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MicroRNA: Mechanism of gene regulation and application to livestock

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MicroRNA: Mechanism of gene regulation and application to livestock[1]

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ABSTRACT: Posttranscriptional regulation of gene expression plays a role in multiple cellular pathways. MicroRNA (miRNA) are an emerging class of small RNA that regulate gene translation. However, the mechanisms by which miRNA regulate this process remain controversial. By altering posttranscriptional regulation, miRNA have a role in guiding developmental decisions, including cell fate, cell cycle progression, apoptosis, adipocyte differentiation, and processes that alter muscle development and growth. The role of miRNA in developmental decisions that affect animal biology is of significant interest, yet the current literature is limited in livestock models. Therefore, a review of the mechanisms by which miRNA alter gene translation and the current research evaluating miRNA in production livestock is needed.

Key words: development, livestock, microRNA, translation

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INTRODUCTION

MicroRNA (**miRNA**) are noncoding small RNA, 18 to 26 nucleotides (**nt**) long, that regulate gene expression by altering translation of protein-encoding transcripts (Hutvágner, 2005). Functionally important small RNA were first described in nematodes in 1993 (Lee et al., 1993; Wightman et al., 1993). However, it was not until 2001 that researchers began to understand the function of this family of RNA that includes miRNA and to recognize that their significance was not confined to lower-order organisms (Lau et al., 2001; Lee and Ambros, 2001).

With the increase in identified miRNA sequences, a public database dedicated to the cataloging of predicted and experimentally observed miRNA has been developed (miRBase; http://microrna.sanger.ac.uk/ last accessed Apr. 2008). To date, a total of 6,396 (Release 11.0, April 2008) sequences have been submitted to miRBase including sequences for most major livestock species, but their role in biological processes has not been fully determined (Coutinho et al., 2007; Gu et al., 2007; Feng et al., 2008). Comparative analysis of miRNA sequences indicates they are highly conserved among species as diverse as nematodes and mammals, supporting the hypothesis that they are of central importance to biology and developmental decisions (Zhao and Srivastava, 2007; Ibáñez-Ventoso et al., 2008; Kedde and Agami, 2008).

Although progress in our understanding of miRNA biogenesis, function, and posttranscriptional regulation has been made, the mechanisms of posttranscriptional regulation remain unclear, and, more importantly, the role of specific miRNA in biological functions is just now becoming established. This review will address biogenesis and processing of miRNA, regulation of miRNA transcription, and 3 leading theories of how miRNA alter gene translation, including a discussion of current concepts that support and challenge these theories. In addition, the potential role for miRNA to influence expression of genes important for livestock production will be addressed.

MICRORNA TRANSCRIPTION AND BIOGENESIS

Transcriptional Control of miRNA Genes

MicroRNA genes are predominately located in intergenic regions. However, they have also been identified in introns and exons of protein-coding genes (Saini et al., 2007). Transcription of miRNA genes is tightly regulated spatially among tissues and temporally during development within tissues in all species studied (Aboobaker et al., 2005; Wienholds et al., 2005). In addition, transcription is regulated by multiple mechanisms including transcription factors (Rosenberg et al.,

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2006; Liu et al., 2007), epigenetic silencing (Lujambio and Esteller, 2007), and even other miRNA (Tuccoli et al., 2006). For example, muscle-specific transcription factors, myocyte-enhancing factor-2, and myoblast-determining protein have been identified as activators of muscle-specific miRNA, miR-1 and miR-133, transcription through an intragenic enhancer (Liu et al., 2007). In addition, myoblast-determining protein increases transcription of muscle-specific miR-206, which targets myogenic-inhibiting genes (Rosenberg et al., 2006).

