

Recent Advances That Impact Skeletal Muscle Growth and Development Research¹

J. M. Reecy², S. A. Miller, and M. Webster

Department of Animal Science, Iowa State University, Ames 50011

ABSTRACT: Numerous technological advances and scientific insights have had profound effects on our understanding of skeletal muscle growth and development. The objective of this review is to highlight a new technology and recent findings on the functional responses of skeletal muscle/satellite cells to physiological stimuli. First, recent technological advances have facilitated global gene expression profiling experiments. This type of research has, for the first time, provided researchers with insights into cell/tissue-wide response to a given treatment. These experiments have dramatically increased our understanding of the extent to which cells/tissues respond. Furthermore, these experiments have implicated previously underappreciated genes as playing potentially vital roles in biological events. Secondly, recent advances have suggested that the cell culture model utilized can greatly influence the

results and conclusions obtained from an experiment. Under standard culture conditions, satellite cells obtained from aged rats are capable of only a few rounds of replication before becoming senescent. Under conditions of reduced oxygen content, the number of rounds of replication is greatly increased. These results demonstrate that experiments using traditionally accepted in vitro culture conditions might be flawed. Finally, recent studies have identified a population of pluripotent stem cells in skeletal muscles termed side population cells. These cells possess the ability to efflux Hoechst dye, which distinguishes them from all other cells that cannot efflux the dye. These cells are capable of differentiating into many other tissue types in vitro and in vivo. With these new technologies and insights, our portrait of skeletal muscle growth and development continues to evolve.

Key Words: Cells, Cell Cultures, Differentiation, Gene Expression, Skeletal Muscle

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Introduction

The growth and development of skeletal muscle has long been of interest to animal scientists. Not only will a better understanding of this process lead to improved strategies to increase the efficiency of lean tissue deposition in domestic animals, but it also has human health implications. Thus, there is an ever-growing need to define the molecular mechanisms controlling embryonic and postnatal skeletal muscle growth and development.

Throughout the history of skeletal muscle growth and development research, there have been a number of landmark discoveries. The ability to grow myoblast/myotubes in cell culture (Rinaldini, 1959), the discovery

of the satellite cell (Mauro, 1961), and the discovery of myoD (Lassar et al., 1986) to name a few. Our intent here is to discuss one technology and two research advances that we believe will have dramatic effects on how skeletal muscle growth and development research is conducted. First, we will discuss several microarray-based gene expression profiling experiments that have been conducted with skeletal muscle. Traditionally, researchers have taken a reductionist approach (i.e., one gene/protein at a time) to studying skeletal muscle growth in vivo and in vitro. However, with the advent of high-throughput technologies, such as complementary DNA (cDNA) microarrays (Schena et al., 1995), more systems-based biological studies are becoming increasingly possible (Kitano, 2002). Second, we will discuss the affects that atmosphere may have on in vitro experiments. Cell culture has long been used as a technique to study skeletal muscle growth and development in vivo. Recent research suggests that atmospheric conditions can dramatically affect cell culture results. Finally, we will discuss some recent findings with skeletal muscle-derived stem cells. Recent studies have suggested that not only satellite cells, but also stem cells,

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²Correspondence: 2255 Kildee (phone: 515-294-9269; fax: 515-294-2401; E-mail: jreecy@iastate.edu.

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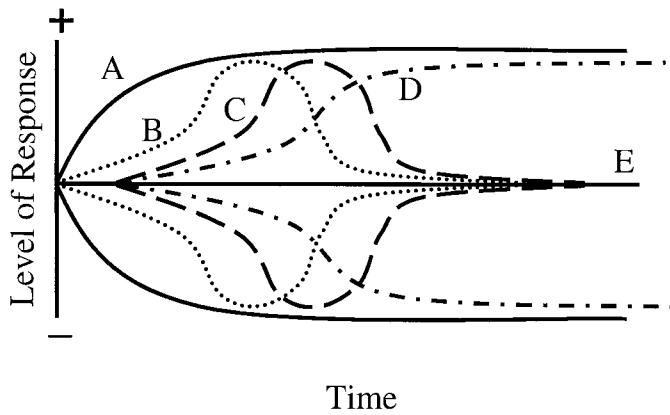


