Leucine/glutamic acid/lysine protein 1 is localized to subsets of myonuclei in bovine muscle fibers and satellite cells


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ABSTRACT: Skeletal muscle growth is accomplished chiefly through the actions of satellite cells, a heterogeneous population that includes the adult muscle stem cell. Located adjacent to a mature muscle fiber, satellite cells typically reside in a quiescent state. Little information exists detailing satellite cell regulation of reversible G0. One member of the mitosin family of centromere proteins, LEK1 (leucine/glutamic acid/lysine protein 1), is present in the nucleus of nondividing mouse satellite cells. The objective of this study was to evaluate LEK1 as a marker of quiescent bovine satellite cells (BSC) in vitro and in vivo. The BSC were isolated from young bull calves (≤7 d) and cultured in vitro for up to 9 d before fixation and immunostaining for LEK1. Results demonstrated that all myogenic cells contain the protein, with immunostaining primarily within the nucleus and immediate perinuclear region. Immunocytochemical detection of LEK1 in cryosections of mature cows revealed that the protein was present in a fraction of satellite cells and muscle fiber nuclei. Approximately 20% of Pax7-expressing satellite cells contained LEK1. An equivalent percentage of myonuclei, as defined by nuclei within a dystrophin boundary, contained nuclear LEK1. To gain insight into the functional role of LEK1, BSC were transiently transfected with plasmids coding for putative dominant inhibitory LEK1 proteins [ΔLEK1(991) and ΔLEK1(911)] and evaluated for cell proliferation. Both forms of ΔLEK1 inhibited (P < 0.05) BSC proliferation, as indicated by a decrease in Ki67 immunopositive cells. In C2C12 myoblasts, ΔLEK1(911) inhibited (P < 0.05) myoblast determination protein 1 (MyoD)-directed muscle gene transcriptional activity; ΔLEK1(991) had no effect on TnI-Luc transcription. By contrast, both ΔLEK1 fusion proteins inhibited myogenin expression in BSC without disrupting myoblast fusion. These results provide evidence that LEK1 serves to coordinate proliferation and differentiation in myogenic cells. Coupling the immunostaining pattern and functional data, we propose that LEK1 may serve as a useful marker for satellite cells that are preparing to fuse into adjacent fibers as well as an indicator of recently added myonuclei.

Key words: bovine, cell cycle, differentiation, leucine/glutamic acid/lysine protein 1, myonuclei, satellite cell

INTRODUCTION

Satellite cells, which include the resident myogenic stem cell, lie adjacent to the mature muscle fiber in a quiescent state until required for damage repair or the support of fiber hypertrophy. Bovine satellite cells (BSC) activate, proliferate, and differentiate in vitro, similar to their rodent contemporaries (Allen et al., 1991; Kamanga-Sollo et al., 2004). The cells express the full complement of myogenic regulatory factors, with myogenin prevalent in the myonucleus of fibers (Mu-roya et al., 2005; Kook et al., 2008). Importantly, BSC are denoted in vivo by their expression of the lineage marker Pax7 (Gonzalez et al., 2007). Although much is known regarding BSC myogenesis, the mechanisms controlling their self-renewal and return to G0 remain poorly defined.

Leucine/glutamic acid/lysine protein 1 (LEK1) was identified in a screen for protein markers of reversible quiescence in myoblasts (Reed et al., 2007). The protein persists in the nucleus of G0 arrested mouse satellite cells and translocates to the cytoplasm during activation. As a kinetochore binding protein, LEK1 participates in the regulation of cell division (Ma et al., 2006). The protein is proteolytically processed to yield a nuclear-retained subunit that contains docking sites for pocket-binding protein transcriptional regulators (Goodwin et al., 1999; Evans et al., 2007). However, nuclear LEK1 does not appear to directly bind chro-
matin and elicit an effect on gene transcription (Ashe et al., 2004; Evans et al., 2007). The cytoplasmic N-terminal portion of LEK1 is involved in microtubule assembly and function and plasma membrane recycling (Soukoulis et al., 2005; Pooley et al., 2006).

Because the distribution of LEK1 changes during mouse satellite cell activation in vitro, the protein may serve as a useful marker of quiescent bovine satellite cells. The objective of the study was to characterize the subcellular localization of LEK1 during BSC myogenesis in vitro and to evaluate the protein as a marker of quiescent BSC in vivo.

**MATERIALS AND METHODS**

Euthanasia and harvest of muscle tissue were approved by the University of Florida Institutional Animal Care and Use Committee.

