed in fusion potential by more than 10 fold; differences in fusion were maintained for two subpassages. Twenty-one additional satellite cell strains were allowed to proliferate, differentiate and form myotubes. Isoforms of myosin heavy chain (MYHC) expressed by myotubes were determined by immunocytochemistry and Western blotting using monoclonal antibodies specific for fast and slow MYHC isoforms. Twelve strains formed myotubes that co-expressed fast and slow MYHC isoforms. Nine strains produced fast MYHC but failed to express detectable quantities of slow MYHC. Collectively, these data suggest that satellite cells from sheep skeletal muscle are likely present as a heterogeneous population.

Key words: Satellite cells, clones, growth, differentiation, myosin.

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Satellite cell nuclei appear to exist in three physiologic states. First, satellite cell nuclei are wedged between the muscle fiber and basal lamina. In this position, satellite cells are considered to be quiescent [27, 33]. Second, following activation, satellite cells may proliferate, differentiate and add nuclei to myofibers through cellular fusion [5]. Third, after satellite cells are incorporated into the myofiber, satellite cell-derived myonuclei likely join with other myonuclei in regulating the synthesis of muscle proteins [45]. Numerous studies suggest that satellite cells are able to respond to multiple signals characteristic of the physiological state of the myofiber [1, 20, 34]. However,

the extent to which satellite cells are homogeneous in their response to regulation prior to, or following fusion, remains speculative [7, 38].

Satellite cells proliferate differently in response to different types of myofiber growth. During postnatal growth of the myofiber, satellite cells proliferate and concomitantly donate nuclei to maintain the DNA unit within the enlarging myofiber [42, 6]. When repairing damaged muscle tissue, satellite cells proliferate rapidly, and fuse with existing myofibers or fuse together to form nascent myofibers [37, 8, 43]. The environment associated with each type of muscle growth may account for differences in

satellite cell proliferation. However, some studies suggest that satellite cells may intrinsically differ in their ability to proliferate. For example, on the basis of proliferation, two populations of satellite cells were identified in both rat soleus and extensor-digitorum longus muscles *in vivo* [35]. Within both muscles, a significant proportion (20-30 %) of satellite cells failed to divide during a 7 d period of rapid muscle growth. *In vitro* studies commonly demonstrated differences between other myogenic cells based on proliferation. During embryonic development, populations of myoblasts were present simultaneously in developing chick and human limb buds that differed in media requirements [14, 36]. Also, satellite cell clones isolated from chicken skeletal muscle can be classified on the basis of proliferative capacity *in vitro*. The majority (90%) of chicken satellite cells formed colonies with more than 16 cells. In contrast, 10% of the satellite cells failed to proliferate beyond 16 cells [32]. Collectively, these studies suggest that satellite cells are not equivalent in their ability to proliferate. However, it is not known whether these differences in satellite cell proliferation relate to specific physiological growth states in skeletal muscle such as maintenance of the DNA unit or regeneration.

Following fusion with myofibers, satellite cell-derived myonuclei apparently become positioned adjacent to other myofiber nuclei and begin to synthesize muscle-specific proteins [45]. It is well documented that the type and amount of protein produced by the myofiber can be modulated by numerous environmental factors [23, 30]. However, the synthesis of some muscle specific proteins may be regulated at a more dominant level such that the environment may no longer change their pattern of expression [40]. For example, satellite cells from avian skeletal muscle differ depending upon the muscle fiber type from which they are isolated [21, 39]. Satellite cells from a fast muscle fiber form myotubes *in vitro* that express only the fast isoform of myosin heavy chain (MYHC). In contrast, satellite cells from a slow muscle fiber form myotubes *in vitro* that express both fast and slow MYHC isoforms. Moreover, clonal studies suggest that individual nuclei express different isoforms of MYHC in a similar *in vitro* environment [12]. *In vivo* studies also demonstrate that satellite cells maintain an intrinsic level of regulation independent of the environment. When jaw-closing muscles from cats are transplanted to an ectopic muscle bed that differs in the pattern of innervation, myofibers regenerating from satellite cells continue to express an isoform of MYHC characteristic of jaw muscle [15]. In a similar experiment, transplantation of chicken breast muscle into the leg musculature resulted in the expression of troponin isoforms (following regeneration) characteristic of the breast muscle [44]. Finally, data suggest that satellite cell nuclei may carry positional differences based on the muscle from which they were isolated [21, 39]. Satellite cells from a more caudal position in the muscle, form myofibers *in vitro* that express higher levels of a reporter

gene relative to satellite cells collected from a more rostral position within the muscle [13].

