ABSTRACT Delta-like protein 1 (DLK1) is involved in adipose and muscle development as shown by the reduction of fat mass in DLK1 transgenic mice and in muscle hypertrophy of callipyge sheep. However, no study on DLK1 has been investigated in avian species. Cloning and sequencing of a full length of chicken DLK1 (gDLK1) complementary DNA revealed that gDLK1 contains a total of 1,161 bp, encoding 386 amino acids. The similarity of gDLK1 nucleotide and protein sequences was over 50% compared with other mammalian species. In addition, chickens only express one full length of gDLK1 in various tissues at different ages without the alternative splicing variants of DLK1 found in mammalian species. This suggests that the full-length form of gDLK1 may be sufficient for normal development in the chicken. In adipose tissue, the gDLK1 gene was highly expressed in preadipocytes as compared with adipocytes (P < 0.05), whereas expression levels of adipogenic marker genes such as stearoyl-coenzyme A desaturase 1 (SCD-1) and fatty acid binding protein 4 (FABP4) were higher in mature adipocytes than in preadipocytes (P < 0.05 and P < 0.01, respectively). Expression of gDLK1 in adipose tissue tends to decrease with age. The expression of gDLK1 gene in the pectoralis major muscle was significantly higher in 13- and 17-d-old embryos (P < 0.05), decreased in 1- and 5-d-old chicks (P < 0.05), and further decreased in 11- and 33-d-old chickens (P < 0.05). This expression pattern of gDLK1 was very similar to the expression patterns of myogenin and Pax7 genes, suggesting a close association with myogenic activities. In conclusion, the developmental regulation of gDLK1 expression might play an important role in the early stages of adipose and muscle tissue development.

INTRODUCTION

Delta-like protein 1 (DLK1) has been known as Pref-1, SCP-1, FA-1, Zog, and pG2. The DLK1 is a transmembrane glycoprotein containing 6 epidermal growth factor repeats homologous to the Notch/Delta/Serrate family. The DLK1 gene has been widely studied in embryo 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), and ear wound repair (Samulewicz et al., 2002). The DLK1 is an imprinted gene that is expressed from the paternal allele in mammals (Sutton and Shaffer, 2000; Lin et al., 2007). Recent studies using DLK1 transgenic mice models have provided direct evidence that DLK1 is a promoting factor of muscle development (Davis et al., 2004) and an inhibitory factor of adipose development (Lee et al., 2003). These characteristics of DLK1 may be useful for producing maximum meat yield from animals by increasing muscle mass and decreasing fat content.

The callipyge phenotype in sheep shows an inherited muscular hypertrophy with larger longissimus muscle area and leg scores, resulting in a 42 to 50% increase in the muscles compared with those of normal sheep (Cockett et al., 1996; Jackson et al., 1997). The gene responsible for this increased muscle mass in sheep was recently identified as DLK1 with the phenotype inherited from the paternal allele (Takeda et al., 2006). Recent studies on transgenic mice overexpressing the DLK1 gene confirm the pro-myogenic function of DLK1 (Davis et al., 2004). Although the DLK1 gene expression is associated with muscle growth and development, the DLK1 gene has not been reported in the avian species.

The DLK1 gene has been extensively studied in the areas of adipocyte development and obesity. For example, previous studies demonstrated that DLK1 gene expression is decreased during adipocyte differentiation in vivo and in vitro (Smas and Sul, 1993; Deiuliis et
al., 2006). Our previous in vivo studies clearly indicate that overexpression of the DLK1 gene in adipose tissue of transgenic mice inhibited adipocyte development (Lee et al., 2003) and that the knockout of the DLK1 gene induced the obesity phenotype in mice (Moon et al., 2002). An increasing number of studies show that the hormonal and genetic modulation of adiposity successfully used DLK1 as a marker of preadipocyte and adipocyte development. However, the DLK1 gene has never been studied in adipose development in poultry.

