The Myogenic Satellite Cell: Discovery, Function, and Recent Advances in Understanding its Role in Growth and Development

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Prior to the 1960s the source of nuclei or cells for postnatal skeletal muscle growth and regeneration was unknown. In 1961, Alexander Mauro wrote a brief note describing electron microscopic observations he had made with frog skeletal muscles (Mauro, 1961). He noted what appeared to be a previously unrecognized cell type residing between the plasma membrane and the basement membrane. The cell contained little cytoplasmic structure and the majority of mass consisted of nucleus, leading to the belief that they were dormant. He conferred with several other colleagues and found that they had also seen similar cells in rat skeletal muscles, but not cardiac muscle. Due to the peripheral location of the cells on muscle fibers, Dr. Mauro termed them "satellite cells." While the source and function of the satellite cells was unknown, Dr. Mauro speculated that they might become activated or "mobilized" to repair damaged muscle fibers. At this time it was not known whether nuclei within the muscle fiber proper (i.e. myonuclei) were capable of producing new DNA or whether another mononucleated cell was required to fuse with the existing fiber to contribute new DNA. To answer this question, Stockdale and Holtzer (1961) utilized chick embryo muscle culture to look for the appearance of mitotic figures and examine incorporation of tritiated thymidine into DNA using autoradiography. Embryonic myoblasts were allowed to fuse to form multinucleated myotubes in culture. There were no mitotic figures observed within the myotubes and administered tritiated thymidine was not incorporated into myonuclei. This demonstrated that myonuclei were incapable of cell division and that another cell type must be responsible for growth of the myotubes. Administration of tritiated thymidine to cultures of proliferating embryonic myoblasts, however, did result in labeling of the nuclei. Furthermore, during differentiation to form myotubes, muscle cells synthesized myosin, the meromyosins, and actin, while proliferating cells did not. It appeared clear that in the developing embryo, muscle fibers likely developed from the fusion of mononucleated myoblasts, but what was the source of nuclei in the postnatal or posthatch animal? Was it the satellite cell? Evidence pointing to the satellite cell's role in muscle growth was presented by Moss and Leblond (1971). Following the injection of tritiated thymidine into growing rats, the disposition of the label was tracked by autoradiography over time in the skeletal muscle. One hour following injection, labeled thymidine was identified in satellite cells but not myonuclei. Between 1 and 24 hours the number of labeled satellite cells doubled, demonstrating that the satellite cells had undergone mitosis. Following this, labeled myonuclei began to appear, demonstrating the fusion of satellite cells with the adjacent fiber and establishing the role of the satellite cell in DNA accretion in muscle fibers.

To put these findings in perspective it is necessary to review some of the earlier work describing postnatal/posthatch skeletal muscle growth. Studies by Smith (1963) using growing chickens determined that increases in cell number (i.e. fiber number) occurs prior to hatch. Following hatch, increases in musculature resulted from increases in fiber size. A growth-selected heavy breed of chicken (White Gold) had a greater number of fibers that were slightly smaller than a layer breed (White Leghorn) at hatching. At 10 weeks of age the heavy breed had considerably larger muscle fibers than the layer breed. This demonstrated the importance of fiber number at hatch in determining muscle growth potential. In addition to this observation, Moss (1968) determined that there was a constant ratio of the fiber cross-sectional area and the DNA content of the fiber. The cross-sectional fiber area increased proportionally to the muscle weight, suggesting that the width and length of the fibers maintained a constant ratio. These findings demonstrated that DNA accretion was necessary for growth of musculature.

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Although Mauro's initial report identified what he described as dormant or quiescent satellite cells, many investigators have identified metabolically active and dividing satellite cells and described their properties. Snow (1977) using electron microscopic methods examined the satellite cell population of muscles of young growing rats (1-2 months of age). Most of the satellite cells had a euchromatic nucleus with a prominent nucleolus. These cells revealed a high nuclear to cytoplasmic ratio and the cytoplasm contained polyribosomes, mitochondria, some rough endoplasmic reticulum and Golgi apparatus. Some of the satellite cells showed evidence of undergoing mitosis. It was evident that these cells were metabolically active and not quiescent. However, when examining muscles from older rats (8-30 months of age), there were significant changes in satellite cell morphology. Nuclei exhibited heterochromatin and visible nucleoli were rarely seen. The nuclear to cytoplasmic ratio was higher in these cells compared to those found in young animal muscles. The rough endoplasmic reticulum with dilated cisternae were sparce and the Golgi apparatus was poorly developed. Additionally, microtubules and microfilaments were rare and glycogen was not observed. No dividing satellite cells were detected. Similar results were reported by Schultz (1976) while tracking the morphology of mouse satellite cells from 7 days to 50 weeks. Furthermore, Schultz and coworkers (1978) demonstrated that tritiated thymidine was not incorporated into the nuclei of satellite cells of mature mice and that the frequency of satellite cells along the fibers declines significantly with age. Although fewer in number and largely in a quiescent state, satellite cells from older animals do have the capacity to proliferate and differentiate (Allen et al., 1980), but the proliferation potential is diminished (Schultz and Lipton, 1982).

