The ontogeny of delta-like protein 1 messenger ribonucleic acid expression during muscle development and regeneration: Comparison of broiler and Leghorn chickens

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ABSTRACT Delta-like protein 1 (DLK1) has been implicated in the muscle hypertrophy observed in DLK1 transgenic mice, callipyge sheep, and mouse paternal uniparental disomy 12 and human paternal uniparental disomy 14 syndromes. The current study was aimed to determine chicken DLK1 (gDLK1) mRNA expression during primary muscle cell differentiation and during muscle regeneration after cold injury and to compare gDLK1 mRNA expression during skeletal muscle development in layers and broilers. In chicken primary muscle cell culture, gDLK1 mRNA expression was significantly increased from 12 to 48 h \( (P \leq 0.05) \) when the nascent myotubes were actively formed at d 2 to 3. Myogenin, a late myogenic marker gene, mRNA expression peaked at 36 to 48 h. Myogenic differentiation 1 (MyoD) and paired box gene 7 (Pax7), early myogenic marker genes, mRNA expression gradually decreased during myogenic differentiation. During muscle regeneration, the expression of MyoD and Pax7 peaked at d 2 \( (P \leq 0.05) \), and myogenin mRNA expression peaked at d 4 \( (P \leq 0.05) \). The induction of gDLK1 gene appeared between d 7 to 10 postinjury \( (P \leq 0.05) \) when myotubes were actively formed as also demonstrated in histological sections. The expression of gDLK1 was slowly downregulated to the control levels at d 14 when the damaged muscle appeared nearly healed. These data suggest that gDLK1 may be involved in the late myogenic stages of primary muscle cell differentiation and muscle regeneration. The gDLK1 mRNA in the muscle tissues was very abundant at embryonic ages but decreased after hatching in both broiler and layer chickens. Compared with layers, broiler muscle at embryonic d 13 had a 3-fold greater expression of DLK1 \( (P \leq 0.01) \). In addition, the gDLK1 mRNA expression at d 1, 11, and 33 posthatch was significantly higher in broilers than layers \( (P \leq 0.05) \). Therefore, the relatively greater expression of the gDLK1 gene in muscles of broilers compared with layers suggests that gDLK1 may serve as a new selection marker for high muscle growth in chickens. These findings may provide new insight into chicken muscle development and regeneration.

Key words: chicken delta-like protein 1, muscle, differentiation, growth, development

INTRODUCTION

The stages of embryonic myogenesis are determination of myogenic precursor cells, proliferation and migration of myoblasts, fusion of myoblasts into myotubes, and terminal differentiation into functional myofibers. In addition, muscle regeneration from muscle stem cells or satellite cells after muscle injury or exercise partially recapitulates the process of embryonic myogenesis (Schultz and McCormick, 1994; Zhao and Hoffman, 2004; Parise et al., 2006). These developmental processes, accompanied by morphological and biochemical changes of muscle cells, are governed by a complex orchestration of signals that are mediated by myogenic regulatory transcription factors (MRF; Schultz and McCormick, 1994; Buckingham, 2006), the extracellular matrix (Velleman, 1999), and growth factors (McFarland et al., 1993; Tatsumi et al., 1998; McFarland, 1999). The MRF include myogenic factor 5, myogenic differentiation 1 (MyoD), myogenin, and MRF4 (Schultz and McCormick, 1994; Buckingham, 2006). These genes have been used as myogenic markers for indicating different stages of myogenesis. Numerous studies have revealed the expression and involvement of new factors, their functions, and their signaling at specific stages of muscle development (Li and Johnson, 2006; Dogra et al., 2007; Ochi et al., 2008). One of these factors is delta-like protein 1 (DLK1).
Delta-like protein 1 has been referred to as preadipocyte factor 1, mouse stromal cell-derived protein 1, fetal antigen 1, rat adrenal zona glomerulosa, and human adrenal specific cDNA (pG2). Delta-like protein 1 is a glycosylated protein homologous to the Notch/Delta/Serrate family. Delta-like protein 1 has been studied in various areas such as embryonic development (Georgiades et al., 2000), obesity (Moon et al., 2002; Lee et al., 2003), human genetic disease (Berends et al., 1999), skeletal stem cells (Abdallah et al., 2004), ear wound healing (Samulewicz et al., 2002), and muscle development (Davis et al., 2004). Delta-like protein 1 is an imprinted gene paternally expressed in mammalian species and located in syntenic chromosomes 14, 12, and 18 in mice, humans, and sheep, respectively (Berends et al., 1999; Georgiades et al., 2000; Takeda et al., 2006). Dysregulation of DLK1 gene expression in human and mouse genetic diseases leads to developmental abnormalities including either muscle hypotonia in the case of the absence of DLK1 expression or muscle hypertrophy in the case of abnormally high levels of DLK1 expression (Berends et al., 1999; Georgiades et al., 2000; Davis et al., 2004). In addition, callipyge sheep expressing high levels of DLK1 gene expression in postnatal ages are characterized by muscle hypertrophy (Cockett et al., 1996, Takeda et al., 2006). Although the DLK1 gene is involved in muscle development, little is known about the developmental and physiological regulation of chicken DLK1 (gDLK1) during muscle cell differentiation, muscle development, and regeneration.