It has been reported that DLK1 has several isoforms generated by alternative splicing in the tissues of various species (Smas et al., 1994, 1997; Vuocolo et al., 2003; Deiuliis et al., 2006). Therefore, the first aim of this study was to clone and sequence chicken DLK1 gene (gDLK1), compare its nucleotide and deduced protein sequences with mammalian species, and investigate the possible alternative splicing of gDLK1 transcript. A second objective was to investigate developmental regulation of the DLK1 gene in chickens and assess how specific developmental patterns of gDLK1 expression correlate with the specific stages of chicken muscle and adipose development. Our current studies on chicken DLK1 were initiated to enhance our basic understanding of the DLK1 gene in avian muscle and adipose development.

MATERIALS AND METHODS

Experimental Birds

Animal care and procedures were approved by the OSU Institutional Animal Care and Use Committee. A total of 40 chicken eggs (White Leghorn) were incubated and sampled at various developmental times [embryonic day (E) 13, 17 and posthatch day (P) 1, 5, 11, and 33]. Hatched chicks were fed a standard diet ad libitum throughout the growth period. At each developmental time, pectoralis major muscle and fat tissues were collected from 4 or 5 chicks per group and kept at −80°C for total RNA isolation, regular or quantitative real-time PCR (qRT-PCR), and Western blotting. To investigate alternative splicing variants of gDLK1, chicken pectoralis muscle, heart, fat, liver, lung, and intestine tissues at E17 were snap frozen in liquid nitrogen and kept at −80°C for total RNA isolation, reverse transcription-PCR, and qRT-PCR.

Cloning of Chicken DLK1

Chicken DLK1 was cloned using a set of forward and reverse primers designed according to 2 predicted DLK1 sequences: The Institute for Genome Research (TIGR) database sequence (TC214622) and National Center for Biotechnology Information (NCBI) database sequence (XM_421369.1). Briefly, chicken DLK1 gene was amplified by using 2 primer sets: gDLK1-F1 (5’-CCA GAG GCC CCA ACA TGA G-3’) and gDLK1-R1 (5’-ACC TGC ACC AAT ATC TGT GCA CG-3’) as shown in Figure 1. Chicken complementary DNA (cDNA) from embryonic muscle tissue total RNA was used as a template for PCR. The putative gDLK1 cDNA was ligated to the PCR 2.1 vector after gel extraction using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer. The TOP 10 chemical competent cells (Invitrogen) were transformed with the PCR 2.1 plasmid including the putative gDLK1 insert. Transformants were grown on a kanamycin agar plate containing X-gal. Plasmids isolated from positive colonies were sequenced by The Ohio State University Sequencing Core Facility using an Applied Biosystems 3730 DNA Analyzer.

Chicken Stromal-Vascular and Adipocyte Cell Fractionation

Chicken stromal-vascular (SV) and adipocyte cell fractionation was performed by following the cell fractionation procedure of Deiuliis et al. (2006, 2008). Briefly, adipose tissue (3 to 5 g) was isolated from 20-d-old chickens (n = 4), minced, and incubated with 3.2 mg/ml of collagenase II (Sigma-Aldrich, St. Louis, MO) for 1 h in a shaking water bath (180 rpm, 37°C) to separate adipose tissue into SV and adipocyte fractions. The suspension was passed through a 100-μm nylon cell strainer (BD Falcon, Franklin Lakes, NJ) to remove undigested tissue. The filtrate was centrifuged at 200 × g for 5 min. The top layer (adipocyte fraction) and the pellet (SV fraction) were collected for total RNA isolation.