We have previously demonstrated that primary cultures of sheep satellite cells formed myotubes that expressed fast and slow MYHC isoforms [26]. Limitations of primary culture prevented drawing firm conclusions concerning differences between satellite cells based on their expression of MYHC. In the current study, satellite cell strains formed from the progeny of cloned satellite cells were categorized on the isoform (fast or slow) of MYHC expressed following myotube formation. Also, satellite cell strains were used to determine how similar satellite cells were in their ability to proliferate and differentiate. The overall hypothesis tested in the current study is that satellite cells are present in sheep skeletal muscle as a heterogeneous population.

Materials and Methods

Cell culture

The semimembranosus and semitendinosus muscles were aseptically removed from a black face wether lamb weighing approximately 100 pounds and cells were isolated as previously described [11]. Costar® cultureware, coated with 0.5% (w/v) pig skin gelatin (PSG) was used for all studies [10]. Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% v/v fetal bovine serum, antibiotics (penicillin, streptomycin), and fungizone was used for cell population expansion. For differentiation studies, myotube formation was promoted by using DMEM supplemented with 2% v/v horse serum. Pig skin gelatin (swine-skin, type 1) was purchased from Sigma (St. Louis, MO). All other cell culture reagents were purchased from GIBCO (Grand Island, NY).

Clonal cultures were established by plating 62-125 mg of muscle mass equivalence (wet weight) onto 15 cm culture plates. In a previous study, it was estimated that an average of 5964 cells (per gram of tissue) were present in culture 12 h after isolation. Using this estimation, between 372 and 745 cells were plated on each 15 cm plate for clonal establishment. An individual cell was sequestered within a plastic cloning ring as described [22]. Cloning rings (1 cm diameter) were used at the single cell stage to prevent the mixing of detached cells between clonally-derived colonies. By using 1 cm diameter cloning rings, growth of colonies were similar to colony growth in the absence of a cloning ring (unpublished observation). Approximately 5 clones were established on each 15 cm culture plate. Cell proliferation within cloning rings was monitored by cell count determination on a daily basis until colonies reached 100 cells. When colonies were composed of 300-400 hundred cells, colonies were liberated using 0.025% trypsin/1 mM ethylenediamine tetraacetic acid (EDTA). Cells were then subpassaged onto three wells (60 mm) of a six-well plate. One well contained three circular glass coverslips (12 mm diameter) coated with 0.5% pig skin gelatin. Following the formation of myotubes, coverslips were processed for immunocytochemistry as pre-

viously described [23], using monoclonal antibodies F-59 and S-58 (kindly provided by Dr. Frank Stockdale, Stanford University) which were specific for fast and slow MHC isoforms, respectively [24, 25]. Prior to confluency, cells on the remaining two wells were liberated using 0.025% trypsin/1 mM EDTA, filtered through 40 μ m Nytex, pooled, and frozen into one cryovial with approximately 6×10^5 cells/cryovial. Freezing media consisted of DMEM, supplemented with 20% horse serum and 10% dimethyl sulfoxide [9].

Satellite cell proliferation

Six cell strains (5 myogenic and 1 non-myogenic) representing the culture passage number three (PN3) were thawed, counted using a hemacytometer, and plated onto two 10 cm plates at 10^5 cells/plate. Four days after plating, cells were liberated with 0.025% trypsin/1 mM EDTA, filtered through 40 μ m Nytex, counted, and plated onto 24-well plates for proliferation studies (see below). Cells were also plated onto 2-10 cm plates (10^5 cells/plate), allowed to proliferate for 4 d, liberated, counted, and used for proliferation studies in passage number six. For both experiments using cultures representing passage numbers 4 and 6, proliferation studies were conducted as follows: each strain was plated at high density (50 cells/mm²) or low density (5 cells/mm²), onto PSG-coated 16 mm diameter culture wells (four wells/strain/density/time). Following 12, 48, 72, 96, and 120 h, cultures were fixed in absolute methanol and stained with 0.025% giemsa. Total nuclei/mm² were determined by counting stained cells (ten random areas per well) [10].