Figure 1. Potential changes in messenger RNA (mRNA) levels in response to a stimulus. A) The mRNA level may rise and remain elevated. B) The mRNA level may rise then return back to baseline levels (i.e., immediate response gene). C) The mRNA level may rise in a delayed fashion and then return to a baseline level of expression. D) The mRNA level may rise in a delayed manner and remain elevated. E) Alternatively, the mRNA level may not change. The mirror image of each of these response lines is also possible.

reside in skeletal muscle (Jackson et al., 1999; McKinney-Freeman et al., 2002).

Microarray Gene Expression Analysis

To further our understanding of skeletal muscle growth and development, we need to identify genes that play critical roles in the different stages of skeletal muscle growth and development. Pinpointing these genes has traditionally been done on a gene-by-gene basis (i.e., a single gene is identified as suggestive and studied independently). This systematic evaluation has several benefits: 1) experiments can be designed so that each gene can be thoroughly investigated, and 2) the idea that a single gene(s) controls an important trait is compelling. Furthermore, if a trait like muscle hypertrophy is regulated by a small subset of genes, it should be relatively easy to design strategies to manipulate these genes for medical and commercial use.

Until recently, scientists were constrained by a lack of technology allowing analysis of global changes in messenger RNA (mRNA) levels. Today, advances in gene transcript profiling techniques, such as microarray and similar technologies, allow for genome-wide or tissue-specific examination of global changes in gene transcription in response to an experimental stimulus (Figure 1). The promise of these new technologies lies in their potential to tie specific changes in gene expression to a phenotype of interest. It is beyond the scope of this review to discuss issues related to the way microarray experiments are conducted, or to discuss the different platforms utilized. For those discussions, we would direct you to a number of recent articles (Strausb-

erg and Austin, 1999; Hedge et al., 2000; Brazma et al., 2001). For those readers interested in the fundamental differences in different gene expression profiling arrays, such as macroarray vs microarray and cDNA spotted vs oligonucleotide array, we would direct you to Freeman et al. (2000). Furthermore, it is beyond the scope of this discussion to evaluate different approaches to the statistical analysis of microarray experiments (see Wolfinger et al., 2001; Kerr and Churchill, 2001a,b). However, by no means are these lists complete, as this field is quickly evolving. It is also important to note that microarrays are not the only high-throughput gene expression profiling system. Other systems, such as serial analysis of gene expression (Velculescu et al., 1995), subtractive hybridization, CuraGen GeneCalling (New Haven, CT), or Lynx Therapeutics Megasort (Hayward, CA) are also used. However, to date, microarray technology has been utilized to the greatest extent.

In this section, we will review the results of several studies that have utilized microarrays to gain a greater understanding of the changes in gene expression that accompany 1) aging of skeletal muscle, 2) muscle fiber type differences, and 3) physical activity level. We will finish this section with a discussion of the availability of microarrays for livestock species and the need for complementary technologies, such as proteomics.

Effect of Age on Skeletal Muscle Gene Expression

Although the changes in gene expression during aging do not directly impact animal agriculture, it is an important area of research. In humans, identifying genes that are involved in the diminished muscle mass and impaired muscle function seen during aging is critical to combating these problems. To analyze important questions about the effects of aging on skeletal muscle, several microarray studies have been conducted.

Jozsi et al. (2000) used Atlas human macroarrays from Clontech (Palo Alto, CA) to examine the changes in gene expression in the vastus lateralis that accompanies aging. Their results suggest that skeletal muscle in older men had a similar baseline stress-response expression profile to exercised muscle from young men. This study indicated that genes differentially expressed in older muscle had an attenuated response to resistance exercise in senior men. Not only is aged skeletal muscle stressed at basal activity levels in comparison to young skeletal muscle, but it also cannot respond to the extent to which younger muscle responds to increased physical activity.