RNA Isolation, Regular PCR, and Quantitative Real-Time PCR

Chicken pectoralis muscle, heart, fat, liver, lung, and intestine tissues were snap frozen in liquid nitrogen and homogenized using a Tissuemiser homogenizer (Fisher Scientific, Pittsburgh, PA). Total RNA from the tissue was isolated using Trizol (Invitrogen) following the manufacturer’s instructions, and RNA quality was assessed by electrophoresis. Reverse transcription was performed using 1 μg of total RNA and M-MLV reverse transcriptase (Moloney murine leukemia virus RT, Invitrogen). Reverse transcription conditions for each cDNA amplification were 65°C for 5 min, 52°C and 70°C for 15 min. To examine whether chickens have alternative splicing isoforms of gDLK1, the regular PCR was performed with gDLK1-F1 and gDLK1-R1 primer set to amplify the full length of gDLK1 for various tissues and ages. Regular reverse-transcription-PCR conditions were 95°C for 2 min and 37 cycles of 94°C for 15 s, 60°C for 1 min, and 72°C for 1 min. The PCR products were separated in 1% agarose gel. In addition, qRT-PCR using gDLK-F2 and gDLK1-R3 primer set was performed to measure the relative levels of gDLK1 expression in muscle and adipose tissues. The gyleraldehyde-3-phosphate dehydrogenase (GAPDH)
gene served as a housekeeping gene. The gene expression was quantified by SYBR green real-time PCR as described previously (Deiuliis et al., 2006, 2008). The 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; Figure 1) to quantify total

**Figure 1.** The putative nucleotide sequence of chicken delta-like protein 1 (gDLK1) complementary DNA cloned from embryonic muscle tissue at d 14 with a length of 1,161 bp. The boldfaced nucleotides indicate the start codon and the stop codon of gDLK1. The arrows indicate the primer sets for the cloning and quantitative real-time PCR (qRT-PCR). The primers (gDLK1-F1/R1) were designed for cloning the full length of gDLK1, whereas the primers (gDLK1-F2/R3) were designed to perform qRT-PCR for the gDLK1 mRNA amplification.
gDLK1 gene expression were designed to align to the sequences in exon 4 and 5 of the chicken DLK1 gene, respectively. Therefore, the primers that span genomic intron (2.1 kb) between exon 4 and 5 could avoid amplification of contaminated genomic DNA during the PCR reactions. Conditions for the 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. Real-time PCR was performed in duplicate in 25-μL reactions on 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 $2^{-\Delta\Delta C_{T}}$ method for relative quantification (Livak and Schmittgen, 2001).

Bioinformatics, Sequence Analysis, and Statistical Analysis

Applied Biosystems Sequence Scanner v1.0 was used to analyze the chromatogram. Homology analysis was performed using BLAST at NCBI as described previously (Deiuliis et al., 2006). Sequence alignment and comparison in addition to protein translation were done using the ClustalX and GeneDoc software (Deiuliis et al., 2006, 2008). Results are presented as mean ± SEM. Comparison of 2 means was accomplished by a Student’s $t$-test at $P < 0.05$ and 0.01. Comparisons among gene expression data were performed using 1-way ANOVA followed by the Tukey’s test at $P < 0.05$. Statistical analysis was performed using Minitab software (version 15.0).

RESULTS

Chicken DLK1 Gene Cloning and Sequence Comparisons

The gDLK1 cDNA was cloned and sequenced (GenBank # EU_288039), which is 1,161 bp and encodes 386 amino acids (Figures 1, 2, and 3). The multiple nucleotide sequence comparison between species (using ClustalX and GeneDoc software programs) showed that gDLK1 has 55, 55, 56, 56, and 55% similarity to that of the mouse, rat, human, pig, and cattle, respectively (Figure 2). In addition, the alternative splicing sites such as acceptors or donors of B, C, C2, D, D2 forms of gDLK1 gene at the fifth exon were not conserved across mammals (Figure 2). Putative protein sequence comparison also showed that the amino acid sequence of gDLK1 has 48% (65%; similar amino acid group), 49% (65%), 51% (65%), 51% (65%), and 50% (65%) similarity to that of the mouse, rat, human, pig, and cattle, respectively (Figure 3). Interestingly, the gDLK1 protein sequence contains a 7-cysteine repeat (C11 to C17 from N terminus), which is not contained in mammalian species (Figure 3). The first 2 N-linked glycosylation sites (black arrow) across species also were present in the chicken; however, the third located in the juxtamembrane region of the protein was substituted with glutamine (Figure 3). Furthermore, the alternative splicing donor or acceptor sites (black diamond arrow) for DLK1-B and C2 forms among others were not conserved in those of gDLK1 amino acid sequence (Figure 3).