Satellite cell differentiation

Three of the strains used in the proliferation study were tested for their ability to differentiate, fuse and form myotubes. Each strain representing PN3 was thawed, counted, and plated onto two 10 cm plates at 10^5 cells/plate. Proliferation and subpassaging of each strain was repeated exactly as previously described in the proliferation studies. For subpassages 4 and 6, each clone was plated at three different densities (50 cells/mm², 100 cells/mm² and 200 cells/mm²) onto eight 16 mm wells/density. Four wells representing each clone were fixed in methanol and stained with giemsa at 72 h and 144 h after the addition of fusion medium. Fusion medium (DMEM + 2% horse serum) was added 48 h after initial plating of cells. Classification of cells as myotubes required at least three nuclei within a discernible continuous cell membrane [10].

Myosin collection and Western blotting

Myosin was collected from satellite cell-derived myotubes (10 d post fusion) and Western blotted, as previously described [26]. Monoclonal antibodies F-59 and S-58 were used to differentiate between fast and slow MYHC isoforms. Reagents used to detect antibody binding were purchased from Vector Laboratories (Burlingame, CA).

Results

Colony Growth and Development

Satellite cells are activated to proliferate after being liberated from skeletal muscle and subsequently cultured [2]. To determine if satellite cells from the semimembranosus and semitendinosus muscles of sheep were similar in their ability to proliferate *in vitro*, the number of days required for clonally-derived satellite cells to proliferate to 100 cells was determined by microscopic counting (table 1). Frequency distributions for days required to proliferate to 100 cells and proportion of myogenic cells were nearly identical ($\chi^2 = 0.568$ $P = 0.966$) for clones originating from the semimembranosus ($n = 28$) and semitendinosus ($n = 24$) muscles. Four satellite cell clones required ≥ 16 d to proliferate to a colony size of 100 cells. In contrast, 16 clones proliferated to 100 cells in ≤ 10 d. Also, five clones failed to proliferate to 100 cells, of these clones, 3 remained at the single cell stage.

In addition to proliferation, the myogenic potential of progeny from each clone was determined to estimate the number of satellite cells within the original muscle isolates. Clones were classified as satellite cells only if progeny formed discrete myotubes. Eighty-seven percent of the clones that grew to 100 cells were satellite cells.

Proliferation of satellite cell clones

In vivo, satellite cells must respond to various physiological growth states. If satellite cells are equivalent in their capacity to respond to different microenvironments, then growth *in vitro* should result in similar proliferation rates between satellite cells. Within the current *in vitro* environment, satellite cell strains were highly divergent in proliferation potential (table 2). The relative ranking of satellite cell strains ($N_3 > O_1 > J_6 > I_1 > I_2$) were the same for subpassages four and six. However, a large decrease in

Table 1. Twenty-eight cell clones from the semimembranosus (S.M) and 24 cell clones from the semitendinosus (S.T) were characterized on the number of days required for each clone to proliferate to 100 cells and the percentage of the clones that were myogenic (i.e. satellite cells). Forty-seven of the 52 clones proliferated to 100 cells with a mean of 12.6 and a standard deviation of 3.0. Seventy-eight percent of all clones isolated were satellite cells and 87% of the clones that proliferated to 100 cells were satellite cells.

days to 100 cells	# of clones		# of satellite cells	
	S.M.	S.T.	S.M.	S.T.
8-10	9	8	8	8
11-13	8	6	8	5
14-16	5	5	4	5
≥ 17	3	3	1	2
Failed	3	2	-	-

Sheep satellite cell clones

Table 2. The proliferation and differentiation potential (% fusion) of satellite cell strains and one non-myogenic strain 10A were determined in separate experiments for passage numbers four (PN4) and six. For proliferation studies, cells were plated at 50 cells/mm² (H) and 5 cells/mm² (L) and enumerated at 120 h. The percentage of fusion was determined at 120 h after the addition of fusion media. Data are presented as the mean standard error of four 16 mm wells/clone/passages/density and 10 random observations/well.