Delta-like protein 1 mRNA is abundantly expressed in the fetal muscle of mammalian species as well as chickens (Floridon et al., 2000; Shin et al., 2008; White et al., 2008), suggesting that DLK1 may be actively involved in fetal muscle development. Previously, we cloned chicken gDLK1 cDNA, compared its nucleotide and protein sequences to those of mammals, and showed the developmental regulation of gDLK1 mRNA during chicken (White Leghorn) muscle development (Shin et al., 2008). The aims of this study were: 1) to examine whether the gDLK1 mRNA expression is induced at specific stages of myogenesis during chicken primary muscle cell differentiation, 2) to further examine gDLK1 mRNA expression during muscle regeneration, and 3) to investigate if the difference in muscularity between broiler- and Leghorn-type chickens correlates with different levels of gDLK1 mRNA expression during development.

MATERIALS AND METHODS

Experimental Animals

Animal care procedures were approved by The Ohio State University Agricultural Care and Use Committee. A total of 40 layer (White Leghorns) and 40 broiler (Ross 708) eggs were incubated. Five muscle tissues of layers and 5 of broilers were collected at embryonic day (E) 13 and 17. Thereafter, 5 muscle samples of layers weighing ~39 g at posthatch day (P) 1, ~54 g at P5, ~99 g at P11, and ~302 g at P33 and 5 of broilers (weighing ~48 g at P1, ~100 g at P5, ~243 g at P11, and ~1,508 g at P33) were collected after chicks were killed with CO2. Chickens were fed a standard diet ad libitum throughout the growth period. At each ontogenic time point, pectoralis major muscle tissues from layers and broilers were kept in −80°C for isolating total RNA and performing quantitative real-time PCR (qRT-PCR).

The Injury of Chicken Pectoralis Major Muscle

A total of 42 layer chickens weighing ~473 g (White Leghorns, 40 d old) were used to study muscle regeneration because our previous study showed the very low expression of myogenic marker genes [myogenin and paired box gene 7 (Pax7)] and gDLK1 was shown at P33 (Shin et al., 2008), indicating that activities of satellite cells will be low at d 40. Therefore, it was hypothesized that muscle damage induces gDLK1 mRNA during muscle regeneration. The chickens were anesthetized after inhalation of isoflurane, and their skins were incised for exposure of the right breast muscle surface. The tip of a metal rod with a flat 5-mm² surface area was dipped in liquid nitrogen for 1 to 2 min and then held gently against the left breast muscle surface (2 to 3 cm) for 4 to 5 s. The injured chickens were kept warm during recovery from anesthesia. Samples were collected at d 0, 2, 4, 7, 10, and 14) after cold injury for histological analysis and measurement of gene expression levels by qRT-PCR. The undamaged left side of the muscle was used as a control.