To determine if satellite cells were involved in regeneration of skeletal muscle following damage, Bischoff (1975) developed a culture system using teased-out individual rat muscle fibers maintained in a fibrin clot overlain with culture medium. During the first several hours degenerative changes were observed within the basement membrane (endomysial tube). While the basement membrane remained intact, contraction clots formed and myonuclei did not appear to undergo mitosis. However, satellite cells enlarged and began to proliferate during the second day of culture forming small clones. After 5-7 days, these presumptive myoblasts began to fuse to form myotubes within the endomysial tube of the original fiber and exhibited contraction. These and other studies (Carlson and Faulkner, 1983; Schultz, 1989; Ontell, 1986) demonstrated the important role of satellite cells in the regeneration of skeletal muscle damaged by trauma or muscular dystrophy. In addition to functioning in the repair of damaged muscle fibers they are associated with, Hughes and Blau (1990) demonstrated that satellite cells can cross the basement membrane barrier and contribute to the repair of other fibers damaged by trauma or disease. Recovery from muscular atrophy resulting from spaceflight or muscle unweighting results in the activation of satellite cells and their participation in this process (Riley et al., 1990; Darr and Schultz, 1989).

Satellite cell activation state is also responsive to nutritional status and exercise. During examination of the skeletal muscle biopsies from malnourished children (Hansen-Smith et al., 1979) demonstrated that the proportion of nuclei within satellite cells in relation to myonuclei was significantly diminished compared to age-matched well-nourished control children. Satellite cells in malnourished children were generally heterochromatic and were characteristically quiescent. Upon nutritional recovery, many of the satellite cells became enlarged and morphologically appeared active. Furthermore, recovery was characterized by an increase in the proportion of satellite cells compared to muscles of malnourished children (Hansen-Smith et al., 1978). Darr and Schultz (1987) utilizing untrained exercised rats as a model, demonstrated that eccentric treadmill running caused activation of satellite cells as measured by incorporation of tritiated thymidine into nuclei. While a small percentage of necrotic fibers resulted from the exercise (<3%), the extent of satellite cell activation exceeded that needed to support fiber regeneration. Additionally, the presence of newly formed or repaired fibers was noted by there being fibers with centrally-located nuclei.

Much progress has been made in understanding the physiology of satellite cells through the use of cell culture. Isolation of satellite cells for culture was first described by Bischoff (1974) using adult rat muscles. Since then, investigators have isolated and cultured satellite cells from agriculturally-important animals (for review: see Dodson et al., 1996) and humans (Blau and Webster, 1981). Following removal of skeletal muscles, procedures usually required removal of visible connective tissue and fat, mechanical dissociation and liberation of satellite cells from beneath the basement membrane using a protease. This is followed by separation of the suspended cells from the muscle fiber debris. Cells may then be plated directly into substratum-coated cultureware or frozen with the cryoprotectant, dimethylsulfoxide and stored in liquid nitrogen for later use. Care must be taken to assure that cultures are not contaminated with non-myogenic cells. However, the ability of satellite cells to take alternative developmental pathways under particular conditions (see below) can provide a challenge to validating the purity of cultures. Optimization of basal medium, serum (type and level), and other components has been done for most of the species studied. For growth, sera are typically included in media at a level of 10-20%. Following proliferation, lowering of sera to 1-3% will often induce cells to differentiate and fuse to form multinucleated myotubes. Figure 1 illustrates representative proliferating turkey satellite cells and Fig. 2, multinucleated turkey myotubes. For more intensive examination of growth requirements, serum-free defined media formulations have been devel-

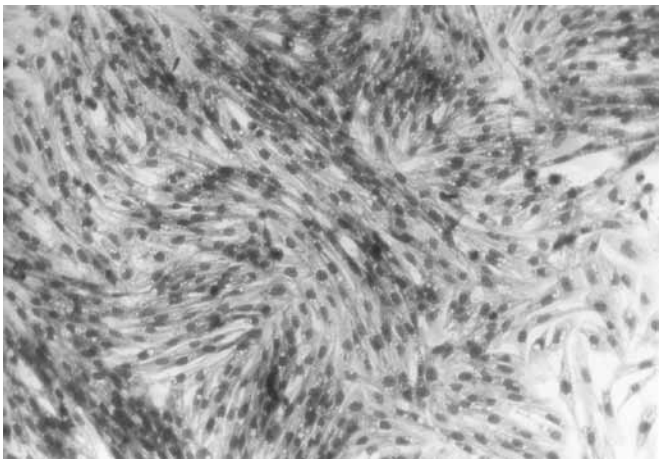

Fig. 1. A near confluent culture of turkey satellite cells stained with Giemsa to better visualize the nuclei. Note the pattern of alignment of satellite cells prior to differentiation and fusion to form myotubes.