**Bovine Satellite Cell Isolation**

After captive bolt stun and exsanguination, the right hindlimb semimembranosus muscle was removed. All animals (n = 4) were Holstein bull calves >7 d of age. The muscle was dissected free of fascia, connective tissue, and adipose and finely ground with a commercial meat processor. The tissue (approximately 10 g) was digested with 35 mL of 1 mg/mL of pronase E (Sigma Aldrich, St. Louis, MO) in PBS (pH 7.4) for 45 min at 37°C with agitation at 10-min intervals. The slurry was pelleted at 1,500 × g for 4 min at 22°C, and the proteinase was decanted. Fresh PBS was added to the tissue, vortexed for 5 min, and centrifuged (500 × g) for 10 min. The supernatant containing the satellite cells was retained. The process was repeated, and a total of 3 supernatants were collected. The cells were pelleted (10 min, 1,500 × g) and resuspended in growth media before sequential filtration through 70- and 40-µm sieves (Invitrogen, Carlsbad, CA). Cells were collected by centrifugation at 1,500 × g for 5 min at 22°C, resuspended in growth media supplemented with 10% dimethylsulfoxide, and stored in liquid nitrogen until needed. Clonal analysis of the BSC indicates that 98% of the cells are myogenic as measured by immunofluorescent detection of Pax7 and Myf5 (data not shown).

**Cell Culture, Plasmids, and Transfection**

The BSC were cultured in low-glucose Dulbecco’s modified Eagle medium (DMEM; Invitrogen) supplemented with 10% horse serum (HS), 1% penicillin-streptomycin, and 0.5% gentamycin on entactin-collagen-laminin (Invitrogen) coated tissue cultureware. Proliferation was measured by incorporation of 5-bromodeoxyuridine (BrdU, 10 µg/mL, 2 h pulse) into DNA. The C2C12 myoblasts were cultured on gelatin-coated surfaces in growth media (high glucose DMEM, 15% fetal bovine serum, 1% penicillin-streptomycin, 0.5% gentamycin). The C3H10T1/2 fibroblasts were cultured in basal Eagle medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Differentiation of C2C12 myoblasts and C3H10T1/2 fibroblasts was initiated by treatment with differentiation medium composed of low glucose DMEM supplemented with 2% HS for 48 h. The C2C12 myoblasts and C3H10T1/2 fibroblasts were transiently transfected with FuGene6 (Roche, Nutley, NJ) using a plasmid DNA:reagent of 3:2 in serum-containing media. Plasmids included pAc green fluorescent protein (pAcGFP; Clontech, Mountain View, CA) and pAcGFP-ΔLEK1(991), −ΔLEK1(911), and −ΔLEK1(685) coding for the C-terminal 108, 188, and 413 AA of mouse LEK1, respectively. The plasmid CMV-MyoD contains the cytomegalovirus (CMV) immediate early promoter driving expression of a cDNA coding for mouse MyoD (Johnson et al., 2002). The reporter plasmid, TnI-Luc, contains the quail fast troponin I internal response element driving expression of firefly luciferase (Johnson et al., 1996). Muscle reporter gene activity was normalized to the transcriptional activity of pRL-tk-Luc, a reporter plasmid containing the minimal thymidine kinase promoter driving expression of a Renilla luciferase cDNA. Luciferase activities were measured using the Dual-Luciferase Reporter Assay system (Promega, Madison, WI).

**Immunocytochemistry**

Cell cultures were fixed with 4% paraformaldehyde (Polysciences, Warrington, PA) in PBS for 10 min at room temperature. Fixed cells were rinsed with PBS followed by incubation in 10% HS, 0.1% Triton X100 in PBS for 30 min at room temperature. Subsequently, the cells were incubated in primary antibody diluted in 1.0% HS, 0.01% Triton X100 in PBS for 1 h at room temperature. Antibodies and dilutions were anti-MyHC (1:5 hybridoma supernatant MF20, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City), anti-myogenin (F5D, 1 µg/mL, Developmental Studies Hybridoma Bank, anti-Ki67 (1:200, AbCam, Cambridge, MA), pre-immune LEK1, and anti-LEK1 [1:200 (Reed et al., 2007)]. Before incubations with anti-BrdU (1:100, Invitrogen), cells were incubated with 2 N HCl for 1 h at 37°C followed by acid neutralization (2 × 10 min) with TBE (89 mM Tris-borate, 2 mM EDTA, pH 8.3). After extensive washing with PBS, the cells were incubated with donkey anti-rabbit-AlexaFluor488, goat anti-rabbit-AlexaFluor568 or donkey anti-mouse-AlexaFluor488 (Invitrogen, Carlsbad, CA) diluted 1:250 in PBS containing 1% HS for 45 min at room temperature. Hoechst 33245 (10 µg/mL) was included as a nuclear counterstain. After washing with PBS, cells were visualized by epifluorescence on a Nikon TE2000 and representative images captured with a CoolSnap EF camera (Nikon, Lewisville, TX). Images...
were compiled with NIS Elements (Nikon) and Adobe PhotoShop (Adobe, San Jose, CA).