Clone	PROLIFERATION				% FUSION	
	PN4 (H)	PN4 (L)	PN6 (H)	PN6 (L)	PN4	PN6
I ₂	95 ± 1	17 ± 4	83 ± 7	8 ± 1	---	---
J ₆	249 ± 9	62 ± 3	119 ± 4	15 ± 3	---	---
N ₃	511 ± 18	137 ± 4	252 ± 22	55 ± 9	3 ± 1	2 ± .4
I ₁	203 ± 5	55 ± 11	99 ± 12	10 ± 13	---	---
O ₁	335 ± 28	112 ± 13	168 ± 18	21 ± 4	35 ± 2	19 ± 2
10A	290 ± 23	47 ± 7	348 ± 12	53 ± 10	0	0

proliferation for 3 of the 5 satellite cell strains occurred from passage number four to six. This decrease could not be accounted for by different initial plating densities. Finally, plating density (5 cells/mm² or 50 cells/mm²) did not affect proliferation between satellite cell strains.

Differentiation of satellite cell clones

Immunocytochemistry studies conducted during the second subpassage suggested that colonies derived from strains O₁ and N₃ formed myotubes that expressed MYHC. Moreover, it was apparent from visual observation that the extent of myotube formation differed between strains O₁ and N₃. This prompted the following study to evaluate the ability of each strain to differentiate following several subpassages in vitro. Since satellite cell strains O₁ and N₃ differed in their rate of proliferation, cells were initially plated at three densities (50 cells/mm², 100 cells/mm² and 200 cells/mm²) to compensate for the possible affects of cell density on the fusion process [19]. Cell fusion data for satellite cell strains O₁, N₃ and one non-myogenic clone 10 A (for subpassage numbers 4 and 6) are provided in table 2. The percentage of fusion was eleven-fold higher for cell strain O₁ relative to cell strain N₃. Figure 1 further demonstrates the discrepancy in myotube formation between cell strains O₁ and N₃. Cell density and time in culture had no affect on the percentage of fusion for cell strain N₃. In contrast, both density and time in culture affected the fusion process in strain O₁ (table 3). Time allowed for myotube formation (72 vs 144 h) affected the fusion of strain O₁ more than cell density. However, increasing the cell density also seemed to affect the potential of strain O₁ to form myotubes.

Synthesis of myosin heavy chain isoforms

In a previous study [26], it was demonstrated that myotubes derived from sheep satellite cells expressed fast and slow MYHC isoforms in primary culture. In the current study, monoclonal antibodies F-59 and S-58 were used to determine if myotubes derived from satellite cell

strains co-expressed fast and slow MYHC isoforms, or if strains formed myotubes that expressed only fast, or only slow MYHC. Using immunocytochemistry, 12 satellite cell strains formed myotubes that co-expressed fast and slow MYHC, and 9 satellite cell strains expressed only the fast isoform. Western blots (figure 2) confirm the ability of myotubes derived from satellite cell strain O₁ to co-express fast and slow MYHC isoforms. However, if MYHC collected from strain O₁ (34 % fusion) is loaded at a similar concentration to N₃ (4% fusion), no binding of S-58 is detected in myosin extracted from strain O₁ myotubes. Finally, in relation to the biotinylated molecular weight standard (lane 1), the slow MYHC isoform (produced by sheep satellite cell-derived myotubes) seemed to migrate faster relative to the fast MYHC isoform.

