Application of Satellite Cell Research: Models for Post-Differentiation Regulation and Development of Applied Molecular Approaches to Enhance Muscle Growth of Meat Animals

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Abstract

Increasing the rate of lean muscle growth is an issue of significant importance to commercial producers of agriculturally-important animals. To address this issue, present research efforts with satellite cells, as reported in this issue of BAM, have focused on: I, optimization of methods to isolate and characterize satellite cells; II, definition of regulatory factors that influence satellite cell proliferation and differentiation *in vitro*; and III, determination of the effect of satellite cell-derived myonuclei on postnatal myofiber hypertrophy. Future research efforts need to implement the information from the present types of *in vitro* studies to applied approaches for enhancing muscle growth and development *in vivo*. This review places emphasis on application of satellite cell technology and molecular biological approaches to enhance postnatal skeletal muscle hypertrophy.

Key words: satellite cells, muscle growth, hypertrophy, protein metabolism, genetic markers, animal breeding, domestic animals.

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With the consumer desire for leaner red meat products and the increased demand for poultry, fish and alternative meat products, it is imperative to have a comprehensive understanding of myogenesis and the elements influencing postnatal muscle growth. As described in the previous articles, satellite cells have been identified as being a crucial factor in facilitating postnatal muscle hypertrophy. The proliferation, differentiation, and fusion of satellite cells to existing myofibers are requisite for myofiber hypertrophy [7, 24] and myofiber regeneration [6, 9, 18, 25]. Following postnatal stimulation, or the removal of developmental inhibition, satellite cells proliferate and/or differentiate; subsequent satellite cell-derived myonuclei join existing myonuclei to help regulate growth or development of existing myofibers. The increased number of myonuclei endows the muscle fiber with a greater protein synthesis potential. It is now well understood that a primary role during growth of satellite cells *in vivo* is to supply extra DNA to the polynucleated myotubes to maintain a relative constant cytoplasmic protein/DNA ratio. Indeed, the incorporation of satellite cell nuclei into myofibers has been correlated with increased muscle weight and myofiber cross-sectional area [7].

Developing approaches for the *in vivo* manipulation of satellite cells resulting in maximal fusion with existing myofibers will be of economic benefit to commercial producers of agriculturally-important animals. In addition to previous papers of this volume, this paper describes the general role of satellite cell-derived myonuclei in hypertrophic muscle growth and potential technological innovations to enhance post-fusion skeletal muscle protein hypertrophy in meat animal-species.

Use of Satellite Cells in Research on Hypertrophic Muscle Growth

In recent years, researchers have developed media recipes to successfully grow satellite cells from various species and study regulation of proliferation [12, 13, 14, 21]. Establishment and maintenance of post-differentiation cultures of myotubes/muscle cells arising from satellite cells has been less successful. One goal of studying muscle fiber hypertrophy, *in vitro,* would be to emulate conditions

of accelerated muscle protein deposition and growth exhibited by rapidly growing animals.

The essence of food animal production is to constantly achieve greater and more efficient rates of protein synthesis and skeletal muscle protein deposition. To pursue such goals, an understanding is needed as to which step(s) or factors in the overall array of polypeptide synthesis and myofibrillar protein assembly processes are rate-limiting. Primary factors that determine rate of protein synthesis such as: ribosomal RNA, mRNA, tRNA and amino acyl ligases, amino acids, ATP, and initiation, elongation, and termination factors of transcription have been identified [1]. Determining which of these factors are rate-limiting in satellite cell-derived myotubes from meat animals, could then be utilized to help construct breeding plans as well as develop animal production systems to increase skeletal muscle protein deposition.

From protein synthesis/breakdown studies using *in vivo* ^{14}C amino acid infusions or urinary N^T methylhistidine excretion [3, 20, 22] and protein accretion studies [2], it can be calculated that the ratio of muscle protein synthesis to muscle protein accretion (protein deposition efficiency) is much narrower in poultry (2:1) and pigs (4:1) than in ruminants (sheep/cattle 8-10:1). These ratio differences mean that skeletal muscle protein deposition is energetically more costly in ruminants than poultry or pigs [3]. As it is not known whether the whole animal or species variations in protein synthesis efficiency are reflected at the cellular level; this topic should be amenable for study in satellite cell-derived myotube cultures.