Chicken Primary Muscle Cell Culture and Differentiation

White Leghorn eggs were provided by The Ohio State University Poultry Center. Chicken primary muscle cells were isolated from pectoralis major muscles in 13-d-old embryos. The cells were cultured for primary cell muscle differentiation as previously reported (Yablonka-Reuveni and Paterson, 2001). Briefly, the muscle tissues were finely minced and dissociated with 0.05% trypsin-EDTA for 20 min at 37°C (Invitrogen, Carlsbad, CA). The cells were sedimented at 1,500 × g for 5 min at room temperature and subsequently seeded onto 0.01% calf skin collagen-coated plates in Dulbecco's modified Eagle's medium, supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/mL), and streptomycin sulfate (100 µg/mL; Invitrogen). After 2 d, differentiation was induced by incubation in primary muscle cells with differentiation media (Dulbecco's modified Eagle's medium with 2% horse serum) for 3 d. The cells were collected at 0, 12, 24, 36, 48, and 72 h for total RNA isolation, qRT-PCR, and cell morphological images.
**Immunostaining of Myosin Heavy Chain and Staining of Nuclei with 4',6-Diamidino-2-Phenylindole**

Fluorescent immunostaining of myosin heavy chain (MyHC) and nucleus staining were performed in chicken primary muscle cells from d 0 to 3 after differentiation as described previously (Li et al., 2009). Briefly, the cells were rinsed with PBS, fixed in 10% buffered formalin, and rinsed with PBS again. After the cell fixation, the primary antibody [pan sarcomeric MyHC (NA4), 1:500: The Developmental Studies Hybridoma Bank, Iowa City, IA] was incubated with PBS in the fixed muscle cells overnight at 4°C. After several rinses in PBS, the cells were incubated with rhodamine-conjugated goat anti-mouse IgG (1:200, Jackson ImmunoResearch, West Grove, PA) for 1 h at room temperature. In addition, the cells were nuclear-stained with 300 nM of 4',6-diamidino-2-phenylindole (DAPI, Invitrogen) in PBS. The cells were rinsed 5 times with PBS and subsequently imaged using an inverted fluorescence microscope at 100× magnification (Olympus IX50, Olympus, Melville, NY).

**Total RNA Isolation and qRT-PCR**

Chicken pectoralis muscle tissue at selected ages was snap-frozen in liquid nitrogen and homogenized using a Tissuemiser homogenizer (Fisher Scientific, Pittsburgh, PA). Briefly, total RNA isolation and qRT-PCR were performed as described previously (Shin et al., 2008). Total RNA from the tissue was isolated using Trizol (Invitrogen) following the instructions of the manufacturer, and RNA quality was assessed by electrophoresis (1% agarose gel). Reverse transcription was performed using 1 μg of total RNA and Moloney murine leukemia virus reverse transcriptase (Invitrogen). Reverse transcription condition for cDNA amplification was 65°C for 5 min, 37°C for 52 min, and 70°C for 15 min. The qRT-PCR using gDLK1-F2 and gDLK1-R3 primer set was performed to measure the relative levels of gDLK1 expression in muscle tissues. The glyceraldehyde-3-phosphate dehydrogenase gene served as a housekeeping gene as described previously (Lowe and Alnys, 1999; Shin et al., 2008). The qRT-PCR was performed using AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA), and SYBR Green was used as the detection dye. Primer sequences used for the qRT-PCR are shown in Table 1. Primers (gDLK1-F2 and gDLK1-R3) to quantify total gDLK1 gene expression were designed to align with the sequences in exon 4 and 5 of the gDLK1 gene, respectively. Therefore, the primers that span the genomic intron (2.1 kb) between exon 4 and 5 could avoid amplification of contaminated genomic DNA during the PCR reactions. Conditions for qRT-PCR were 95°C for 10 min and 40 cycles of 94°C for 15 s, 60°C for 40 s, 72°C for 30 s, and 82°C for 32 s. Quantitative real-time PCR was performed in duplicate on 25-μL reactions using an ABI 7300 Real-Time PCR Instrument (Applied Biosystems). The relative level of target gene expression, as determined by ABI software, was calculated using the comparative 2−ΔΔCt method for relative quantification (Livak and Schmittgen, 2001).