Primary cultures of satellite cells expressed a protein that co-migrated with MYHC following SDS-PAGE but was not detectable with McAbs F-59 or S-58 [26]. Moreover, this protein was shown to be produced by mononucleated cells and considered likely to be a non-sarcomeric isoform of MYHC. Using satellite cell strains, the origin of this unknown protein could be better defined in the absence of other contaminating cell types. The Coomassie stained gel (CM) in figure 2 shows that satellite cell strains also produce a similar protein (top band of the doublet) that co-migrates with MYHC, but is unreactive with antibodies F-59 and S-58. This unidentified protein migrated at the same molecular weight as the protein extracted from the non-myogenic strain 10 A, which was also not detected using current antibodies.

Discussion

To accommodate the various physiological requirements of adult skeletal muscle, satellite cells, or nuclei derived from satellite cells, must be flexible in their ability to participate in myofiber growth [16, 30]. However, the extent that satellite cells are equivalent in their ability to respond to different physiological demands of the

Sheep satellite cell clones

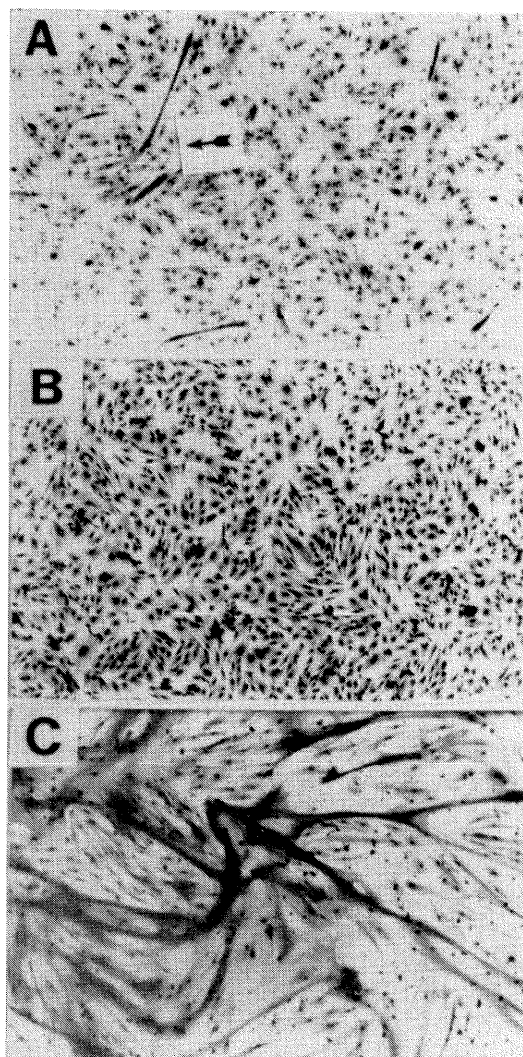


Figure 1. Photomicrographs (100 x) of cell strains N_3 (A), 10A (B) and O_1 (C). Each strain was plated at 50 cells/mm² and fusion media was added 48 h after initiation of the cultures. Cells were fixed with methanol and stained with Giemsa at 144 h. Arrow in (A) denotes area of myotube formation.

myofiber is not known. The current studies use clonally-derived strains of sheep satellite cells to determine how homogeneous these cells are in their ability to proliferate, differentiate, and express MYHC isoforms following myotube formation *in vitro*.

Initial characterization of cell clones ($n = 52$) was based on the time required for a single cell (i.e. clone) to proliferate and form a colony composed of 100 cells. Using this criterion, three cell types were identified: 1) cells that failed to proliferate (6%); 2) cells that did not proliferate to 100 cells (4%) and 3) cells that did proliferate to 100 cells (90%). These results are similar to others [32], and suggest that the majority of satellite cells in sheep skeletal muscle reside within the stem cell compartment and are not re-

Table 3. Effect of density (Nuclei/mm²) and time in culture after the addition of fusion media (72 vs 144 h) on the differentiation of clone O_1 . Initial plating densities were 50, 100 and 200 cells/mm². Cultures were switched to fusion media 48 h after plating, fixed, and stained at the times indicated in the text. Data are presented as the mean $\pm$ standard error of four 16 mm wells/density/time and 10 random observations/well.