Epigenetic changes have also been reported to regulate miRNA transcription. Most recently, silencing of miRNA via hypermethylation of the sequence and subsequent decrease in transcription has been linked to an increase in expression of genes that promote cancer progression (Saito et al., 2006; Lujambio and Esteller, 2007; Fabbri, 2008). As a result, epigenetic silencing of miRNA is emerging as a potential biomarker to identify cancerous cells and has even allowed for preliminary classification of cancers based on the transcriptome profile of methylated miRNA (Ahmed, 2007; Mulero-Navarro and Esteller, 2008; Taylor and Gercel-Taylor, 2008).

MiRNA Biogenesis

Biogenesis of functional miRNA sequences (18 to 26 nt) begins in the nucleus with transcription of the miRNA gene by RNA polymerase II, generating the long primary miRNA that contains the mature miRNA as an RNA hairpin (Figure 1; Lee et al., 2002; Murchinson and Hannon, 2004). Once transcribed, the primary miRNA is cleaved by Drosha, an endonuclease, to create an approximately 70-bp precursor miRNA. The precursor miRNA is then exported out of the nucleus to the cytoplasm by exportin-5 for further processing by a second endonuclease, Dicer. This step in the processing excises the ~21 nt miRNA:miRNA* duplex by cutting near the hairpin loop. The miRNA:miRNA* duplex contains the miRNA strand identified by the RNA-induced silencing complex (**RISC**) and the complementary strand (miRNA*) that is generally degraded. During processing, the miRNA strand with less stability for base pairing is identified and loaded into the RISC (Hutvágner, 2005; Lai, 2005). The miRNA loaded in the complex targets the RISC to specific binding sites in the 3′ untranslated region (**UTR**) of mRNA transcripts by base-pairing interaction across the seed sequence, the 7- to 8-nt sequence of the mature miRNA that dictates miRNA:mRNA binding.

The RISC is a ribonucleoprotein complex required for miRNA:mRNA interaction and miRNA-mediated gene silencing (Hutvágner, 2005). Multiple proteins have been identified as components of the RISC including argonaute protein (AGO1 thru AGO4; Liu et al., 2004), Dicer (Hutvágner et al., 2001), and human immunodeficiency virus-1 transactivating response element RNA-binding protein (Haase et al., 2005), with the ribonuclease Dicer and argonaute protein receiving the most attention. The argonaute protein is a major player in the RISC, functioning as the catalytic component of endonucleolytic cleavage (Liu et al., 2004). Dicer, as discussed previously, functions as a ribonuclease in miRNA biogenesis leading to release of the mature miRNA duplex. In addition to their role in the RISC, expression of argonaute protein and Dicer differs among tissues, and posttranscriptional modification alters argonaute protein stability (González-González et al., 2008; Qi et al., 2008).

MiRNA Control of Posttranscriptional Gene Regulation

It has been well documented that miRNA decrease translation of protein-coding genes (Jackson and Standart, 2007). However, controversy still surrounds the proposed mechanisms of action. Evidence has also emerged that contradicts the current dogma of decreased gene translation (Buchan and Parker, 2007; Vasudevan et al., 2007). In lieu of space, this review will focus on the 3 predominate theories for miRNA control of posttranscriptional regulation in animal models. Additionally, challenges to these theories will be addressed.

Degradation of mRNA Sequence

Regulation of translation via degradation of the mRNA sequence by small RNA has been well characterized, because use of small interfering RNA (**siRNA**) to decrease gene expression has been widespread over the past 10 yr (Tomari and Zamore, 2005). Unlike miRNA, which are produced from genome-encoded precursors as discussed previously, siRNA are short RNA sequences (~21 nt) produced from long double-stranded RNA precursors (Rana, 2007). Shared homology across the complete siRNA:mRNA sequence results in posttranscriptional regulation of gene expression through endonucleolytic cleavage of the target mRNA sequence at the binding site by utilizing components of the miRNA machinery, including Dicer (Elbashir et al., 2001). A similar mechanism for miRNA regulation of translation emerged as studies evaluating miRNA-mediated mRNA decay in multiple species came to light (Lim et al., 2005; Wu et al., 2006; Shyu et al., 2008). However, unlike siRNA that rely on complete homology across the binding site of the target mRNA, miRNA dictate mRNA targeting and translation based on the number and type of base pair matches in the 3′ UTR and binding site (Bagga et al., 2005; Alemán et al., 2007). In addition, although siRNA decay of mRNA is initiated by endonucleolytic cleavage, repression of translation via miRNA is a result of deadenylation of the poly-A tail and subsequent decapping of the mRNA sequence (Figure 2A; Giraldez et al., 2006; Wu et al., 2006). As a result, the mRNA sequence becomes unstable and is susceptible to degradation, resulting in decreased mRNA abundance and subsequent decrease translation.