A further key component to skeletal muscle protein hypertrophy is the process of protein degradation. Protein accretion represents the difference between synthesis and degradation [4]. Since lowered degradation may translate into a real saving in animal energy protein needs, many studies on muscle protein degradation have been reported. Such studies range from observations on total muscle breakdown rate *in vivo* [4, 20, 30] to laboratory activity assays of proteases from skeletal muscle samples [16]. *In vivo* studies on protein breakdown provide a limited range of data. Furthermore, the role of protease inhibitors in muscle proteolysis, the actual substrate as well as cellular location of proteases in muscle cells under physiological conditions are also poorly understood. Although there are some excellent studies on proteases in *post mortem* muscle tissue, such results do not *a priori* reflect protein breakdown in living muscle. Post-fusion satellite cell culture studies may provide an ideal experimental system to unravel the mechanisms of degradation in skeletal muscle development. Data derived from these studies could then be applied to the direct *in vivo* manipulation and genetic selection of satellite cell populations and genetic elements that enhance muscle growth in agriculturally-important animals.

Molecular Biological Approaches to Enhance Skeletal Muscle Hypertrophy

Developing technological approaches to select for inherent differences in satellite cells to enhance muscling in food animals is one of the most exciting and economically beneficial challenges facing researchers. Advances in molecular biology provide the means for the genetic improvement of agriculturally-important animals. Traits like muscle growth potential are difficult to identify by traditional phenotype records as their expression is modified by environmental factors and regulated by a large number of different genes (quantitative trait) which include myogenic regulatory factors, growth factors, myofiber specific genes, and extracellular matrix genes. The identification of a genetic marker or markers segregating with the rate of muscle gain will permit the selection of breeding stocks by allelic variants which will lead to time- and cost- efficient breeding practices. This is particularly valuable to the beef, swine, and poultry industries. With a known DNA marker, animals can be selected by progeny testing at a younger age by both genotype and ancestral records. For genetic marker identification to be successful, it is necessary to have DNA probes isolated for the genes involved in the regulation of skeletal muscle hypertrophy. *In vitro* satellite cell studies offer the greatest potential in identifying and characterizing DNA probes to be used in the identification of gene markers (DNA polymorphisms).

Although, genotype-based selection will undoubtedly enhance current selection methods, the identification of genetic markers is not trivial. The following is a brief description of several methods that are currently used to identify DNA polymorphic gene markers.

Restriction Fragment Length Polymorphism

Most DNA polymorphism studies to identify both single gene defects and microsatellite DNA variability have used the method of restriction fragment length (RFLP) analysis. In RFLP analysis, genomic DNA fragments are generated by restriction endonuclease cleavage. The restriction fragments are separated using agarose gel electrophoresis. Cloned DNA for a specific gene is then used to detect the separated genomic DNA fragments. Although many genetic markers have been identified by RFLP analysis, RFLP analysis detects only polymorphisms resulting from large base insertions or deletions, or alterations in restriction enzyme recognition sites. Small base substitutions in DNA are undetected by RFLP analysis. Therefore, many potentially useful DNA polymorphisms remain undetected. These limitations have led to the development of other methods to detect subtle DNA modifications which are likely to occur in single gene DNA polymorphism studies.

Denaturing Gradient Gel Electrophoresis

Denaturing gradient gel electrophoresis (DGGE), originally described by Fischer and Lerman [15], functions by separating DNA fragments according to their melting

domain behavior. Single base modifications will alter DNA melting domains and change the migration of a DNA fragment through a gel matrix. This property makes the DGGE methodology extremely sensitive. Furthermore, a much larger percentage of potential DNA polymorphisms may be resolved by DGGE technology than by RFLP analysis.