Identification of Alternative Splicing in Tissue Distribution and In Vivo Fat and Muscle Developmental Expression of gDLK1

Reverse-transcription-PCR was performed for tissue distribution of a full length of gDLK1 mRNA using total RNA isolated from the pectoralis major muscle, heart, fat, liver, lung, and intestine collected at E17. As shown in Figure 4A, only a single PCR product was detected in these tissues of egg-type chickens. Next, alternative splicing transcripts of gDLK1 were investigated in fat and muscle tissue from E13 to P33. There were no alternative splicing variants of the gDLK1 gene during chicken adipose and muscle development (Figure 4B and 4C). Therefore, it was confirmed that chickens express only the A form of gDLK1 mRNA in multiple tissues and ages in adipose and muscle tissues.

Table 1. Primer sequences for quantitative real-time PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Product size (bp)</th>
<th>GenBank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>gDLK1-F2</td>
<td>5'-TGT GTG CCC AGG GAT TTA CAG GA-3'</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>gDLK1-R3</td>
<td>5'-ACC TGC ACC AAT ATC TGT GCA CG-3'</td>
<td>262</td>
<td>NM_204305</td>
</tr>
<tr>
<td>gGAPDH-F1</td>
<td>5'-CTC TGT TGT TGA CCT GAC CTG-3'</td>
<td>216</td>
<td>NM_204290</td>
</tr>
<tr>
<td>gGAPDH-R1</td>
<td>5'-CAA GTC CAC AAC ACA GGT GCT-3'</td>
<td>180</td>
<td>X60465</td>
</tr>
<tr>
<td>gFABP4-F</td>
<td>5'-TCA CAT CAG AAA GAA GTA CCT TCA A-3'</td>
<td>342</td>
<td>D90157</td>
</tr>
<tr>
<td>gFABP4-R</td>
<td>5'-GTC ACG ATT CAT GGT GCA T-3'</td>
<td>206</td>
<td>NM_205065</td>
</tr>
<tr>
<td>gMyogenin-F</td>
<td>5'-CAG TCC CAT GCA GCC TCA ACC AGC A-3'</td>
<td>342</td>
<td>D90157</td>
</tr>
<tr>
<td>gMyogenin-R</td>
<td>5'-ACT CCT CCA GCA TCA CCA CCA T-3'</td>
<td>206</td>
<td>NM_205065</td>
</tr>
</tbody>
</table>

$^1$gDLK1 = chicken delta-like protein 1; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; FABP4 = fatty acid binding protein 4; SCD-1 = stearoyl-Coenzyme A desaturase 1; Pax7 = paired box transcription factor; F = forward; R = reverse.
Figure 2. Alternative splicing variants of delta-like protein 1 (gDLK1)-A between the chicken and selected mammalian species. The nucleotide alignments of gDLK1 with mammals. The known 5′ (arrow) and 3′ (diamond) splice sites of DLK1 B, D, C2, D, and D2 forms in the mouse (GenBank no. BC052159), rat (GenBank no. NM053744), human (GenBank no. BC007741), pig (GenBank no. NM001048187), and cow (GenBank no. AF181466) are illustrated. Black shading indicates identical nucleotides, and gray shading indicates highly conserved nucleotide sequence stretches.
Figure 3. Putative amino acid sequence comparison of chicken delta-like protein 1 (gDLK1; A-form) among species. The arrows indicate the deduced N-linked glycosylation sites in the protein. Black shading indicates identical amino acids and gray shading indicates highly conserved amino acid sequence stretches. Chicken DLK1-A lacks the third N-linked glycosylation site at amino acid 302, which is highly conserved in the human, rat, mouse, and cow. Instead, the putative third N-linked glycosylation site is at amino acid 259, unique to the chicken.
The Relative gDLK1 Gene Expression in the Stromal-Vascular and Adipocyte Fractions, and During Chicken Adipose Development