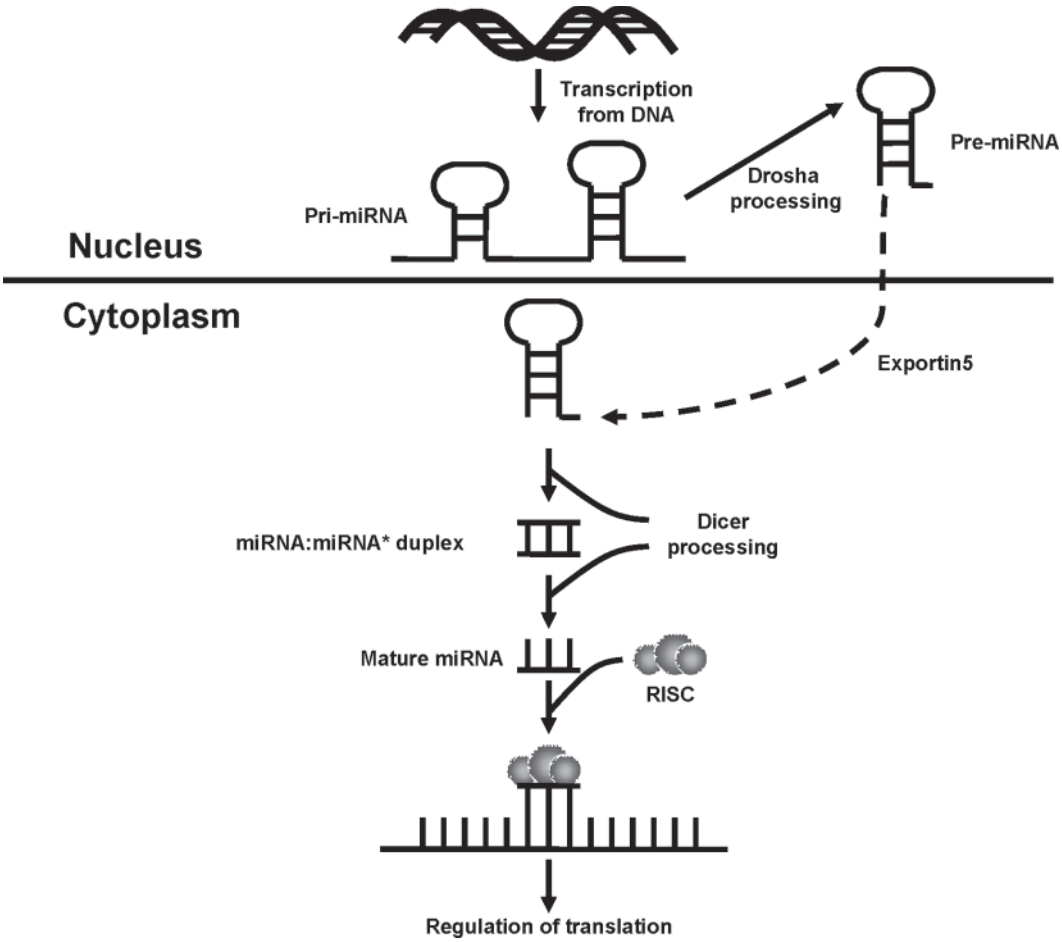

Figure 1. MicroRNA (miRNA) biogenesis in the animal cell. Biogenesis of mature miRNA begins in the nucleus with transcription of the miRNA gene generating the primary RNA (pri-miRNA). An endonuclease, Drosha, cleaves the pri-miRNA creating the precursor RNA (pre-miRNA). The pre-miRNA is exported out of the nuclease to the cytoplasm by exportin-5 for further processing by a second endonuclease, Dicer, resulting in a miRNA duplex (miRNA:miRNA*). The mature miRNA strand with less stability for base pairing (miRNA) is identified and loaded into the RNA-induced silencing complex (RISC) for specific binding to the 3′ untranslated region of messenger RNA transcripts. As a result, translation of the targeted gene product is altered.

Blocking of Initiation

An alternative mechanism of posttranscriptional control by miRNA began to emerge as research revealed that mRNA abundance did not always decrease with gene translation, suggesting that miRNA regulate translation through multiple mechanisms (Wang et al., 2006; Thermann and Hentze, 2007). Similar to the previously discussed mechanism, the miRNA binds to the RISC and targets the 3′ UTR of the mRNA sequence. However, instead of inducing degradation of the mRNA by deadenylation, targeting the miRNA:RISC complex to the 3′ UTR is thought to activate a sequence of events that block initiation proteins from binding to the 5′ cap of the mRNA (Figure 2B; Chendrimada et al., 2007; Kiriakidou