**Immunohistology**

Tissues were collected from adult cows (n = 4) at slaughter and frozen in optimum cutting temperature compound freezing medium. Cryosections (12 μm) were collected onto SuperFrost microscope slides and stored at −80°C until needed. Tissue cryosections were fixed with 4% paraformaldehyde for 10 min at room temperature. Nonspecific binding sites were blocked by incubation in 5% HS, 0.1% Triton X100 in PBS for 20 min at room temperature. Cryosections were incubated in rabbit anti-LEK1 (1:200) and mouse anti-dystrophin (1:200, AbCam, Cambridge, MA) or mouse anti-Pax7 (Developmental Studies Hybridoma Bank) for 1 h at room temperature. After washing with PBS, cryosections were incubated with donkey anti-rabbit-AlexaFluor488 (1:250) and donkey anti-mouse-AlexaFluor568 (1:250) for 40 min. Hoechst 33345 (10 μg/mL) was included as a nuclear counterstain. After washing with PBS, the slides were coverslipped, and immune complexes were visualized with a Nikon TE2000 equipped with epifluorescence. Representative images were captured with a Cool Snap EF camera (Nikon), and morphometrics were measured using NIS Elements (Nikon) and Adobe Photoshop (Adobe).

**Western Blot**

Total protein lysates were prepared from 1 × 10⁶ cells by scraping into 4 × SDS-PAGE buffer (250 mM Tris, pH 6.8, 8% SDS, 40% glycerol, 0.4% β-mercaptoethanol) and heating at 95°C for 7 min. An equivalent amount of cellular protein extract was electrophoretically separated through denaturing 10% polyacrylamide gels followed by semi-dry transfer to nitrocellulose. Blots were incubated with 10% nonfat dry milk in 10 mM Tris, pH 8.9, 150 mM NaCl, 0.1% Tween20 (TBST) for 30 min at room temperature to remove nonspecific antigen sites. Subsequently, blots were incubated with anti-GFP (1:500 in TBST, Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature with rocking. Blots were washed 3 times for 5 min each with TBST before incubation with goat anti-mouse-peroxidase (1:2,000, Vector Laboratories, Burlingame, CA) for 40 min at room temperature with rocking. After extensive washing with TBST, immune complexes were detected by chemiluminescence (enactin-collagen-laminin, GE Healthcare, Pittsburgh, PA) and exposure to x-ray film (Kodak Biomax XAR, Sigma Aldrich, St. Louis, MO).

**Statistics**

All data were analyzed by 1-way ANOVA followed by t-test (SAS Institute Inc., Cary, NC). Results are presented as the mean ± SEM. A P-value < 0.05 was considered to be significant.