**Histological Analysis**

The skin was immediately removed from the breast region after euthanizing the birds, and a sample of the pectoralis major muscle, approximately 0.5 × 3.0 cm, was obtained by carefully dissecting muscle fibers following the orientation in a manner to prevent contraction (Velleman et al., 2003). Injury samples were taken from the area (right side of pectoralis major) of muscle injury of the chicken breast muscle, whereas control samples were taken from the uninjured left side of pectoralis major muscle. The tissue samples were dehydrated, cleared, embedded in paraffin, sectioned at 5 μm, and mounted on slides as previously reported (Velleman et al., 2003). Before staining with hematoxylin and eosin, the tissue sections were incubated and rehydrated (Velleman et al., 2002). The stained sections were viewed for muscle morphological characteristics with an Olympus XL 70 microscope and digitally recorded with an Olympus Magna Fire digital camera. Four sections from each bird were placed on a slide. Five fields of each section were viewed.

<table>
<thead>
<tr>
<th>Primer&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Sequence</th>
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<td>gPax7-R</td>
<td>5'-CAC CGT GAG CAC ATG TCC AT-3'</td>
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<sup>1</sup>gDLK1 = chicken delta-like protein 1; gGAPDH = chicken glyceraldehyde-3-phosphate dehydrogenase; gMyoD = chicken myogenic differentiation 1; gMyogenin = chicken myogenic factor 4; gPax7 = chicken paired box gene 7; F = forward; R = reverse (Shin et al., 2008).
Statistics

Results are presented as mean ± SEM. Comparisons between Leghorn and broiler chickens were performed using 1-way ANOVA followed by Tukey’s test at $P \leq 0.05$. Pairwise analysis was performed using Student’s $t$-test at a significant level set at $P \leq 0.05$. All statistical analyses were performed using Minitab software (version 15.0, Minitab Inc., State College, PA).

RESULTS

Induction of gDLK1 mRNA Expression During Chicken Primary Muscle Cell Differentiation

The chicken primary muscle cells were cultured to examine the gene expression of gDLK1 along with the expression of myogenic marker genes and the morphological changes of primary muscle cells during differentiation (Figures 1 and 2). Myosin heavy chain, a marker for nascent myotubes, was not detected at d 0 but started to show as early as d 1. Its staining increased until d 3 after differentiation. The staining of nuclei with DAPI showed that myotubes exhibit multiple nuclei. These observations demonstrate that primary myoblasts were quickly fused into myotubes at d 1 and myotubes became longer and thicker during d 2 to 3 (Figure 1).

The ontogeny expression of myogenic marker and gDLK1 genes are shown in Figure 2. After induction of myogenesis on the chicken primary muscle cells by treatment with differentiation media, MyoD mRNA, an early marker of myogenesis, was gradually downregulated from 0 to 72 h ($P \leq 0.05$). Similarly, before the induction of myogenesis, the mRNA expression level of Pax7, another early marker gene or lineage, was also high at 0 h, significantly decreased to 12 h ($P \leq 0.05$), and remained at low levels to 72 h. On the other hand, myogenin, a late marker of myogenesis, mRNA expression was low at the early time points (12 and 24 h). However, when the early myogenic markers were downregulated at 36 to 48 h, the levels of myogenin were increased by 10- to 20-fold, compared with those at 12 and 24 h ($P \leq 0.05$). The level of myogenin mRNA expression then decreased to a level similar to that at 0 h ($P \leq 0.05$). Similar to the pattern of myogenin mRNA expression from 0 to 12 h, the high expression level of gDLK1 mRNA at 0 h was significantly decreased at 12 h ($P \leq 0.05$). The mRNA expression of gDLK1 was gradually upregulated from 12 to 48 h (around 2.5-fold induction, $P \leq 0.05$) when the myotubes are actively formed and enlarged as shown in Figure 1.

The Temporal Expression of gDLK1 mRNA During Chicken Muscle Regeneration

Along with changes in the morphology of muscle fibers after injury, the expression of gDLK1 gene and myogenic marker genes (MyoD, myogenin, and Pax7) were measured to investigate the coordination of gDLK1 expression with developmental stages of muscle regeneration. Histological analysis at d 0 showed that 40-d-old layer chickens had well-organized myofibers. At d 5 after muscle damage, the injured muscle contained unorganized small fibers with centered nuclei. In addition, an increased cell number caused by infiltration and proliferation of cells was shown in the damaged area at d 5 (Figure 3), indicating the early processes of muscle regeneration. Through d 10 to 14 after muscle injury, the muscle fibers returned to a normal shape as seen in the control tissue at d 0, indicating that the injured muscle was almost recovered (Figure 3).