Previously, several studies have demonstrated that reduced caloric intake could increase life expectancy in rats and mice (Weindruch and Walford, 1988; Fishbein, 1991). Microarray analysis has been conducted with Affymetrix (Santa Clara, CA) GeneChip technology. See Lockhart et al. (1996) for a description of Affymetrix-based gene expression analysis on the impact of caloric restriction on aging in the skeletal muscle of the mouse (reviewed in Weindruch et al., 2001). A compari-

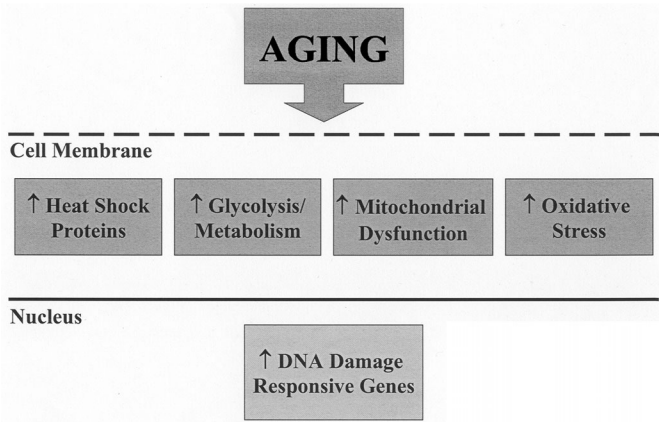


Figure 2. Changes in gene expression in skeletal muscle associated with aging. Differentially expressed genes were involved in DNA damage, metabolism, and stress response. Each system is shown relative to its location within the cell.

son of gastrocnemius muscle from 5- (adult) and 30-month-old (old) mice indicated an increased stress response (i.e., increased heat shock protein, DNA damage, inducible and oxidative stress, inducible gene expression, decreased energy metabolism (i.e., reduced glycolysis), increased neural damage and repair, and increased mitochondrial dysfunction in aged skeletal muscle (Figure 2). In contrast, caloric restriction increased protein metabolism (i.e., increased protein synthesis and degradation), energy metabolism (i.e., increased glycolysis, gluconeogenesis and pentose phosphate shunt), and biosynthesis of fatty acids and nucleotide precursors, and decreased macromolecular damage (i.e., decreased heat shock factors, detoxification systems, and DNA repair systems). Caloric restriction appears to completely reverse or at least alleviate many of the changes in gene expression observed in aged skeletal muscle. It is important to note that tissues capable of cellular proliferation do not respond identically to postmitotic skeletal muscle (Weindruch et al., 2001).

As it is with many diseases, the mouse is a commonly used model for aging in humans. Recent work by Welle et al. (2001) compared Affymetrix oligonucleotide microarray analysis results of aging in human skeletal muscle to that in mice. In that study, they examined the changes in gene expression between eight healthy young men (mean: 23-yr-old) and eight healthy old men (mean: 71-yr-old) to the changes in gene expression reported by Lee et al. (1999) between 5- and 30-month-old mice. The results of this comparison demonstrated that the effects of aging were often not the same between the two species. This finding underscores the need for caution in the use of the mouse as a model for other species. However, it is difficult to document that the physiological age of the tissues examined in humans and mice were identical. Thus, the observed differences

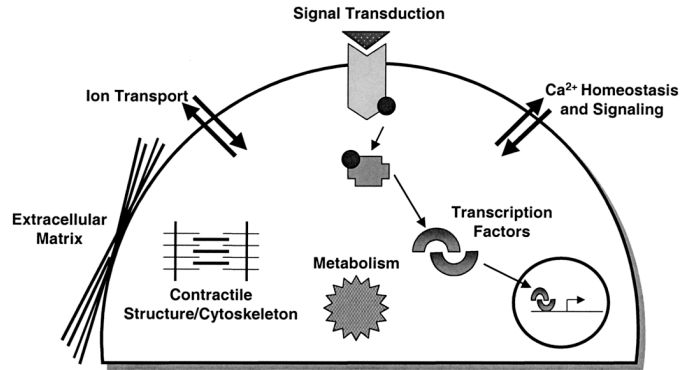


Figure 3. The changes in gene expression in the red soleus vs white quadriceps muscles. Differentially expressed genes were involved in Ca^{2+} homeostasis and signaling, contraction and cytoskeletal structure, extracellular matrix, ion transport, metabolism, signal transduction and transcriptional regulation. Each system is shown relative to its location within the cell.