et al., 2007). This theory has been supported by research demonstrating interaction of RISC components with proteins that initiate translation (Chendrimada et al., 2007; Kiriakidou et al., 2007). For example, AGO2 contains a similar binding domain as the eukaryotic translation initiation factor 4E (Kiriakidou et al., 2007). These data suggest that AGO2 competes with eukaryotic translation initiation factor 4E for binding to the 5′ cap of the mRNA sequence, resulting in a binding loop that blocks initiation and subsequent translation (Figure 2B). The argonaute protein has also been reported to bind proteins of the 60S ribosomal subunit including eukaryotic translation initiation factor 6 (Chendrimada et al., 2007). As a result of this proposed mechanism, the mRNA sequence cannot be translated and protein concentrations decrease, whereas mRNA abundance is not altered.

Translocation to Processing Bodies

A third proposed mechanism of miRNA action involves posttranscriptional regulation by translocation of the miRNA:mRNA complex to cytoplasmic foci in the cell, known as processing bodies (**P-bodies**), after the miRNA:RISC complex binds the mRNA target (Figure 2c; Chan and Slack, 2006). This theory is supported by research demonstrating that P-bodies contain components of the miRNA:RISC complex including AGO2, miRNA, and miRNA targets (Liu et al., 2005). In addition, P-bodies do not contain ribosomal proteins for translation but possess enzymes and fac-