**RESULTS AND DISCUSSION**

In a proteomic screen for markers prevalent in non-dividing myoblasts, LEK1 was discovered as a novel indicator of reversible quiescence (Reed et al., 2007). As a member of the mitosin family, LEK1 participates in the ordered process of DNA replication and microtubule arrangements during cell division (Varis et al., 2006). Thus, LEK1 may be a useful determinant of mitotically inactive myogenic cells in vivo. As a first step, a crude activation time course was established for BSC. In brief, BSC were cultured in growth media for 4 d with BrdU incorporation monitored daily. Few BSC were actively proliferating after 24 h in culture with significantly more (P < 0.05) BrdU-immunopositive cells found at 48 h (Figure 1A). By 72 h in culture, approximately 50% of the cells were traversing S-phase as measured by BrdU incorporation into DNA. From these results, we conclude that BSC are mitotically active within 48 h postseeding. If LEK1 denotes quiescence, we hypothesized that the protein should be prevalent in the nucleus at 24 h but localized in the cytoplasm and nucleus at subsequent time points. As such, BSC were isolated and parallel plates were fixed and immunostained for LEK1 after 24 h, 72 h, or 9 d in culture (Figure 1B). Cell compartments were defined with phalloidin-Texas Red and nuclear Hoechst 333425 staining. After 24 h in culture, the satellite cells exhibit a round morphology with little cytoplasmic volume and few membrane extensions. The LEK1 immunostaining is primarily within the nuclear compartment with diffuse staining in the perinuclear region. By 72 h, the cell is firmly attached to the substratum surface and filamentous actin fibers are located throughout the cell. Many cells exhibit distinct leading edges and ruffled borders, as detected by phalloidin dye. Again, LEK1 immunofluorescence is contained within the nucleus with diffuse perinuclear staining present. The protein is not localized throughout the cell cytoplasm and is not present at the plasma membrane. In addition, all myoblasts express the protein. By contrast, fully differentiated myofibers (>7 d) exhibit distinct changes in LEK1 subcellular location. The protein is predominantly nuclear in the fiber with surrounding mononucleated myoblasts exhibiting noticeably weaker immunofluorescent intensity. Closer examination demonstrates that LEK1 is not present in all nuclei and that myoblasts nearest to a mature fiber appear to contain nuclei with a more intense staining pattern.

The presence of nuclear LEK1 in satellite cells and myonuclei was examined further using cryosections isolated from adult cows. Twelve-micron cryosections from the infraspinatus of mature beef cows (>36 mo, n = 4) were immunostained for LEK1 and dystrophin or Pax7. Nuclei contained adjacent to the dystrophin borders were classified as myonuclei; anti-Pax7 was used to denote satellite cells. Results indicate that fewer than 20% of the myonuclei exhibited LEK immunostaining (Figure 2). Colocalization of Pax7 and LEK revealed...
that approximately 17% of the satellite cell expressed the mitosin family member. These results clearly establish that LEK1 is a poor indicator of myonuclei or satellite cells. It is interesting to note that in vitro all myogenic cells contain LEK1 immunoreactivity, but only a fraction of myonuclei in vivo express the protein. These results argue that the local environment is involved in LEK1 subcellular distribution in vivo. A host of growth factors and morphogens is present in adult skeletal muscle fibers. Monitoring LEK1 distribution in response to soluble peptide agonists, growth factors, and extracellular matrix-associated molecules

Figure 1. Bovine satellite cells (BSC) express LEK1 (leucine/glutamic acid/lysine protein 1) in vitro. A. The BSC were isolated and pulse-labeled with BrdU for 2 h before fixation at 24-h intervals for 96 h. 5-Bromodeoxyuridine (BrdU) was detected with immunocytochemistry, and the numbers of immunopositive cells were enumerated. BrdU% = (BrdU nuclei/total nuclei) × 100. Means and SEM are shown. B. Parallel plates of BSC were fixed and immunostained for LEK1. Actin filaments were detected with phalloidin-conjugated Texas Red. Nuclei were detected with Hoechst 33325 dye. Representative photomicrographs at 630× are shown. 3× = 3-fold enlargement of upper myofiber photomicrograph.
will provide clues into the subtle regulation of myogenic cell behavior that is independent of increased rates of proliferation and differentiation.

The mitosin/CENP-F/LEK1 family member, cardiac muscle factor 1 (CMF1) is developmentally regulated in the embryonic chick heart (Wei et al., 1996) and is expressed in avian skeletal muscle (Dees et al., 2000). Embryonic d 10 chick myoblasts cultured in vitro demonstrate differential localization of CMF that is reflective of the stage of differentiation (Dees et al., 2000). Early myoblasts, defined as 24 h postseeding, contain immunoreactive CMF1 in the nucleus with very little cytoplasmic CMF1. As the myogenic cells fuse into multinucleated muscle fibers, CMF1 is retained primarily in the cytoplasm in a diffuse localization pattern. By contrast, BSC in vitro retain the majority of LEK1 in the nuclear compartment throughout myogenesis. With increasing developmental age of the bovine myoblast, an increase in cytoplasmic LEK1 is evident but the protein tends to return to the nuclei during fiber maturation. The disparate results between chick and bovine myoblasts may reflect specie differences, subtle differences between CMF1 and LEK1, time in culture, or a combination of these.