could be due to physiological age differences between species.

Effect of Red vs White Skeletal Muscle on Gene Expression

A gene expression profiling experiment that has implications on animal production is a study conducted by Campbell et al. (2001). They investigated the changes in gene expression between the white quadriceps muscle, which is comprised of 100% type-IIb muscle fibers, and the red soleus, which is comprised of 70% type-I and 30% type-IIa muscle fibers in female ICR mice. Using Affymetrix GeneChips, they identified 49 differentially expressed genes, 27 of which were expressed at a higher level in the quadriceps muscle, and 22 genes of which were higher in the soleus muscle. In general, these genes could be subclassified into the following categories: 1) Ca^{2+} homeostasis and signaling, 2) contractile structure/cytoskeletal, 3) metabolism, 4) extracellular matrix, 5) ion transport, 6) signal transduction, 7) transcription factor/coregulator, and 8) miscellaneous (Figure 3). As expected, the group with the greatest number of genes, whose expression differed, was that of energy metabolism. The next largest group was that of the transcription factors and coregulators, which suggests that regulation of fiber type at the transcriptional level may be greater than previously appreciated. Furthermore, these candidate genes offer new inroads necessary to define the transcriptional controls underlying the differences between red and white skeletal muscle.

In terms of animal agriculture, we know that muscle that is composed of predominately white/glycolytic/type IIb myofibers grows at a faster rate than muscle composed of red/oxidative/type I myofibers (Swatland, 1994). Alternatively, we know that the meat-processing characteristics of glycolytic myofibers are inferior to

that of oxidative myofibers (Xiong, 1999). Any strategy devised to either increase skeletal muscle growth or improve meat-processing characteristics by changing skeletal muscle fiber-type composition would need to bring about reciprocal changes in gene expression. It is distinctly possible that information provided by this experiment has supplied us with a starting point to begin to break the antagonism between increased skeletal muscle growth rate and decreased meat quality. If we could identify strategies to increase oxidative myofiber growth rate without converting it to a glycolytic myofiber, we could truly benefit the meat industry by dramatically improving meat quality.

Effect of Increased Physical Activity on Gene Expression

The structural adaptation of skeletal muscle to work overload is well characterized (Goldberg, 1967). However, a comprehensive understanding of the molecular events underlying skeletal muscle hypertrophy remains elusive. To investigate the global changes in gene expression induced by work overload, microarray analysis of gene expression was performed on overloaded rat skeletal muscle. Rats were randomly assigned to work overload, which was induced in the soleus muscle by gastrocnemius ablation, or to a control group, which received a sham operation. The soleus was collected after 3 d of work overload from each rat and total RNA was isolated and analyzed (Carson et al., 2002).

In the past, many microarray experiments have not been statistically analyzed. This needs to change and progress is being made. We statistically analyzed our microarray data in two different ways. First, we performed an ANOVA test coupled with the bootstrapping of residuals to determine a *P*-value for each gene. This analysis provided us with a list of 19 genes with a type I error rate of 5%. Due to the cost of conducting the experiment and the small number of animals utilized in this study, we wanted to be as comprehensive as possible in identifying differentially expressed genes, so we also performed a false discovery rate analysis. This returned 125 genes, of which 5% were false positives (Carson et al., 2002).