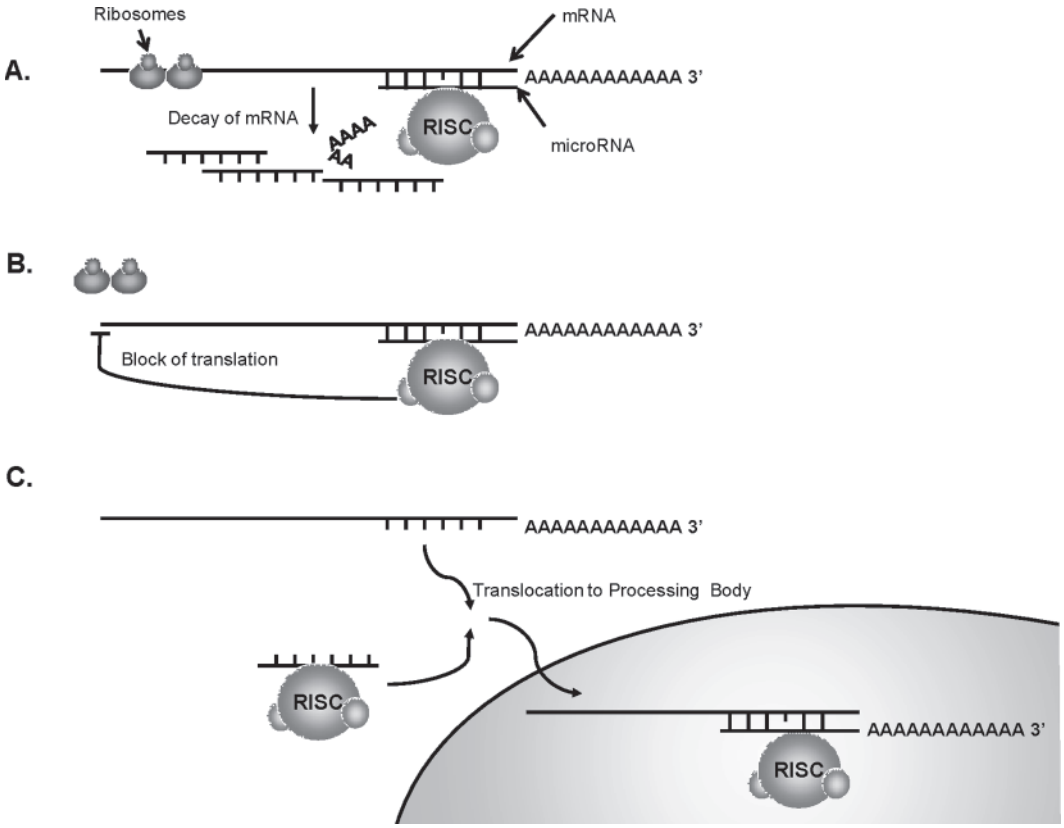

Figure 2. Mechanisms by which microRNA (miRNA) regulate translation of the targeted gene product. MicroRNA regulate translation through (A) degradation of the mRNA, (B) blocking of initiation, and (C) translocation to processing bodies (P-bodies). Initiation of these mechanisms involves miRNA binding to the RNA-induced silencing complex (RISC) and targeting the 3′ untranslated region (UTR) of the mRNA sequence. After the miRNA:RISC complex targets the mRNA sequence, degradation of the mRNA sequence (A) is triggered by deadenylation of the poly-A tail resulting in decreased stability of the mRNA sequence and subsequent decrease in mRNA abundance and translation. Binding of the miRNA:RISC complex to the 3′ UTR may also result in blocking of initiation proteins from the 5′ cap of the mRNA sequence (B). As a result, the mRNA sequence cannot be translated and protein concentrations decrease. Translocation of the mRNA to P-bodies after targeting by the miRNA:RISC complex (C) may also inhibit translation. Processing bodies lack ribosomal components, and therefore, they may serve as a storage site for mRNA.

tors for mRNA turnover and repression of translation (Liu et al., 2005). For example, trinucleotide repeat containing 6A (GW182) and a dipeptidyl carboxypeptidase (Dcp1/Dcp2) decapping complex colocalize in P-bodies and bind to argonaute proteins (Rehwinkel et al., 2005; Behm-Ansmant et al., 2006; Ikeda et al., 2006). The GW182 protein is an RNA-binding protein, whereas the Dcp1/Dcp2 decapping complex allows for removal of the 5′ cap and degradation of the mRNA sequence (Rehwinkel et al., 2005; Behm-Ansmant et al., 2006; Ikeda et al., 2006). However, evidence also indicates that P-bodies may merely serve as a storage unit for mRNA, because disrupting the P-bodies does not alter the miRNA pathway or the degree of translational repression (Chu and Rana, 2006; Eulalio et al., 2007). Therefore, P-bodies might have an intermediate role in miRNA regulation of translation and serve as a site of temporary storage before mRNA degradation (Eulalio et al., 2007).