It is of interest to examine the gene expression of gDLK1 with other adipogenic marker genes such as stearoyl-Coenzyme A desaturase 1 (SCD-1) and fatty acid binding protein 4 (FABP4) in 2 types of fractionated cells, SV and adipose cells. The SV cells contain mostly preadipocytes, whereas adipose cells contain mostly mature adipocytes. The levels of FABP4 and SCD-1 mRNA expression were significantly greater in mature adipocytes than those levels in preadipocytes ($P < 0.01$ and $P < 0.05$, respectively) as shown in Figure 5A. However, the level of gDLK1 mRNA expression was significantly higher in preadipocytes than in mature adipocytes ($P < 0.05$). The in vivo temporal expression of gDLK1 during adipose tissue development was further investigated. The gDLK1 mRNA expression tended to be low at E17 and tended to increase at P1 as shown in Figure 5B. Thereafter, it seemed gradually downregulated until P33 during adipose development, although there was no statistical significance in differences ($P = 0.18$).

The Relative Temporal Expression of gDLK1 Gene During Chicken Muscle Development

The gene expression of gDLK1 with other myogenic marker genes such as Pax7 and myogenin during chicken muscle development was shown in Figure 6. The mRNA expression of gDLK1 was gradually downregulated during muscle development. First, it was significantly expressed at E13 and E17 ($P < 0.05$) and thereafter, 4-fold decreased at P1 and P5 ($P < 0.05$) until P11 and P33 ($P < 0.05$). The gene expression of Pax7 was significantly greater at E13 and E17 than at other time points, 34-fold decreased after posthatch until P33 ($P < 0.05$). Myogenin mRNA was highly expressed at E13 and significantly decreased until P1 ($P < 0.05$), and slightly increased at P5 and downregulated up to P33 ($P < 0.05$).

DISCUSSION

Improving muscle growth and reducing the amount of fat in avian species are essential in maximizing poultry production. Understanding the role of factors and their interplay in muscle and adipose development will lead to strategies to ultimately improve muscle growth and decrease fat deposits. The pro-myogenic and anti-adipogenic functions of DLK1 have been known in mammalian species (Lee et al., 2003; Davis et al., 2004). Here, we report for the first time cloning, sequencing, alternative splicing, and temporal expression of gDLK1 during muscle and adipose development in the chicken.

The DLK1 gene has several spliced transcripts generated by alternative splicing in the fifth exon across various species. A total of 6 different isoforms of DLK1 such as DLK1-A, B, C, C2, D, and D2 have been identified in mice (Smas et al., 1994, 1997). Pigs, cattle, and sheep have mainly the DLK1-C2 isoform. Humans express only the A form of DLK1. Moreover, the role of DLK1 isoforms has been studied in cell culture as well as in animals (Smas et al., 1997, 1994; Davis et al., 2004). Overexpression of A and B forms of DLK1 inhibits adipocyte differentiation (Mei et al., 2002; Lee et al., 2003). Callipyge sheep primarily express the C2 form with minor expression of the A form (Davis et al., 2004). Overexpression of the C2 form of mouse DLK1 in the muscle of transgenic mice appears to recapitulate...
the callipyge phenotype. Our previous studies with pig DLK1 showed that the C2 form was the most abundant isoform, whereas the B form was present to a lesser extent (Deiuliis et al., 2006). Similar to humans, chickens express only the A form, which is a full-length form of chicken DLK1. In addition, no detectable amplification of other splicing transcripts was shown in various tissues including adipose and muscle tissues at different ages of chickens (Figure 4). An absence of alternative splicing of gDLK1 transcripts can possibly be explained by a comparative point of view in their sequences across the species. Our comparative analysis of the multiple nucleotide sequences of DLK1 genes (Figure 2) revealed that the alternative splicing donor or acceptor sites of B, C, C2, D, and D2 of DLK1 shown in mammalian species were not conserved at the fifth axon of chicken DLK1 nucleotide sequences. In addition, the obligatory adenosine residue [CT(A/G)A(C/T)] of a lariat structure or a branch point conserved in mammalian species (Figure 2), which is required for steps of alternative splicing, was not found in chickens. Therefore, a lack of conserved sequences for the formation of a branch point as well as splicing donor and acceptor sites may result in chickens expressing only the A form of DLK1. From the evolutionary point of view, it appears that chickens have evolved to have only the A form of DLK1, which is sufficient for normal tissue and organ development.