	72 h		144 h	
Density	Nuclei/mm ²	% Fusion	Nuclei/mm ²	% Fusion
50	94 $\pm$ 7	11 $\pm$ 1	117 $\pm$ 9	36 $\pm$ 3
100	147 $\pm$ 6	12 $\pm$ 3	153 $\pm$ 16	35 $\pm$ 2
200	249 $\pm$ 23	25 $\pm$ 1	175 $\pm$ 11	19 $\pm$ 5

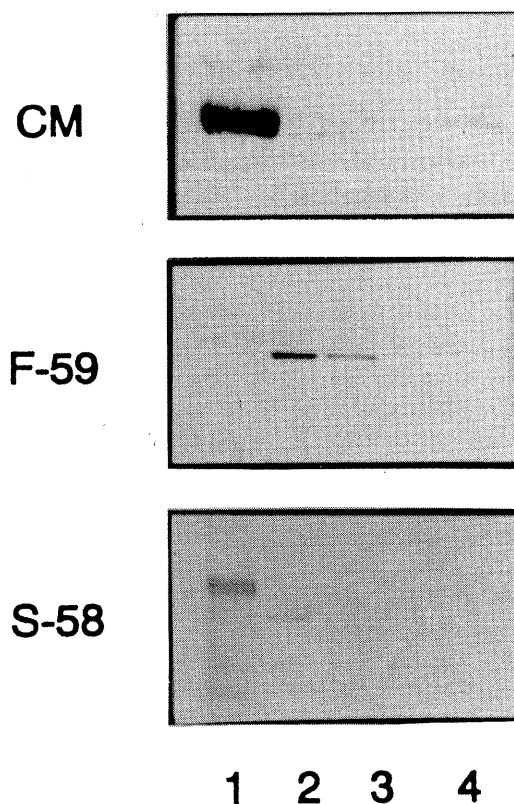


Figure 2. Coomassie stained gel (CM) and immunoblots using monoclonal antibodies F-59 and S-58 specific for fast and slow MYHC isoforms, respectively. Lane 1 contains a MYHC molecular weight standard. Lanes 2-4 are myosin extracted from cell strains O_1 , N_3 , and 10A, respectively.

stricted in the number of cell divisions prior to differentiation [31].

Based on the number of days required to obtain a colony size of 100, satellite cells ($n=43$) were normally distributed ($\bar{x} = 12.6$ $sd = 3.0$) and can be categorized as belonging to a single population. However, within this population there was divergency: 4 satellite cell clones required ≥ 16 d to obtain 100 cells, in contrast, 15 satellite cell clones proliferated to 100 cells in ≤ 10 d. Since these satellite cell clones attached, proliferated, and formed myotubes, it was unlikely that differences in proliferation to 100 cells could be accounted for by damage incurred during the isolation procedure. However, it was possible that a satellite cell could co-localize with a small (less than 40 μm) section of tissue, and factors derived from the tissue might have increased and/or decreased the proliferative status of the nearby satellite cell [3].

Several other explanations are possible which may explain the differences in satellite cell colony growth. First, some satellite cell clones could have required 5-7 d longer to initiate proliferation. Second, some clones could have longer cell cycle times (most likely remain in G1 longer). Finally, following cell division, some daughter nuclei could have differentiated and no longer remained mitotically active. Clones derived from human skeletal muscle showed similar heterogeneity in their initial growth patterns [4]. This heterogeneity in growth was shown to result from differences in the time required for clones to initiate cell proliferation. Once proliferation was initiated, growth kinetics between clones were similar [4].

This study was not designed to elucidate the mechanism(s) responsible for the initial differences in colony growth. However, differences in proliferation between satellite cell strains in mass culture, cannot entirely be explained by differences in the initiation of proliferation. For example, some satellite cell strains had similar numbers of cells at 48 and 72 h, but differed at 96 and 120 h (data not shown). These data could indicate that some satellite cell strains produce progeny that withdraw from the cell cycle (possibly in preparation for differentiation) sooner than progeny of other cell strains. Since differentiation and proliferation are thought to be mutually exclusive events [28], it is not surprising that the satellite cell strain with the highest rate of proliferation had a low level of differentiation. Finally, each satellite cell strain maintained its relative ranking (based on proliferation) from the fourth to the sixth subpassage. Since different culture plates and media preparations were used between subpassages, these observations could be interpreted to suggest that differences in proliferation were intrinsic to the satellite cell strain in question.