DGGE has been used successfully to detect DNA polymorphisms linked to human genetic disorders [8, 10, 23, 26], to monitor intragenic recombination effects in *Drosophila* [11], and to map point mutations [17]. The only work to date using DGGE in agriculturally-important animals is in poultry where a DNA polymorphism in a cartilage extracellular matrix gene has been linked to the inheritance of a lethal defect [28, 29]. Although the potential power of DGGE can be easily visualized for the identification of muscle-specific gene markers linked to enhanced muscle growth, DGGE is not without its methodological limitations. It is often difficult to optimize DGGE electrophoresis conditions to strand-separate DNA fragments. Hence, a DNA polymorphism could go unresolved without the appropriate electrophoresis conditions [27]. Subtle differences in DGGE electrophoresis variables significantly affect the resolution of DNA polymorphisms. This negative aspect of DGGE has resulted in investigators pursuing other approaches.

Single-strand Conformation Polymorphism Analysis

Single-strand conformation polymorphism (SSCP) is a technique coupled to the polymerase chain reaction to detect DNA polymorphisms. SSCP analysis is an ideal methodological approach to detect allelic differences in small amplified regions of exonic DNA (100-250bp). In brief, SSCP analysis is a PCR amplification of a desired DNA region and then the amplified DNA is denatured and electrophoresed through an acrylamide gel matrix. This method is ideal for single gene analyses distinguishing DNA homoduplexes from heteroduplexes.

In terms of genetic marker identification, this method is outstanding in that 90% or more of the DNA polymorphisms contained in a PCR product of less than 200bp are detected [19]. However, if the goal of identifying a gene marker is not to use a defined gene approach then other methods must be evaluated.

Random Amplification of Polymorphic DNA

Random amplification of polymorphic DNA (RAPD) utilizes short oligonucleotide primers of an arbitrary nucleotide sequence to amplify genomic DNA. The use of arbitrary primers combined with PCR amplification of genomic DNA permits the identification of a large number of DNA polymorphisms in random segments of genomic DNA. This methodological approach permits the rapid identification of DNA difference between animals from unique genetic lines. RAPD polymorphisms can be very useful in animal breeding as it permits an efficient means for identifying and isolating chromosome-specific DNA fragments. However, RAPD-PCR is limited in that homozygosity or heterozygosity of polymorphic DNA fragment can not be determined. This is a particular problem for a gene marker inherited in a dominant fashion. In time, RAPD analysis will be replaced by newer methods because loci identified by RAPD analysis are often difficult to reproduce between different laboratories and PCR machines.

Although the use of recombinant DNA techniques like the ones described above are at an early stage of development, the combination of molecular genotyping with conventional breeding will play a significant role in the future improvement of animal breeding stocks for commercially-important traits like postnatal muscle growth potential.

Application of Molecular Biological Methods

Once a desirable gene marker has been identified, the next step is the introgression of that gene marker into breeding populations and eliminating less desirable gene arrangements. The creation of transgenic lines perhaps holds the most promise in this regard. Although the methodology still requires refinement in regard to agriculturally-important animals, transgenic breeding lines establish the stable expression of a targeted gene whose DNA sequence is known into offspring according to Mendelian inheritance. For the development of transgenic meat producing animals to reach its full potential, a comprehensive understanding of gene coding regions and regulatory elements for genes involved in muscle growth and development must first be developed [5] . Such research must be followed by development of more efficient gene targeting methods [5].

Conclusion

Improvement of efficiency of meat-animal production continues to be one of the highest priority areas of research in animal and food science. An additional goal is enhancing lean gain and lowering fat accumulation in meat producing livestock. To develop the next level of production enhancement, including genotype-based breeding systems, an in-depth knowledge of muscle metabolism at the genomic and cellular level for food animal-species must be acquired. A comprehensive understanding of food animal-specific satellite cells are key for this type of research to be successful. This new body of knowledge will be crucial in the further refinement of animal production technology and ultimately improve production efficiency and product quality.

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