Differential Expression Levels of Genes Related to Myogenesis During Embryogenesis of Quail and Chicken

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ABSTRACT

The present study was designed to investigate the expression dynamics of genes during myogenesis in quail and chicken. Real-time PCR was used to detect mRNA expressions of MyoD, MyoG, MLP and MSTN in breast muscle of quail and chicken embryos during the period of embryonic days E7-17. Results showed that expression profiles of each gene displayed similar trend in the experiment period between quail and chicken, however, the expression concentration between the two species differed at the same time detected. MyoD mRNA expression in quail was significantly lower in the early phase of the experiment period (E7-9) (P<0.01 on E7; P<0.05 on both E8 and E9). For MyoG and MLP, the mRNA expressions were both lower in quail than that in chicken during the experiment period. Additionally, the embryonic day when quail reached its peak expression was earlier than that in chicken (MyoG: quail E12 vs. chicken E13; MLP: quail E14 vs. chicken E15), and the peak expression for both in quail was significantly lower than that in chicken (P<0.01 for both). For MSTN, expression in quail was significantly higher in quail than that in chicken at each time detected (P<0.01). It is concluded that differential expression of these genes might or at least partially contributed to the different development of muscle development in quail and chicken.

INTRODUCTION

Muscle development, myogenesis, is a complex process, which can be divided into two phases: the embryonic and the postnatal phase. Compared with the poorly understood postnatal phase, the embryonic myogenesis received much more attention in the past decades either in domestic animals or in avian (Bentzinger et al., 2012). During the process of embryonic myogenesis, the myogenic progenitor cells (myoblasts) firstly proliferate and differentiate extensively, but then decrease because the number of myoblasts fused into multinucleated myotubes and myofibers, finally the muscle maintained its growth and maturation until postnatal (Davis and Fiorotto, 2009; Zhao et al., 2011).

It has been reported that a broad spectrum of genes regulated myogenesis during the embryonic phase, such as the myogenic regulatory factors (MRFs) including MyoD (Liu et al., 2011a), myogenin (MyoG) (Liu et al., 2011b), Myf5 (Braun et al., 1992) and MRF4 (Braun et al., 1990; Liu et al., 2011b), myostatin (MSTN) (McPherron and Lee, 1997; Huang et al., 2011; de Santis et al., 2012) and muscle LIM protein (MLP) (Kong et al., 1997; Swali et al., 2011). Extensive data on the expression pattern of these genes that regulate the development of muscle in embryogenesis have been obtained in avian like chicken (Gabriel et al., 2011; Saxena et al., 2007), duck (Li et al., 2010; Huang et al., 2011). However, there were few reports in the literature describing alterations in the expression pattern of these genes in quail myogenesis, although sequential activation of three myogenic regulatory genes during somite morphogenesis in quail embryos by in situ hybridization has been reported as early as 1992 (Pownall and Emerson, 1992). Furthermore, there was seldom comparison about the molecular genetic regulatory of myogenesis between chicken and quail, in other words, whether the expression profile of these genes in the two species has some differences is still not clear, although the quail (Coturnix coturnix) belongs to the same order (Galliformes) and

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family (Phasianidae) as the chicken (Gallus gallus), and comparison studies for the two species, on expressions of gene related to other organs development during embryogenesis, like reproductive organ development during sex differentiation, have received much more attention, which is reviewed by Brunstrom et al. (2009). It is, therefore, necessary to understand the dynamics of genes during myogenesis between quail and chicken, which could be able to partially uncover the complex mechanism underlying muscle development in avian.

The present study, therefore, was designed to investigate the dynamics of gene expressions during myogenesis in quail and chicken. Real-time PCR was used to detect mRNA expressions of MyoD, MyoG, MLP and MSTN in breast muscle of quail and chicken embryos during the period of E7-17.