We hoped to identify potential pathways and specific genes that may play important roles in skeletal muscle hypertrophy. As with any broad-based analysis, microarray data is most useful as a tool to locate candidates for detailed investigation. Work overload altered the mRNA levels of metabolism and intracellular genes and increased the expression of transcription factors, extracellular matrix, and immune response genes (Carson et al., 2002). In addition, the expression level of genes involved in cell-cycle regulation and protein metabolism increased (Figure 4).

One of the advantages of global gene expression analysis is the identification of potential pathways that were not previously implicated. The practical value of global analysis lies in its ability to gather individual, differen-

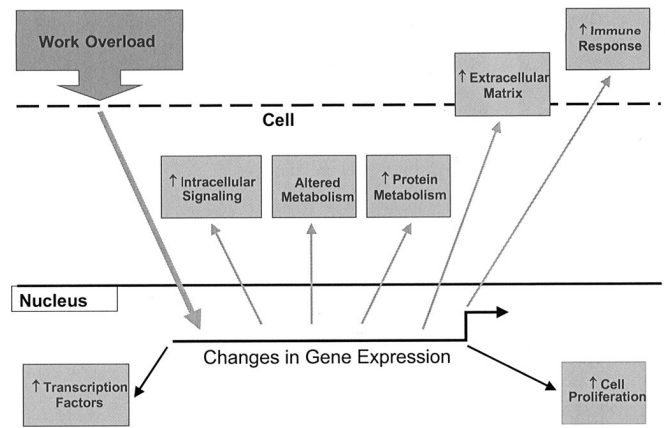


Figure 4. The changes in gene expression in the rat soleus in response to 3 d of work overload can be parsed into different cellular responses. Physical activity is converted into a chemical signal, which results in changes in gene expression. These genes, whose expression level changed, play important roles in the physiological response of skeletal muscle, such as altered metabolism and increased cellular proliferation.

tial expression changes into an overall portrait of a biological event. This overview can identify interactions that previously may have been overlooked. However, such an overview is not possible with traditional molecular techniques. The number of transmembrane and intracellular signaling genes that were differentially expressed as a result of work overload intrigued us—particularly the number of genes whose expression level increased, which can increase Janus kinase/signal transducers and activator of transcription pathway signaling activity (Figure 5) (Carson et al., 2002). Alternatively, other labs may be more interested in the number of immune related genes whose expression levels change in response to work overload.

An important point to consider is that although the microarray analysis was conducted with skeletal muscle, this tissue contains a variety of cell types. Muscle cells, such as myofibers and satellite cells, are highly

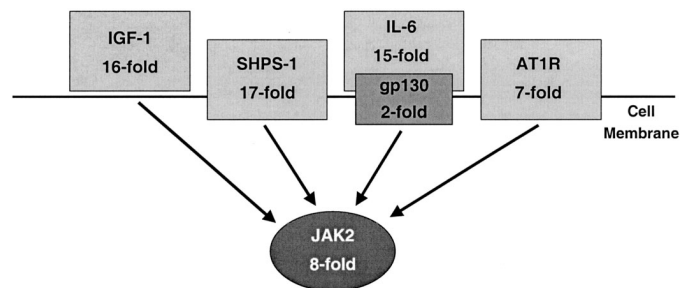


Figure 5. Some identified changes in gene expression in the rat soleus in response to 3 d of work overload. The arrows represent pathways that have been reported to occur within at least one cell type.

abundant, but other cell types, such as fibroblasts, nervous tissue, smooth muscle, and endothelial cells, are also present. Work overload is an insult to the muscle tissue and results in immune cell infiltration. Since all of these cell types contribute to muscle hypertrophy, it is impossible to determine precisely which cells account for specific mRNA changes without performing in situ gene expression analysis of each gene. This raises an interesting problem of systems-based research. Once an experiment is completed, future individual gene-based experiments are often required to better understand the molecular mechanisms involved.