Activation of Translation

At the end of 2007, our current understanding of post-transcriptional regulation was challenged by reports that miRNA also increased gene translation (Buchan and Parker, 2007; Vasudevan et al., 2007). Vasudevan et al. (2007) identified a miRNA, miR-369-3, with divergent effects on gene translation based on cell cycle stage. Actively proliferating cells exhibit decreased translation when miR-369-3 is paired with the target mRNA. However, cells entering a quiescent stage due to stimuli including contact inhibition and serum depletion exhibit an increase in protein translation when miR-369-3 is paired with the target mRNA. Stimulation of translation was determined to occur when miR-369-3 bound to the argonaut protein of the RISC and recruited the RNA-binding protein, fragile X related gene 1. To date, an increase in translation has only been reported for a subset of miRNA sequences in a single biological condition of cell quiescence (Vasudevan et al., 2007). However, these results indicate that miRNA have a broad role in gene expression and provide evidence that our understanding of mechanisms that regulate translation is not complete.

Although there continues to be controversy regarding specific miRNA mechanisms of regulation, there are possible reasons for such diversity in regulation of gene expression. First, specific mechanisms may regulate dif-

ferent stages of development, just as miRNA abundance has been reported to change during development (Darnell et al., 2006; Xu et al., 2006). Additionally, mechanism diversity may be the result of posttranscriptional editing of the miRNA sequence that has been reported to change mRNA targeting (Amariglio and Rechavi, 2007) and, therefore, may also change the mechanism by which miRNA control gene expression.

ROLE OF MICRORNA IN PRODUCTION LIVESTOCK

The role of miRNA in cellular processes that affect animal biology is an emerging field of interest for animal scientists. Initial research has focused on expanding the current miRNA database (miRBase) to include sequences for livestock species through evaluation of miRNA transcriptome profiles and homology searches (Kim et al., 2006; Coutinho et al., 2007; Gu et al., 2007; Feng et al., 2008; Shao et al., 2008). In addition, new sequencing technologies have advanced this effort by identifying new miRNA sequences through deep sequencing of the miRNA transcriptome in chicken models (Burnside et al., 2008; Glazov et al., 2008). With the increase in miRNA sequences in the database, interest has expanded to determine what role miRNA have in various areas of animal biology (Clop et al., 2006; Sweetman et al., 2006, 2008). Therefore, a review of the current miRNA research for traits of economical value in livestock species is of importance to determine our current standing and what direction future research needs to take.

Evaluation of regulatory factors that affect development and growth of economically important tissues such as skeletal muscle and adipose tissue is of interest, because profit margin is influenced by nutrient partitioning between these tissues. MicroRNA were initially reported to have a role in skeletal muscle development utilizing mouse, *Drosophila*, and zebrafish models. Three muscle-specific miRNA (miR-1, miR-133, and miR-206) that undergo an increase in abundance during muscle cell differentiation were identified (Brennecke et al., 2005; Chen et al., 2006; Nguyen and Frasch, 2006). However, these miRNA have been reported to regulate different stages of myogenesis (Anderson et al., 2006; Nakajima et al., 2006; McCarthy and Esser, 2007). It was found that miR-133 increases proliferation of C_2C_{12} myoblasts, whereas miR-206 and miR-1 promote differentiation (Chen et al., 2006). Research in livestock models has also begun to evaluate the role these miRNA have in skeletal muscle development. For example, overexpression of fibroblast growth factor-4 has been reported to decrease miR-206 abundance, resulting in developmental changes in the somite of developing chicken embryos (Sweetman et al., 2006). Expression of the muscle regulatory factor, myogenic factor 5, also regulates miR-1 and miR-206 transcription level in a chicken cell culture model (Sweetman et al., 2008).