As shown in Figure 4A, Pax7 mRNA was highly induced at d 2 and 4 by 7- to 10-fold after the injury ($P \leq 0.05$). The high expression level of Pax7 gene was significantly decreased from d 2 to 7 ($P \leq 0.05$). There was no difference in the expression level of Pax7 from d 7 to 14 during the muscle recovery period. In addition, the MyoD mRNA was significantly and highly induced at d 2 by 5-fold after muscle injury ($P \leq 0.05$). Its gene expression level was decreased from d 2 to 4 and significantly downregulated to d 7 when compared with the level of MyoD mRNA expression at d 2 ($P \leq 0.05$). Therefore, the gene expression level of MyoD from d 7 up to d 14 was maintained at the d 0 level (Figure 4B). Unlike early expression of Pax7 and MyoD with peaks at d 2, a relatively late marker, myogenin mRNA expression peaked at d 4 with a 5,000-fold higher level when compared with the level at d 0 ($P \leq 0.05$). Its mRNA expression was significantly decreased from d 4 to 10 ($P \leq 0.05$) but remained over 1,000-fold higher at d 7 and 10, then returned to control levels afterward (Figure 4C).

The mRNA expression of gDLK1 in the uninjured left pectoralis major muscle (control) consistently remained at low levels from d 0 to 14 (Figure 4D). In the injured right pectoralis major muscle, the mRNA expression of gDLK1 was not significantly changed from d 0 to 4. However, it was highly induced from d 4 to 7 ($P \leq 0.05$). At d 7, gDLK1 gene expression in the damaged tissue was about 8-fold higher than the control (Figure 4B, $P \leq 0.05$). The gDLK1 mRNA expression level was gradually downregulated from d 7 to 14 ($P \leq 0.05$), but the level was still highly maintained when compared with the control level.

Comparison of the mRNA Expression Levels of MRF and gDLK1 in the Pectoralis Major Muscles of Leghorns with Those Levels of Broilers

The comparison of the gene expression patterns or levels of MRF and gDLK1 of broilers and layers during embryonic skeletal muscle development and growth after hatching was investigated (Figure 5). As shown in Figure 5A, both layers and broilers expressed similar
levels of Pax7 mRNA at embryonic muscle developmental stages (E13 and E17). In layers, the mRNA expression level of Pax7 was decreased 29-fold from E17 to P1 ($P \leq 0.05$). In contrast, its expression level in broilers was decreased 7.7-fold from E17 to P1 ($P \leq 0.05$). This resulted in a significantly different level of Pax7 mRNA expression between them at P1 ($P \leq 0.05$). At P5, however, the expression of Pax7 from P1 was maintained.

**Figure 1.** Differentiation of chicken primary muscle cells. The microscopic images of primary muscle cells at d 0 to d 3 indicate the morphological changes during cell differentiation. The muscle cells isolated from pectoralis major muscle tissue of a 13-d embryo were cultured in differentiation medium. The differentiated muscle cells at each sampling time were analyzed by the immunostaining myosin heavy chain (MyHC) and staining nuclei with 4,6-diamidino-2-phenylindole (DAPI). The white bar scale = 50 μm.
in layers, whereas its expression level in broilers tended to decrease. There were no significant differences of the Pax7 gene expression level between the 2 selected chick lines from P11 to P33.

A declining pattern of MyoD gene expression in both types of chickens was shown from E13 to P33 (Figure 5B). In layers, MyoD gene expression decreased sharply from E13 to P1 by 5-fold \( (P \leq 0.05) \) and maintained from P1 to P5 until P33. In broilers, however, its sharp decline (25-fold) was found from P1 to P5 \( (P \leq 0.05) \). This resulted in significantly different levels in MyoD mRNA expression between the 2 chickens at P1 (4-fold higher in broilers, \( P \leq 0.001 \)) and P5 (3-fold higher in layers, \( P \leq 0.01 \)), respectively. In addition, a decreased level of MyoD mRNA expression in layers was found from P5 to P11, resulting in a 2-fold lower expression level than broilers \( (P \leq 0.05) \).