In animal agriculture, we know that maximal skeletal muscle growth is repressed in growing animals. Several lines of evidence support this contention. First, the double-muscle phenotype is the result of the inactivation of myostatin (Grobet et al., 1997; Kambadur et al., 1997; McPherron and Lee, 1997). Thus, skeletal muscle growth is inhibited by functional myostatin. Second, work overload can stimulate an increased rate of skeletal muscle growth in a rapidly growing rat (Goldberg, 1967). These two phenotypes suggest there is a large untapped potential for increased skeletal muscle growth. However, reproduction problems will limit the introgression of the myostatin null allele into commercial cattle herds. In addition, myostatin-null alleles do not exist in other livestock species. Furthermore, producers are not going to exercise livestock to increase skeletal muscle growth. However, if we can determine the molecular mechanisms whereby physical activity and/or double muscling alleviate the repression of muscle growth, we should be able to develop strategies to increase postnatal skeletal muscle growth. Obtaining a global picture of gene expression in these phenotypes is an initial step in that direction.

Microarrays for Livestock Species

Information obtained from microarray data offers a unique opportunity to gain a broad sense of an organism's genetic response to stimulus. Microarray analysis could benefit behavioral, genetic, nutritional, physiological, and reproductive evaluation of livestock. Furthermore, microarrays can be used to evaluate the beneficial or detrimental effect of a treatment (i.e., toxicogenomics; Waring and Ulrich, 2000). In livestock species however, the only microarray that is commercially available is a small one for cattle (Band et al., 2002; <http://www.anigenicsinc.com>). The only microarrays for pigs and chickens are those used within individual labs. Today, we are primarily limited to using model organisms in an attempt to answer questions of economic importance to the livestock industry. However, this will rapidly change in the near future.

Although changes in gene expression can be indicative of changes in protein production, it is important not to assume that differential mRNA production will always result in corresponding protein levels and activity. In addition to microarray data, it would be very

informative to have proteomic information. Proteomics is simply defined as the global analysis of changes in protein expression (for reviews, see Honore, 2001; Lawrie et al., 2001; Martin and Nelson, 2001). Proteomics takes advantage of the ability to couple two-dimensional electrophoresis with high throughput technology to separate and identify proteins of interest. Factors such as mRNA stability and transcription, protein translation efficiency, and protein modifications all play a role in the ultimate impact a gene will have. Microarray and proteomic analyses should be viewed as tools to identify interesting areas that warrant further investigation. Although these are exciting molecular advancements, both must be used with caution and with the knowledge that these changes are only suggestive of the ultimate molecular events.

Low Oxygen

Traditional muscle-cell culturing methods have focused on understanding the mechanisms controlling myoblast proliferation and differentiation. However, the atmospheric conditions used during this in vitro period have been largely ignored. Recent experiments have suggested that maintaining cells under more physiological atmospheric conditions may have many benefits. Since the 1930s, cell culture experiments have used a combination of 5% CO₂ and 95% air (Parker, 1938) to help maintain media pH. Cells are exposed to atmospheric conditions that contain approximately 20% oxygen. In contrast, the level of oxygen found in vivo is a magnitude or more less than this. The partial saturation of oxygen found in mature skeletal muscle is reported to be between 1 to 10% (Greenbaum et al., 1997; Richardson et al., 1998). Recently, studies have been performed that have sought to address the effects of culturing cells in a more physiologic environment.

Morrison et al. (2000) and Studer et al. (2000) first reported that central nervous system and neural crest stem cells had increased rates of proliferation, reduced apoptosis, and increased dopaminergic neuron generation when cultured in lowered vs traditional oxygen conditions. Based on these results, Chakravarthy et al. (2001) examined the effect of decreased oxygen on the activation and proliferation of satellite cells from aged rats. Under standard cell culture conditions, satellite cells isolated from aged rats undergo only a couple of cycles of replication. Cells that were cultured under atmospheric conditions containing approximately 3% oxygen had a significant increase in proliferation rate and formed larger myotubes than control cells cultured in 20% oxygen. Proliferating myoblasts cultured in lowered oxygen had increased protein levels of G1/S cyclins and cyclin-dependent kinases, as well as decreased levels of the cell cycle inhibitor p27^{Kip1} (Chakravarthy et al., 2001). These results indicate that even mature satellite cells that are mitotically inactive under traditional culturing conditions can be stimulated to proliferate in the right environment.