Mouse LEK1 participates in the correct positioning of chromosomes and microtubules to ensure proper segregation of the DNA material during cell division (Yang et al., 2003). Disruption of CENP-F function by siRNA-mediated depletion causes chromosome decondensation, indicating that the protein is involved in maintaining a compact DNA structure (Holt et al., 2005; Yang et al., 2005). Altering chromatin accessibility is an effective means of regulating developmental gene expression patterns (Bibikova et al., 2008). The distribution of LEK1 to the nucleus in muscle fibers may signal a change in gene expression dynamics. The exclusive presence of LEK1 in satellite cells in vivo may denote a cell that is committed to fusion into the adjacent fiber with myonuclear LEK1 indicating a recently added satellite nucleus. In this scenario, LEK1 immunoreactivity would represent an invaluable tool for the appraisal of muscle fiber hypertrophy and myonuclear domain kinetics. An alternate possibility is that myonuclear LEK1 may reflect the metabolic properties of the muscle fiber. The cryosections in this study were obtained from the infraspinatus of adult beef cows that contains a 50:50 mix of type I and IIA fibers (Gonzalez et al., 2008). There was no apparent correlation between myonuclear LEK1 content and fiber type (data not shown). However, this does not preclude the possibility that myonuclear LEK1 preferentially demarcates muscle fibers utilizing oxidative metabolism for the generation of ATP.

Proper formation of muscle fibers is accomplished, in part, through coordinated cell cycle arrest and subsequent muscle gene expression. Because LEK1 is linked to cell cycle kinetics, we examined the effects of disrupted LEK1 function on measures of myoblast proliferation and differentiation. Experiments incorporated pAcGFP-ΔLEK1(991), a dominant inhibitory form of LEK fused in frame with GFP (Ashe et al., 2004; Papadimou et al., 2005; Evans et al., 2007). This small peptide of LEK1 contains a nuclear localization signal followed by a retinoblastoma protein binding domain and a farnesylation sequence (Evans et al., 2007). Ectopic expression of ΔLEK1(991) causes cell cycle delay at G2/M in fibroblasts (Zhu et al., 1995) and inhibits differentiation of cardiomyocytes (Papadimou et al., 2005). Additional LEK1 fusion proteins examined were GFP-ΔLEK1(685), a form of LEK1 that contains a leucine-rich motif immediately upstream from the inhibitory region and GFP-ΔLEK1(911), a fusion protein of intermediate size between the aforementioned LEK1 proteins. In brief, mouse C2C12 myoblasts were transiently transfected with pAcGFP, pAcGFP-ΔLEK1(991), pAcGFP-ΔLEK1(911), or pAcGFP-ΔLEK1(685) and maintained in growth medium for 48 h. Myoblasts were lysed for Western blot analysis. As shown in Figure 3A, each fusion protein is expressed in the myogenic cells at the correct size. A parallel set of subconfluent C2C12 myoblasts was transfected with plasmids coding for the GFP-ΔLEK1 fusion proteins, fixed, and immunostained for Ki67 after 48 h. The numbers of GFP expressing cells that contained the proliferation marker were enumerated. As shown in Figure 3B, nearly all myoblasts (98%) are actively dividing and express the proliferation marker Ki67. The percentage of dividing cells is re-
duced ($P < 0.05$) in the presence of GFP-ΔLEK1(991) and ΔLEK1(685). The GFP-ΔLEK1(911) elicited no effect on proliferation. The LEK1 fusion proteins were further evaluated in subconfluent cultures of bovine satellite cells. Again, ΔLEK1(991) inhibited ($P < 0.05$) proliferation by comparison to control myoblasts expressing GFP. Ectopic expression of GFP-ΔLEK1(911) reduced the percentage of cycling satellite cells. The effect of ΔLEK1(685) on BSC proliferation was not examined. It is interesting to note that ΔLEK1(911) is capable of suppressing proliferation in BSC but not in mouse myoblasts. The reason for this remains unknown but may be related to protein stability. The relative amounts of GFP-ΔLEK1(911) are less than the other fusion proteins in C2C12 cells (Figure 3A). Thus, it is possible that insufficient protein is produced to elicit an effect or that the protein is degraded rapidly. Alternatively, the repressive actions of ΔLEK1(911) on BSC proliferation may represent subtle differences between bovine and mouse LEK1. In the experiments described
herein, mouse LEK1 cDNA was used as the template for creation of the GFP fusion proteins. Comparison of the C-terminal AA sequence of mouse LEK1(991) and LEK1(911) with the corresponding bovine region reveals the proteins are 85 and 87% homologous, respectively. Examination of the 80 AA region of interest in LEK1(911) does not indicate the presence of an ubiquitination, sumoylation, or furin protease cleavage site.