Muscle-specific miRNA have also been reported to regulate a gene that directly affects economic traits in livestock (Clop et al., 2006). Specifically, a mutation in the myostatin gene of heavily muscled Belgian Texel sheep creates a target site for miR-1 and miR-206 containing RISC complexes in the exon encoding the 3´ UTR of the transcript, resulting in decreased translation of the myostatin protein and consequent increase in muscle mass.

In addition to skeletal muscle, adipose tissue affects carcass value including meat quality grade and yield. Although the predominant research evaluating the role of miRNA in adipose tissue has involved human and mouse cell lines, miRNA transcriptome profiles in adipose tissue have been created for cattle (Gu et al., 2007). Gu et al. (2007) identified 154 miRNA sequences, of which 133 miRNA sequences either matched or differed by 1 or 2 nt to mammalian miRNA in the database. In addition, 54 miRNA sequences were determined to be adipose tissue-specific when compared with miRNA sequences obtained from bovine mammary gland within the same study. Evaluation of miRNA sequences from adipose tissue revealed several miRNA observed more than once including miR-143, which was evaluated previously in human cell culture models (Esau et al., 2004). Esau et al. (2004) identified a potential role for miR-143 in adipocyte differentiation of human preadipocytes, in which miR-143 abundance increased during differentiation of adipocytes and inhibition of miR-143 decreased differentiation.

To evaluate further the role miRNA may have in development of livestock species, miRNA transcriptome profiles have been created from tissues at specific stages of development in chick embryo and adult chicken (Darnell et al., 2006; Xu et al., 2006; Burnside et al., 2008; Glazov et al., 2008; Shao et al., 2008). Although a large proportion of miRNA are ubiquitously expressed at all stages of the developing embryo and adult, a pattern of increased abundance of miRNA during progression of development is observed, with the greatest expression of most miRNA in the adult tissues compared with the embryo (Darnell et al., 2006; Xu et al., 2006; Shao et al., 2008). Similar to other vertebrate species, tissue-specific miRNA were identified including miR-122 in the liver, miR-132 in the cerebrum, and miR-338 in the cerebrum and cerebellum (Xu et al., 2006; Shao et al., 2008). Together, these data discussed to this point suggest a potential role for both tissue-specific and ubiquitously expressed miRNA in skeletal muscle, adipose tissue, and embryo development of multiple livestock species.

Although a significant amount of research has been dedicated to the role of miRNA in growth and development, emerging research has evaluated miRNA in other areas of interest including reproduction, immunology, feed efficiency, and the mammary system (Barendse et al., 2007; Coutinho et al., 2007; Gu et al., 2007; Carletti and Christenson, 2009). Although the role of miRNA

in reproduction is of interest to livestock production, current miRNA research has yet to evaluate domestic livestock models. However, other biological models have been studied, and a complete review of miRNA in the ovary and female reproductive tract has been completed by Carletti and Christenson (2009).

In addition to the transcriptome profiles discussed previously, profiles for other economically important tissues, including bovine immune and mammary-related tissues, have been created (Coutinho et al., 2007; Gu et al., 2007). MicroRNA motifs have also been identified near SNP associated with residual feed intake in cattle, suggesting that miRNA have a functional role in regulation of genes that affect feed efficiency (Barendse et al., 2007). Combined with the miRNA transcriptome profiles discussed previously, the data to date present a set of transcriptome profiles to evaluate and compare miRNA abundance across specific tissues and species. Specifically, identification of these miRNA provides an initial group of miRNA that may play a vital role in developmental processes.

SUMMARY AND CONCLUSIONS

MicroRNA are important regulators of gene expression that affect biological pathways. The research discussed herein suggests that miRNA function through multiple mechanisms that regulate posttranscriptional processes. Although the function of miRNA is only beginning to be understood, future research evaluating the functional effect of miRNA in gene translation and subsequent biological pathways in livestock is of importance. Although current research in other mammalian species provides us with insight to miRNA function in livestock species, additional research needs to be completed to truly understand the functional role and application of miRNA in animal agriculture.

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