The level and pattern of ontogenic gDLK1 mRNA expression in the pectoralis major muscle in both types of chickens is shown in Figure 5C. Gene expression of the layer gDLK1 at E13 and E17 declined significantly to P5 \( (P \leq 0.05) \) and remained at that level. Similarly, broiler gDLK1 gene expression was the highest at E13, significantly downregulated to E17 \( (P \leq 0.05) \), and remained unchanged until P1. Subsequently, the gene expression of broiler gDLK1 was significantly downregulated from P1 to P5 \( (P \leq 0.05) \), and the expression level of broiler gDLK1 gene remained unchanged from P5 until P33. The gene expression levels of gDLK1 at various sampling times ranged from E13 to P33 when compared between broilers and layers. There were significantly higher expression levels of gDLK1 mRNA in broilers than layers at time points E13 \( (2.5\text{-fold}, P \leq 0.05) \), P1 \( (2.5\text{-fold}, P \leq 0.01) \), P11 \( (2\text{-fold}, P \leq 0.005) \), and P33 \( (3\text{-fold}, P \leq 0.01) \). The exceptions were at E17 and P5.

**DISCUSSION**

Myogenic transcription regulatory factors that function in processing myogenesis and their expression have been used as indicators of muscle development as well as regeneration (Buckingham, 2006; Shi and Garry, 2006). In the current study, a chicken primary muscle cell culture system was used to determine the temporal expression of gDLK1 during muscle development.

![Figure 2](https://academic.oup.com/ps/article-abstract/88/7/1427/1549490)
The gradual reduction of expression of early myogenic marker genes (Pax7 and MyoD) during the differentiation period, and induction of a late marker gene (myogenin) at the time of active myotube formation (Nabeshima et al., 1993; Dedieu et al., 2002), indicates that primary chicken muscle cells can serve as an excellent in vitro system for evaluating stages of muscle development. In addition, immunostaining using MyHC and DAPI staining also confirmed that chicken primary myoblasts successfully underwent myogenesis. The gene expression of gDLK1 (Figure 2) was induced at the late stages of muscle development when the myotubes were actively forming and elongating (Figure 1), suggesting a possible involvement of gDLK1 in these developmental processes.

Muscle regeneration has been used as an excellent in vivo model for investigating muscle growth and developmental biology. Its advantages include the following: 1) reestablishment of the activation of quiescent satellite cells (Halevy et al., 2004), 2) recapitulation of the developmental processes during embryonic myogenesis (Parker et al., 2003), and 3) prediction of myogenic functions of genes at the specific stages of muscle development (Jørgensen et al., 2009). Sequential induction of marker genes during the developmental process of muscle regeneration has been demonstrated in mice (Garry et al., 2000; Goetsch et al., 2003). Based on morphological characteristics of regenerating chicken muscles, the recovery period of chicken muscle is between 2 and 3 wk, which is similar to the mouse. In this study, the sequential induction of marker genes was clearly shown in regenerating chicken muscles: induction of early myogenic gene (Pax7 and MyoD) expression at d 2 to 4 postinjury, the late myogenic gene (myogenin) expression at d 4 to 10 postinjury, and the gDLK1 gene expression at d 7 to 14 postinjury.

The expression patterns of Pax7 and MyoD suggest the potential importance of their roles in the early stages of muscle regeneration. Similarly, the sequential expression of these genes was also found during the differentiation of primary chicken muscle cells. This indicates that in vitro myogenic gene expression patterns are recapitulated during regenerative myogenesis. Importantly, the late induction of gDLK1 during in vitro myogenesis and also muscle regeneration suggests that gDLK1 gene expression is highly associated with the late stages of muscle development including myotube formation. In support of this, our unpublished data and

Figure 3. Histology of skeletal muscles in Leghorn chickens during muscle regeneration. The histological tissue sections for undamaged pectoralis muscle at d 0 and damaged pectoralis muscle 5, 10, or 14 d after muscle injury were stained with hematoxylin and eosin. The white bar scale = 50 μm.
previous reports showed that DLK1 protein was exclusively found in the developing myotubes in the fetus of humans and mice (Floridon et al., 2000; Yevtodyenko and Schmidt, 2006). Our data also suggest that the temporal expression of gDLK1 may be involved in tissue regeneration and differentiation of stem or satellite cells, or both.