Csete et al. (2001) found similar results using whole muscle fiber cultures. There was an increase in the chemotaxis and proliferation of satellite cells cultured in lowered oxygen, as well as increased survival of the fiber itself. It is also interesting that some of the satellite cells that were adherent to the fiber could take on an adipocyte phenotype. Furthermore, the percentage of adipose cells on fibers maintained at 20% oxygen was greater than that on fibers maintained at 6% oxygen. These results imply that there are pathways in the cell-regulating, cell-proliferation, and cell-fate determination that are responsive to the level of oxygen exposure.

The implications of this line of research are quite staggering. Generally, we would like to assume that the results from cell culture experiments could be extrapolated to an *in vivo* situation. In situations where cell culture results could not be recapitulated *in vivo*, it is distinctly possible that traditional atmospheric conditions may have caused these confounding results. It is not easy to culture cells in a lower oxygen environment because special equipment is needed to regulate and monitor oxygen levels. However, we need to seriously consider accepting these challenges as the new standard for cell culture if we are to obtain meaningful data. Acceptance of this new standard should improve the transfer of cell culture-obtained knowledge to an *in vivo* setting. It will still be an absolute requirement to perform *in vivo* experiments to validate cell culture findings as being biologically meaningful. This line of research is new enough that we do not yet know what truly constitutes physiological oxygen concentration vs hypoxia and hyperoxia. Furthermore, there is a question of whether optimal oxygen concentrations are organism specific (e.g., mammalian vs amphibian).

The results of Csete et al. (2001) question whether we can manipulate the differentiation of satellite/stem cells located within skeletal muscle to differentiate into either skeletal muscle or adipose. If we wanted to maximize the production of lean meat (i.e., low marbling score), it would be advantageous to maximize the percentage of cells differentiating into skeletal muscle. Conversely, if we wanted to produce a meat product with high levels of intramuscular fat, it would be beneficial to ensure that adequate numbers of cells were directed to differentiate into adipose cells. Would it be possible to do so in a programmable fashion? Could we maximize skeletal muscle production throughout most of the growing period and then only toward the end of the feeding period switch to adipose production or vice versa?

Skeletal Muscle-Derived Stem Cells

There have been several recent reviews that addressed the physiology (Hawke and Gary, 2001) and stem cell potential (Seale and Rudnicki, 2000; Seale et al., 2001) of muscle satellite cells. Considerable effort has been focused on the role of these cells in modulating skeletal muscle growth and repair. Satellite cells were

the first stem cell-like cells isolated from skeletal muscle, although further potential stem cell populations have since been described. The use of fluorescence-activated cell sorting (**FACS**) has revealed several of these putative stem cell populations, the most interesting of which is a recently discovered subpopulation of muscle cells termed side population (**SP**) cells.

Goodell et al. (1996) first characterized SP cells when they were trying to stain murine bone marrow cells with Hoechst 33324 dye. They used FACS and found that a small portion of the cells could exclude the Hoechst dye. Upon further analysis, these cells had many of the phenotypic markers for hematopoietic stem cells (**HSC**). Hematopoietic stem cells are multipotential and can take on either a myeloid or lymphoid phenotype. The cells contain multidrug resistance proteins that enable them to efflux the dye. The stem cell potential of this population was confirmed *in vivo* in mice that received a lethal dose of radiation. The bone marrow of these mice was destroyed by the radiation treatment, but the intravenous injection of a small portion of the SP cells was able to reconstitute the bone marrow, blood, and lymph systems. SP cells have since been isolated from human, porcine, and rhesus marrow (Goodell et al., 1996).