**MATERIALS AND METHODS**

**Sampling:** Fertilized eggs were obtained from 200 Yellow chicken (Gallus gallus) and 200 Korea quails (Coturnix coturnix), respectively, provided by the animal experiment station, Shihze University. They were incubated in humidified atmosphere (60-70%) at 37.8±0.5°C (control incubation conditions). The embryo age was staged and measured in terms of the days of incubation. Whole embryos were collected through embryonic days (E) 7-17 (n=6 per day), during which, the breast muscle were isolated, frozen immediately in liquid nitrogen, and then stored at -80°C for RNA extraction.

**RNA extraction and reverse transcription:** Total RNA was isolated from the above tissues, according to standard Trizol extraction procedures. The quality of total RNA was examined by ethidium bromide stained denaturing agarose gel electrophoresis. The concentration of RNA target Primer sequence Product size /bp Annealing temperature (°C) Acc. No.
<table>
<thead>
<tr>
<th>RNA target</th>
<th>Primer sequence</th>
<th>Product size</th>
<th>Annealing temperature (°C)</th>
<th>Acc. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MyoD</td>
<td>F: 5′-GAATTTCACAGACACACTCCACAT3′</td>
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<td>55</td>
<td>FJ977569.1</td>
</tr>
<tr>
<td></td>
<td>R: 5′-GAATTCGCGGTCCTCCACTGCACT3′</td>
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<tr>
<td>MyoG</td>
<td>F: 5′-GTTGGGATGGTAGTGGGA3′</td>
<td>109</td>
<td>55</td>
<td>FJ882411.1</td>
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<td></td>
<td>R: 5′-TGGGAGAGGAGTGGGAAGA3′</td>
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<tr>
<td>MLP</td>
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<td>56</td>
<td>XM_420911.2</td>
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<td></td>
<td>R: 5′-GCACATTATTCAGCCACTACAT3′</td>
<td></td>
<td></td>
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<tr>
<td>MSTN</td>
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<td>AY448007.1</td>
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<tr>
<td></td>
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<td></td>
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<tr>
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<td>139</td>
<td>55</td>
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</tr>
</tbody>
</table>

**Table 1:** Primer sequences, Genbank accession number for the target genes, and conditions of Real-time PCR

F: forward primer; R: reverse primer; Acc. No: GenBank accession number. Primers were designed according to the sequence of the target genes by there Acc. No. in GenBank.
cells, especially to specify myoblasts for terminal determination and proliferation of myogenic progenitor upstream and is of significant importance to the MyoD is the first to be expressed, which act genetically MyoD1, Myf5, Mrf and Myogenin. Of these factors, regulatory factors (MRFs) gene family, which includes during this period.

We can also see that at each stage, MSTN expression level in quail reached the top at the stage E14, which was just before the day (E15) when chicken reached its peak expression. Moreover, there was a significant difference in terms of the peak expression between the two species (P<0.05). After reaching its respective peak, MLP levels declined in both of them.

We can also see that at each stage, MSTN expression level in quail (Table 2) was significantly higher than that in chicken (Table 3) (P<0.01). In quail, initial MSTN expression was found on E7, after declining on E8, level then increased gradually and reached the top at the stage E14, and thereafter plateaued (Table 2). In chicken, MSTN expression increased manifolds from E11 onwards as compared that from E7 to E10. Peak expression was noticed at E13, which was almost maintained at this level until E17 (Table 3). The late phase of embryogenesis (E13-17) witnessed a relatively stable MSTN expression level in both quail and chicken, although it was significantly higher in quail as compared with chicken during this period.

**DISCUSSION**

MyoD is an important member in the myogenic regulatory factors (MRFs) gene family, which includes MyoD1, Myf5, Mrf and Myogenin. Of these factors, MyoD is the first to be expressed, which act genetically upstream and is of significant importance to the determination and proliferation of myogenic progenitor cells, especially to specify myoblasts for terminal differentiation (Bentzinger et al., 2012). In the present study, we examined the mRNA expressions of MyoD gene in breast muscle of quail and chicken embryos in the stages of embryogenesis (E7-17). MyoD expression showed a downward trend in both of them, with the expression peak occurring at the initial stage (E7). It is known that a secondary wave of myogenesis occurs in birds after sixth day of incubation (E6) (Fredette and Landmesser, 1991). In this study, the period of E7-17 was chose, and highest MyoD expression was recorded on E7 for both of them, suggesting that the high MyoD expressions probably play an important role in the early phase of myogenesis. In addition, we found that the initial expression of MyoD was significantly lower in quail than that in chicken, although the similar expression trend was observed thereafter. This difference might be due to the reason that on E7, quail and chicken might be at different phase of muscle development, respectively, thereby requiring different MyoD concentrations to involve in the process of myoblast proliferation and differentiation.