The effect of genetic selection on muscle growth and development has been widely investigated in the avian species of chickens (Aberle and Stewart, 1983; Scheuermann et al., 2004), turkeys (Velleman et al., 2003), and quail (Campion et al., 1982; Ye et al., 1999). In chickens, broilers are genetically selected for meat production, whereas Leghorn layers are selected for egg production. Many investigators have reported that broilers have larger diameters of myofibers (Smith, 1963), a more rapid rate of myofiber radial hypertrophy (Aberle and Stewart, 1983), greater numbers of myofibers (Scheuermann et al., 2004), a faster proliferation rate of muscle cells (Blunn and Gregory, 1935; Moss, 1968), and, hence, more muscle mass (Mizuno and Hikami, 1971) than layers. In the current study, we investigated whether the temporal expression of myogenic marker genes and the gDLK1 gene during avian muscle development and growth can account for the distinct different muscle growth characteristics between the 2 lines of chickens. Relatively high levels of gDLK1 mRNA were expressed in developmental muscles of broilers compared with those of layers (Figure 5). The greater expression of gDLK1 in broilers than layers may affect muscle hypertrophy in broilers.

The promyogenic activities of DLK1 were demonstrated by muscle hypertrophy in callipyge sheep (White et al., 2008) and transgenic mice ectopically overexpressing DLK1 (Davis et al., 2004). In addition, a paternally imprinted DLK1 gene has been reported to be involved in genetic diseases associated with muscle development. Absence of the DLK1 gene expression in the human genetic disease (maternal UPD14 chromosome) causing hypotonic muscles suggests that DLK1 plays an important role in the muscular development of humans (Berends et al., 1999). Similarly, absence of DLK1 gene expression in the mouse having maternal UPD12 chromosome caused muscle hypotonia (Georgiades et al., 2000). In contrast, double-dose expression of the DLK1 gene in the mouse paternal UPD12 chromosome exhibited myofiber hypertrophy (Georgiades et al., 2000). The greater expression of gDLK1 in broilers could positively affect muscle growth in broilers, implicating it as a new selection marker for large muscling.

![Figure 4](https://academic.oup.com/jcb/article-abstract/88/7/1471/1549490)
The high levels of Pax7 and MyoD mRNA expression at the embryonic stages suggest that active myogenesis occurs during embryonic development in both Leghorns and broilers (Figure 5). It has been found that Pax7 is required for the specification of satellite cells (Seale et al., 2000) and serves as an early marker for proliferation of satellite cells (Halevy et al., 2004), providing new mononuclei for growing myofibers (Moss and Leblond, 1971). Therefore, the relatively higher mRNA expression level of Pax7 in the muscles of broilers at P1 compared with layers may indicate that broilers have a greater population of reserve satellite cells than layers. In addition, MyoD is required for myoblast differentiation by controlling the cell cycle exit (Zhang et al., 1999). This supports our conclusion that the relatively higher levels of MyoD in broilers from embryonic to posthatching days than layers contribute to the extended differentiation of muscle cells in broilers. Taken together, relatively high expression levels of MyoD and Pax7 after hatching support the conclusion that broilers have a larger population of reserved satellite cells that undergo differentiation for a relatively longer period of time to increase muscle mass.

We report here the temporal expression of gDLK1 mRNA during in vitro muscle cell differentiation and in vivo muscle regeneration in chickens. In addition, the greater expression of the gDLK1 gene along with the higher levels of expression of the myogenic genes (MyoD and Pax7) in broilers seem to contribute to higher myogenic activities and greater muscle mass compared with Leghorns. Furthermore, because of the apparent critical role of DLK1 to muscle hypertrophy, our results suggest that gDLK1 can be a new candidate marker for selection of high muscularity in chickens. The mRNA expression of gDLK1 followed by the peak expression of myogenic markers (MyoD, Pax7, and myogenin) was clearly shown in injured muscle tissue and primary muscle cell differentiation, indicating that gDLK1 may be essential for a specific stage of muscle development. However, the mechanism of gDLK1 role in skeletal muscle development remains to be further investigated.

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