Skeletal muscle also has a small portion of these HSC-enriched SP cells (Gussoni et al., 1999; Jackson et al., 1999). Muscle-derived SP cells from male mice were able to reconstitute the marrow compartment of lethally irradiated female *mdx* mice (Gussoni et al., 1999), a Duchenne's muscular dystrophy model. They also found that 90% of the spleen cells were positive for Y chromosomes and were donor derived. The muscle of *mdx* mice is dystrophin negative, but after the addition of the wild-type male SP cells, there was a small proportion of donor-derived dystrophin-positive myofiber nuclei. The muscle-derived SP cells were not as effective as marrow SP cells at replacing the bone marrow of irradiated mice, however, since it took 10 times more muscle SP cells to achieve effective reconstitution. Donor-derived SP cells from muscle were found in the blood of irradiated mice up to 3 mo after transplantation (Jackson et al., 1999). These results indicate that satellite cells are not the only potential stem cells in mature skeletal muscle and, in fact, are more differentiated than SP cells. In support of this contention, McKinney-Freeman et al. (2002) reported that muscle-derived SP cells could be fractionated into hematopoietic (Sca-1 positive and CD45 positive) and myogenic cell populations (CD45 negative). A note of caution, however: these results question the notion of adult-derived stem cells. These two cell populations had differing abilities to differentiate into different cell types. The CD45 negative cells were myogenic *in vitro* and *in vivo*. In contrast, the CD45-positive hematopoietic cells were only weakly myogenic *in vivo* and not *in vitro*.

Blanton et al. (1998) described two distinct populations of primary pig myoblasts using FACS and culturing. Two days after isolation, there was a homogeneous

population of smaller myoblasts, as well as a population of larger diameter cells that consisted of myoblasts and fibroblasts. They found that at d 2, the predominant population was that of the smaller myoblasts, although since these cells were activated to proliferate, their size increased. None of the smaller myoblasts was visible after two passages; Barraffio et al. (1995) showed that this population of cells could be recovered from myoblast populations that were induced to differentiate. Following differentiation, there is a small portion of cells that fail to fuse with the myotubes, and these cells represent a potential long-term stem cell population.

Skeletal muscle contains not only satellite cells that can differentiate into skeletal muscle, but also a subset of cells that are capable of differentiating into other cell types. This leaves a number of open questions. Are stem cells and satellite cells the same, or are they two separate populations? If they are two separate populations, are satellite cells simply partially differentiated stem cells? How can we identify stem cells and satellite cells in skeletal muscle? How can we independently study stem cells and satellite cells *in vivo*? Regardless of these cell population questions, it appears that we need to change how we study satellite cells in cell culture. Traditional satellite cell studies are conducted by isolation of single nucleated cells from skeletal muscle and subsequent evaluation in cell culture. Thus, these cultures contain stem cells and satellite cells, as well as other cell types (i.e., fibroblasts). Traditionally, we have investigated the activation, proliferation, and differentiation of satellite cells in cell culture. If satellite cells are capable of only so many rounds of replication before reaching senescence, whereas stem cells are capable of multiple rounds of replication, would it not be beneficial to specifically identify strategies to enhance stem cell activation, proliferation, and subsequent differentiation into skeletal muscle? Similarly, we would also want these strategies to maximize satellite cell activation, proliferation, and differentiation. We should then be able to maximize the efficiency of livestock growth.

The use or activation of satellite cells and other potential stem cell populations will continue to be important in the future. The use of pharmacological or other methods to stimulate the activation of muscle stem cells is one potential mechanism to increase muscle mass. *Ex vivo* expansion of myoblast populations and subsequent transplantation is another possibility. These experiments would certainly be aided by the use of a more physiological environment, as described earlier during expansion.

Implications

Great strides have been made in our understanding of skeletal muscle growth and development. These advancements have come about because of technological innovations and scientific discovery. It is our belief that microarray analysis of gene expression, atmospheric

culture conditions, and skeletal muscle-derived stem cells are advances that will have profound influences on our understanding of skeletal muscle growth and development. However, future advances will be necessary if we are to successfully develop new strategies to enhance lean tissue deposition in livestock and/or prevent muscle loss in at risk individuals.

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