Inhibition of cell cycle progression is consistent with mouse fibroblasts and myoblasts expressing the smallest LEK1 fusion protein (Evans et al., 2007). Because cessation of cell division is a requisite for terminal differentiation, we examined the ability of the fusion proteins to alter myogenesis. Mouse C3H10T1/2 fibroblasts were transiently transfected with mammalian expression plasmids coding for MyoD, the GFP-DLEK1 proteins, and a muscle-specific reporter gene. After 48 h in differentiation-permissive media, the cells were lysed and reporter gene activity measured. As expected, MyoD readily directs transcription from TnI-Luc in the myoblasts (Figure 4A). The MyoD-expressing myoblasts are differentiation-competent in the presence of GFP-ΔLEK1(991). However, GFP-ΔLEK1(911) and GFP-ΔLEK1(685) repress \( P < 0.05 \) differentiation as measured by TnI-Luc transcription. No differences in the amounts of \( \text{Renilla} \) luciferase were evident, indicating equivalent transfection efficiencies (data not shown). These results were extended to satellite cell differentiation. Primary BSC were transiently transfected with plasmids coding for GFP, GFP-ΔLEK1(991), and GFP-ΔLEK1(911). After 48 h, the cells were fixed and immunostained for myogenin. Approximately 50% of the BSC expressing GFP contain myogenin in the nucleus (Figure 4B). Ectopic GFP-ΔLEK1(911) reduced \( P < 0.05 \) the percentage of BSC immunopositive for myogenin to less than 10%. By contrast to the mouse myoblasts, GFP-ΔLEK1(991) blocked differentiation as determined by a reduction in nuclear myogenin expression. However, it should be noted that formation of multinucleated muscle fibers was not prevented by any of the GFP-ΔLEK1 fusion proteins (data not shown).

Fusion in the absence of myogenin supports our hypothesis that LEK1 may denote bovine myoblasts that are recently added to the growing muscle fiber. Although this aspect of LEK1 biology requires additional research, our efforts do provide perfunctory evidence that LEK1 actions extend beyond cell cycle control.

Proteolytic processing of LEK1 in vivo creates at least 2 large peptides and possibly more (Goodwin et al., 1999; Reed et al., 2007). The importance of LEK1 cleavage is largely unknown but likely to represent key regulatory events. The C-terminal region of LEK1 contains a nuclear localization signal and a retinoblastoma protein binding domain; the same motifs are present in CMF1. Mutation of the nuclear localization signal resulting in CMF1 cytoplasmic sequestration in quail myoblasts disrupts myofiber formation and is conducive to endoreplication in muscle fibers (Dees et al., 2006). The inability to establish the full myogenic gene program is attributed to disrupted Rb actions. Quail myoblasts overexpressing CMF1 lacking the Rb binding site are differentiation defective with limited expression of myogenin (Robertson et al., 2008). Ectopic expression of a dominant negative nuclear LEK1
Leucine/glutamic acid/lysine protein 1 expression

[GFP-ΔLEK(991)] that contains the Rb binding motif in skeletal muscle myoblasts does not appear to inhibit differentiation. Inhibition of MyoD-directed contractile gene expression requires additional sequences in LEK1 that lie immediately upstream of the Rb binding motif. Computer algorithms indicate the presence of a leucine-rich region in ΔLEK1(685) that may be important for inhibition of differentiation. However, a truncated version of this protein that does not contain the full leucine-rich region is equally effective in repression of MyoD-directed transcription. These results suggest an additional regulatory region is located within the 80 AA region spanning 912 to 990 of nuclear LEK1. Depletion of LEK1 with anti-sense RNA in fibroblasts causes apoptosis (Ashe et al., 2004). Future efforts will involve examination of nuclear LEK1 regions, specifically ΔLEK1(911), for their anti-apoptotic activity.

In summary, LEK1 is present in BSC in vitro throughout the developmental myogenic program. However, the protein is present within a subset of satellite cells and myonuclei in adult cattle skeletal muscle. Ectopic overexpression studies indicate that LEK1 participates in cell cycle regulation as well as myoblast differentiation. Due to the unique in vivo expression pattern of LEK1 and its putative role in vitro, we propose that LEK1 may be a marker of satellite cells that are preparing to fuse, or have recently fused, into the adjacent muscle fiber.

LITERATURE CITED