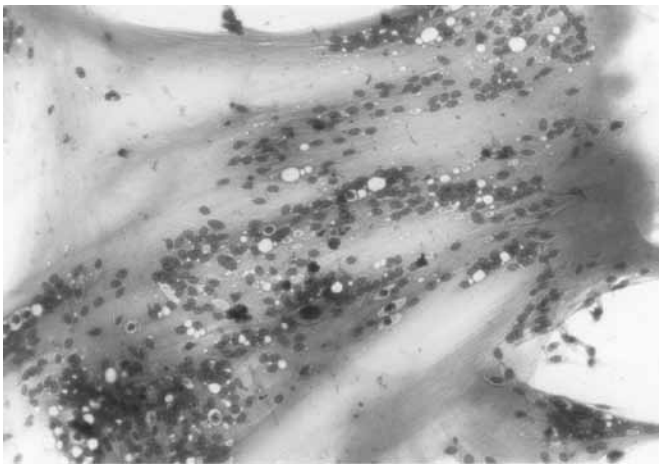

Fig. 2. Multinucleated myotube resulting from differentiation and fusion of turkey satellite cells. Cultures were administered low serum-containing medium for 3 d following proliferation, then stained with Giemsa.

oped for different species (for review see: Dodson et al., 1996). These formulations have been especially important in elucidating the role of growth factors in satellite cell activation, proliferation and differentiation.

Due to its important role in skeletal muscle growth, development and regeneration, satellite cells have become the focus of many researchers in both the agricultural and medical communities. A better understanding of the regulation of satellite cell proliferation and differentiation may lead to methods to improve muscle growth efficiency in meat animals and to facilitate regeneration of muscles of trauma patients and those suffering from muscular dystrophies. In recent years considerable progress has been made in understanding the role of growth factors (for reviews see: Dodson et al., 1996; Rhoads et al., 2009), internal signaling pathways and transcription factors (for reviews see: Zammit et al., 2006; Yablonka-Reuveni, 2011), and the extracellular matrix (For review see: Velleman, 2012) in muscle growth and development. It is also recognized that under certain circumstances skeletal muscle cells may take alternative pathways of development to become non-muscle cells. Using the C_2C_{12} mouse muscle cell line and harvested mouse satellite cells, Teboul and co-workers (1995) demonstrated that rosiglitazone, a PPARγ-selective agonist, prevented differentiation to myotubes and induced the cells to become adipoblasts. Similar findings were reported with porcine satellite cells (Singh et al., 2007). Administration of bone morphogenetic protein to C2C12 myoblasts and primary mouse muscle cells prevented myotube formation and induced alkaline phosphatase and osteocalcin production, markers of osteoblasts (Katagiri et al., 1994). Dispersed muscle fiber explants have also been helpful in recognition of the diversity of satellite cell destiny. Cornelison and Wold (1997) tracked the expression of myogenic regulatory factors following the activation of quiescent satellite cells in situ on muscle fibers. Asakura and coworkers (2001), using a similar procedure, allowed the fibers to attach to a Matrigel substata and recorded the differentiation of satellite cells into myogenic, osteogenic and adipogenic lineages. However, primary muscle cell harvests only resulted in myogenic cultures. From the prospective of meat scientists, these findings may lead to techniques to increase lean muscle accretion and manipulate marbling.

Numerous studies have been conducted to determine the feasibility of using satellite cells in regenerative medicine. Most studies have focused on developing procedures to lessen the impact of age-associated muscle weakening, muscle degeneration caused by the many forms of muscular dystrophy, myoblast transplantation to aid in cardiac muscle regeneration, and in the repair of damaged muscles. Technical challenges include early death of transplanted cells, limited numbers of cell proliferations, immunological rejection by non-autologous transplants, and poor distribution of myoblasts within the muscle beds. Zhu and coworkers (2007) engineered a human satellite cell line that expresses telomerase and cyclin-dependent kinase-4, overcoming the issue of age-related senescence. When transplanted into muscle damaged immuno-compromised mice, these cells contributed successfully to muscle regeneration. The first successful myoblast transfer with humans occurred in 1990 (Hooper, 1990). Satellite cells were isolated and purified from a normal donor skeletal muscle and injected into affected muscles of a boy with Duchenne muscular dystrophy. The immunosuppressant cyclosporine was administered following the therapy to prevent cell rejection. Muscle biopsies taken 6 years following treatment demonstrated that dystrophin production was robust and widespread (Law et al., 1997). However, muscle fiber

diameters were small and oval in shape and the treated muscles did not appear to function properly. Ehrhardt and coworkers (2007) transplanted human fetal muscle precursor cells into cryodamaged muscles of mice and demonstrated that the cells gave rise to satellite cells as well as regenerated muscle fibers. Injection of cultured satellite cells into damaged cardiac muscle has also been seen as a possible strategy for treating heart attacks (Marelli et al., 1992). New muscle fibers were detected in some of these studies several months later. However, a recent report of a phase 1 trial demonstrated that intracoronary infusion of autologous cardiac stem cells improved the ventricular function and decreased infarct size in patients with heart failure following myocardial infarction (Bolli et al., 2011).

From the initial observations by Mauro and others of the existence of skeletal muscle satellite cells, a great amount of research has been done to determine how they become activated, proliferate, differentiate and contribute to the largest organ in the body – skeletal muscle. Attention will continue to be directed on this cell by researchers focused on solving problems in both meat animal agriculture and medicine.

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