Compared with MyoD, MyoG is more directly involved in the myoblasts differentiation process and trigger the expression of myotube specific genes in the myogenesis (Bentzinger et al., 2012). In this study, we detected the mRNA levels of MyoD in breast muscle of quail and chicken embryos during periods of E7-17. MyoG mRNA expression displayed a similar expression pattern between the two species, although it was different at each development stage. Expression of MyoG mRNA rose from the initial stage (E7), after reaching its respective peak (quail at E12 and chicken at E13), it declined significantly. The time of peak expression for MyoG in quail and chicken is consistent with the findings reported by O'Neill (1987), at which, a large number of myotubes could be observed in the breast muscle. It is inferred that MyoG might directly act on the formation of myotubes, or indirectly act on by triggering the expression of myotubes specific genes. MLP belongs to the LIM superfamily of proteins, which plays important roles in a variety of cellular functions. Arber et al. (1994) found that in chicken and rodent muscle cells, MLP is not detected in proliferating myoblasts but is up-regulated during terminal differentiation. In this study, we detected the mRNA expression levels of MLP in breast muscle of quail and chicken embryos during periods of E7-17. We found that in both quail and chicken, MLP mRNA expressions remained lower levels from E7 to E12, but from E12 onward, they rose dramatically, reaching their respective peak, and then still remain their high levels, although a slight decline occurred after the peak. Our findings thus indicated that MLP might be involved in the later stages of muscle development, especially in the formation of myotubes, because from E12 onward, a large number of myotubes began to fuse. This speculation is consistent with the findings reported by previous workers (Arber et al., 1994). In addition, quail and chicken reached to its respective peak expression at different stage. Moreover, significant difference occurred in terms of their peak expression between them. It is inferred that the different MLP expressions at different time might influence the rate of mytute fusion and the thickness of muscle fiber,
thereby resulting in the differences of muscle development between them.

It has been demonstrated in the previous study that MSTN is not the only a major determinant of muscle mass, but also influences early embryogenesis in avian (Saxena et al., 2007). In the present study, the expression of MSTN gene in breast muscle during embryogenesis (E7-17) of quail and chicken was detected, respectively. We found that in the early stage of embryogenesis, MSTN expression displayed relatively lower and plateaued levels, especially for chicken, while in the late phase of (E13-17) embryogenesis, levels rose highly and almost remained stable for both of them. Saxena et al. (2007) reported that higher and almost static MSTN expression was noticed in biceps femoris muscle of Indian broilers during the entire period of myogenesis (E7–E18). Compared with our study, the difference in expression level may be due to the different development between leg muscle and breast muscle in chicken. In addition, in the present study, we found that MSTN mRNA expression reached its first peak on E9 in chicken, which coincides with the periods of primary and secondary muscle fiber formation in chick embryos. While in quail, the first peak expression of MSTN was observed on E7. Compared with chicken, this difference could be due to the different development for embryonic myogenesis between quail and chicken, as the day for hatch out in quail is earlier than that in chicken for about 2-3days, resulting in the earlier muscle development occurred in quail. Hence, MSTN expression reaches its first peak is earlier than that in chicken. Furthermore, MSTN expression differed significantly between quail and chicken, which may be due to the different species in avian.

Conclusion: Expression profiles of each gene detected in the present study displayed similar trend in the experiment period between quail and chicken, however, the expression concentration between them differed at the same time point detected. It is inferred differential expression of these genes might or at least partially caused the different development of muscle development in quail and chicken.

REFERENCES