Glycosylation affects the structure and function of many proteins in eukaryotes. The glycosylation of DLK1 protein has been reported in the mammalian

Figure 5. The chicken delta-like protein 1 (gDLK1) gene expression in preadipocytes and adipocytes fractionated from chicken adipose tissues, and during adipose tissue development. (A) Expression levels of gDLK1, stearoyl-coenzyme A desaturase 1 (SCD-1), and fatty acid binding protein 4 (FABP4) genes in adipose tissue were measured by quantitative real-time PCR (n = 4). The bar represents mean ± SEM. Significant difference is indicated by *, ** between preadipocytes and adipocytes at P < 0.05 and P < 0.01 by using the Student t-test. SV = stromal-vascular fraction; FC = fat cell fraction. (B) Expression levels of gDLK1 gene in adipose tissue at indicated ages of chickens were measured by quantitative real-time PCR (n = 4 to 5 at each time point). The bar represents mean ± SEM. NS: (P > 0.05). GAPDH = glyeraldehyde-3-phosphate dehydrogenase; E = embryonic day; P = posthatch day.
species. As shown in Figure 3, the full length of DLK1 proteins of mice, rats, humans, and cattle have 3 conserved N-linked glycosylation sites at the 105th, 139th, and 301st amino acid residues. However, like the pig, chicken DLK1 protein does not have the third N-linked glycosylation site of DLK1 amino acid sequences. The splicing of DLK1 mRNA into the C2 form generates a major isoform in pigs, cattle, and sheep, resulting in deletion of amino acids from 237 to 312, including the third glycosylation site at 301. The deleted amino acid sequences of DLK1 are most variable across species, conceivably being evolved under less evolutionary pressure to keep a glycosylation site in this residue of gDLK1 protein. However, future studies on the biological function of DLK1 glycosylation in various cells and tissues of many species need to be performed.

Adipose tissue contains many types of cells including mainly adipogenic cells at various stages of adipocyte development. Fractionation of individual cells based on the densities of cells containing various amounts of lipids could separate 2 major populations of cells; the SV fraction contains mostly preadipocytes, and the adipocyte fraction contains adipocytes filled with lipids. Comparing the expression of target genes in 2 different fractions of cells enables us to determine which fraction of cells dominantly expresses the target gene and to understand developmental regulation of gene expression in vivo. Two well-known adipogenic marker genes, SCD-1 and FABP4, were predominantly expressed in the adipocyte fraction (Cohen et al., 2002; Lee et al., 2003), indicating successful fractionation of cells in chicken adipose tissue. In addition, our data showed that DLK1 was highly expressed in the SV fraction of cells in chicken adipose tissue. In general, DLK1 expression was found in many cell types at undifferentiated stages (Carlsson et al., 1997; Costaglioli et al., 2001; Tanimizu et al., 2004; Abdallah et al., 2007), although it is absent at the terminal differentiation stage. Like in the mouse and the human, DLK1 can be used as a marker gene for preadipocytes in chickens.

The function of DLK1 in cellular development has been mostly studied in adipocyte development. Our previous studies clearly indicate that overexpression of the DLK1 gene in adipose tissue of transgenic mice inhibited adipocyte development, and knockout of the DLK1 gene induced the obesity phenotype in mice (Moon et al., 2002; Lee et al., 2003). In chickens, it has been reported that the rapid growth of adipose tissue and its enlargement after the hatching period depends on adipocyte differentiation and fat storing (Leclercq, 1984). Considering the antiadipogenic activities of DLK1, the downregulation of the gDLK1 gene during adipose tissue development indicates that DLK1 is negatively correlated with adipose tissue development in the chicken.