Three of the five satellite cell strains had a two-fold reduction in proliferation from PN4 to PN6. Since the non-myogenic clone used in the study increased in proliferation from PN4 to PN6, and one satellite cell strain remained unchanged, it was unlikely that differences in environmental conditions (media, culture plates, etc.) be-

tween subpassages accounted for this decline in proliferation. The decline in proliferation from PN4 to PN6 has two important implications. First, *in vitro* studies using satellite cells must account for passage number or cell age when interpreting results or designing experiments. Second, this decrease in proliferation provides evidence that these satellite cell strains are not transformed.

Differentiation of specific cell strains was evaluated during culture passage number 4 and 6. Differentiation (% fusion) of satellite cell strain O_1 was 11.6 and 9.5 fold greater than satellite cell strain N_3 for PN4 and PN6, respectively. Differences in myotube formation between these two satellite cell strains (although not quantified) were also detected using immunocytochemistry during the second subpassage. Increased cell density, depletion of nutrients, and decreased proliferation are three culture conditions thought to promote differentiation of myogenic cells [19]. Since satellite cell strain N_3 proliferated faster than O_1 , the increased cell density and depletion of the nutrients within the media, should favor the differentiation of strain N_3 compared to strain O_1 . Further, strain N_3 proliferated slower at PN6 compared to strain O_1 at PN4. It is interesting that the slower rate of proliferation of strain N_3 during PN6 does not change its level of differentiation. Using defined media, cell strains O_1 and N_3 remain divergent in differentiation, but the level of differentiation is increased to 80% and 30%, respectively (manuscript in preparation). Collectively, these data suggest that satellite cell strains N_3 and O_1 intrinsically differ in their capacity to differentiate and form myotubes.

Twenty-one satellite cell strains were screened for their expression of MYHC following myotube formation during PN2. Of the 21 satellite cell strains screened, 12 co-expressed fast and slow MYHC and 9 expressed only the fast isoform. Further, it seemed that all myotubes reacted with McAb F-59. In contrast, few myotubes reacted with McAb S-58. These results suggest that co-expression of fast and slow MYHC by myotubes could account for the fast and slow MYHC isoforms detected in primary culture [26].

The limited amount of slow MYHC produced in myotubes derived from certain satellite cell strains indicated that only a small subset of nuclei were expressing the slow MYHC isoform. This subset of nuclei that express the slow isoform of MYHC could represent the population of satellite cell nuclei that differentiate first. Although a time course experiment was not performed in the current study, a time course experiment was done in primary culture, and no increase in the expression of slow MYHC could be detected as a result of culture age or culture subpassage [26]. Another possible explanation to account for the small number of nuclei that expressed slow MYHC was that a small population of nuclei could be endowed with a "leaky" promoter such that a limited amount of slow MYHC is produced. For example, the expression of myosin light chain [18] and the commitment of mesenchymal cells to a myogenic fate [17] are associated with

decreased methylation in front of specific genes. Since the pattern of methylation can be changed in some daughter nuclei following cell division [29], some satellite cell nuclei could begin to produce the slow MYHC isoform due to hypomethylation of the promoter region in front of the slow MYHC gene. Finally, a recent study demonstrated that clonally-derived satellite cells were able to take on the MYHC expression pattern of the myofiber to which they fused [16]. These results suggested that the satellite cell nucleus was regulated by the myofiber environment to express specific isoforms of MYHC [41]. In the current study, the *in vitro* environment may lack the required factors for efficient production of the slow MYHC protein.

In conclusion, data from the current study suggest that satellite cell strains isolated from sheep skeletal muscle are heterogeneous in their proliferation and differentiation dynamics. Intrinsic divergency between satellite cell strains should provide a suitable model system to study the mechanism(s) responsible for this heterogeneity. Finally, using the current methodology it seems that sheep satellite cells form myotubes that express the fast MYHC isoform and are very limited in their capacity to express the slow MYHC isoform.

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