The importance of DLK1 for the fetal and neonatal muscle development has been demonstrated by the high levels of the DLK1 expression during fetal development and myosregulation of the DLK1 gene in human and

Figure 6. The temporal gene expression of chicken delta-like protein 1 (gDLK1) during muscle development. Expression levels of gDLK1, Pax7, and myogenin gene in muscle tissue at indicated ages were measured by quantitative real-time PCR (n = 4 at each time point). The bar represents mean ± SEM. The letters (a–c) represent a significant difference among groups at various time points by using 1-way ANOVA at \( P < 0.05 \). GAPDH = glyeraldehyde-3-phosphate dehydrogenase; E = embryonic day; P = posthatch day.
mouse genetic diseases affecting muscle development (Berends et al., 1999; Georgiades et al., 2000; Schmidt et al., 2000; Yevtodiyenko and Schmidt, 2006). Like in normal sheep (Fleming-Waddell et al., 2007; White et al., 2008), our current study showed that gDLK1 gene expression in the muscle was the highest in the fetal stages, whereas its expression was decreased during the posthatch period. Therefore, these suggest that high levels of DLK1 expression are generally associated with active myogenesis that occurs during the fetal muscle development.

The processes of embryonic or fetal myogenesis have been characterized with expression of myogenic marker genes such as myogenin and Pax7 (Hasty et al., 1993; Zammit et al., 2006). Myogenin is one of the transcription regulatory factors that plays an important role in myoblast differentiation and myofiber formation (Nabeshima et al., 1993; Yablonka-Reuveni and Patterson, 2001). Along with Pax7 and myogenin, gDLK1 gene expression level was investigated during chicken muscle development. The high expression of myogenin gene in the embryonic muscle may indicate the active processes of chicken myoblast differentiation and myotube formation. During the posthatch period, there was a sharp decline in the expression of the myogenin gene in 1-d-old chicks followed by a rebound of myogenin gene expression in 5-d-old chicks in our study. A similar pattern of myogenin protein levels was reported by Halevy et al. (2004) who showed low levels of myogenin protein at hatch and the increased expression of myogenin in 3-d-old chicks. The expression pattern of myogenin suggests that there is a transient suppression of myogenin expression at hatching because of the possible associated stress of posthatch starvation. It has been reported that posthatch starvation resulted in decreases in myofiber size in avian species (Haley et al., 2000; Moore et al., 2005).

Pax7 has been known as a marker gene required for the specification of satellite cells that are important for postnatal muscle growth and muscle regeneration (Seale et al., 2000; Haley et al., 2004). The high expression of Pax7 gene may be indicative as a proliferating marker of satellite cells at fetal stages (Haley et al., 2004). The levels of Pax7 gene expression during embryonic or fetal skeletal muscle development were seen in normal and callipyge sheep (White et al., 2008), chickens (Otto et al., 2006), and mice (Relaix et al., 2005). Our current study also shows that the Pax7 gene was highly expressed in fetal muscle and dramatically downregulated after hatch. This indicates that satellite cells may be actively proliferating during embryonic stages and inactivated after the hatching period. Alternatively, the downregulation of Pax7 gene expression may represent the loss of mononucleated cells as they fuse into myofibers. Similar to the expression patterns of myogenin and Pax7 during muscle development, the expression levels of gDLK1 gene were very high in embryonic stages, declined during the early posthatch period, and further decreased with increasing age. Taken together, the abundance of gDLK1 mRNA is correlated with the expression levels of myogenin and Pax7 genes and closely associated with myogenic activities.

Here, we report for the first time that chickens express only the A form of gDLK1 (gDLK1-A), which is 1,161 bp, encoding 386 amino acids. The expression of gDLK1-A in various tissues at different ages of chickens suggests that the gDLK1-A is sufficient for normal development of the chicken. The predominant expression of gDLK1 in preadipocytes indicates that gDLK1 gene can function as an early marker gene of chicken adipose development. The downregulation of gDLK1 during adipose development may reflect a negative association with fat cell development and fat deposition. In addition, high levels of gDLK1 gene expression correlate with the high levels of myogenin and Pax7 gene expression in embryonic muscle. However, the precise mechanism of how DLK1 affects specific stages of muscle development remains to be investigated. In addition, it will be interesting to discover if the differences in expression pattern and level of DLK1 gene are associated with the rates of muscle growth in avian species.

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