Centennial Review: Metabolic microRNA - shifting gears in the regulation of metabolic pathways in poultry

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ABSTRACT Over 20 yr ago, a small noncoding class of RNA termed microRNA (**miRNA**) that was able to recognize sequences in mRNAs and inhibit their translation was discovered in *Caenorhabditis elegans*. In the intervening years, miRNA have been discovered in most eukaryotes and are now known to regulate the majority of protein-coding genes. It has been discovered that disruption of miRNA function often leads to the development of pathological conditions. One physiological system under extensive miRNA-mediated regulation is metabolism. Metabolism is one of the most dynamic of biological networks within multiple organs, including the liver, muscle, and adipose tissue, working in concert to respond to ever-changing nutritional cues and energy demands. Therefore, it is not surprising that miRNA regulate virtually all aspects of eukaryotic metabolism and have been linked to metabolic disorders, such as obesity, fatty liver diseases, and diabetes, just to name a few. Chickens, and birds in general, face their own unique metabolic challenges, particularly after hatching, when their metabolism must completely transform from using lipid-rich yolk to carbohydrate-rich feed as fuel in a very short period of time. Furthermore, commercial poultry breeds have undergone extensive selection over the last century for more desirable production traits, which has resulted in numerous metabolic consequences. Here, we review the current knowledge of miRNA-mediated regulation of metabolic development and function in chickens.

Key words: metabolism, microRNA, growth, next-generation sequencing

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INTRODUCTION

Regulation of chicken metabolic development and function has long been an area of intense interest from both a production and biomedical perspective. As one of the most easily produced, readily available, and affordable food sources worldwide, the chicken has undergone intensive selection for faster growth and meat yields (broilers) as well as increased egg production (layers) (reviewed by Buzala and Janicki, 2016). Selection for growth and meat yields in broilers has resulted in a 3.30% increase per year in BW at 42 d of age since the 1950s and conversely a 2.55% decrease per year in feed conversion (Zuidolf et al., 2014). Today's commercial layers produce about twice as many eggs and achieve 50% egg production at a younger age than their predecessors (reviewed by Kidd and Anderson, 2019). As expected, these selection pressures have led to significant metabolic alterations from ancestral breeds and have also produced a number of unintended and often detrimental consequences such as morphological defects and metabolic and immune pathologies (reviewed by Buzala and Janicki, 2016). Chickens seem to be more tolerant to metabolic extremes than other species. For example, during the last days of embryonic development, lipids account for 25–30% of the DM content of the embryo, and ∼2% of the total lipids are located in the liver alone (reviewed by Noble and Cocchi, 1990). However, chick embryos typically do no exhibit any of the pathologies (e.g., nonalcoholic fatty liver disease [**NAFLD**] or hepatosteatosis) found in mammals with

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equivalent hepatic lipid concentrations. Chickens are also naturally insulin resistant (owing to low expression of the insulin receptor) and can tolerate glucose concentrations that are fatal in mammals without developing any of the symptoms associated with diabetes (Wang et al., 2017). Together, these unique features have made the chicken an attractive model for elucidating the underlying regulatory mechanisms of metabolism.

During the transition from embryonic to posthatch life, the chick's metabolism must quickly adapt from a lipid-rich/carbohydrate-poor (yolk) to a lipid-poor/carbohydrate-rich (feed) fuel source. This results in a major metabolic switch from lipolysis (fatty acid oxidation) and gluconeogenesis to glycolysis and lipogenesis (conversion of carbohydrates to fats) within the first few days after hatch (reviewed by Speake et al., 1998). Although the major physiological outcomes of the metabolic switch have been known for some time, the underlying regulatory mechanisms are only now beginning to be discovered.

For many years, the prevailing idea was that only a few forms of functional RNA existed, and the rest was nonfunctional "junk" RNA. However, with the advent of new sequencing technologies, over the last few decades, many new functional RNA species have been and continue to be discovered. One of the more intensively studied of these is a class of small noncoding RNA, termed microRNA (**miRNA**). First discovered in *Caenorhabditis elegans* over 20 yr ago (Lee et al., 1993), miRNA have been discovered in the vast majority of eukaryotic species and are now known to regulate most biological processes, and their dysexpression is linked to numerous pathological conditions. MicroRNAs are short (~22 nt) RNA molecules, which bind to complementary sequences in the mRNAs of protein-coding genes, which are typically located within the 3′UTR and inhibit translation. A single miRNA can regulate the expression of hundreds of genes. MicroRNA biogenesis and function have been extensively reviewed elsewhere; for a recent review, see the study by Bartel 2018.

One of the most dynamic systems of an organism is metabolism, which must rapidly respond to ever-changing nutritional cues and energy demands. Disruption of the homeostatic balance of these processes can often have devastating consequences, including hepatic pathologies, such as NAFLD and hepatosteatosis and other pathologies, for example, obesity and diabetes. Therefore, it is not surprising that regulatory RNAs, including miRNA, not only play a myriad of roles in both maintaining healthy metabolic processes but also contribute to the development of metabolic pathologies (reviewed by Landrier et al., 2019).

There are currently 882 precursor miRNA, which generate 1,232 mature miRNA for *Gallus gallus* in the miRNA database miRBase (Release 22.1; www.mirbase.org). Many of these miRNA are conserved with other species and therefore likely also share conserved functions. Just as with other species, chicken miRNA have been found to regulate many different developmental and physiological processes and contribute to disease development and microbial pathogenesis. Through the use of next-generation sequencing, we and others have discovered that a large and diverse set of miRNA is expressed in metabolically important tissues in poultry (Table 1). Although much remains unknown regarding the specific functional roles these miRNA play, recent studies indicate that miRNA likely contribute to the regulation of most metabolic systems in poultry.

Adipose Tissue miRNA Repertoire

Mammals have several types of adipose tissue; white, brown, and brite/beige (reviewed by Montanari et al., 2017). White adipose tissue (**WAT**) is the main site of lipid storage, while only small depots of brown adipose tissue exist in adults and provide energy in response to thermoregulation (shivering). Meanwhile, brite/beige adipose tissue is the result of browning of white adipose in response to cold exposure. Poultry, however, only develop WAT (Tůmová and Teimouri, 2010). Human and rodent studies have revealed that miRNA-mediated gene regulation contributes to virtually all aspects of mammalian adipose tissue development and function. Both dietary and genetically induced mouse models of obesity have shown distinct miRNA profiles in the adipose tissue of obese individuals from their lean counterparts (Nakanishi et al., 2009). In both leptin-deficient (ob/ob) and leptin receptor–deficient (db/db) genetically obese mice, *mmu-miR-335* adipose-specific levels were significantly higher than in wild-type (**WT**) mice. This increased *mmu-miR-335* expression was associated with both excessive BW and WAT weight. This study also found a correlation between *mmu-miR-335* levels and those of several adipocyte differentiation markers (peroxisome proliferator activated receptor gamma, adipocyte lipid-binding protein, and Fatty Acid Synthase [**FASN**]) in the 3T3-L1 cell line (a mouse embryonic fibroblast–like cell that can be chemically induced into adipocytes) (Nakanishi et al., 2009). These results suggest one function of *miR-335* could be to promote adipocyte accumulation and lipid storage. A later study also linked elevated *mmu-miR-335* expression in WAT to obesity (both genetic and dietary) in mice (Oger et al., 2014). Through the use of an adipose tissue–specific Dicer (**ADicer**) knockout (**KO**) mouse model, Thomou et al. (2017) showed that a significant amount of circulating miRNA-containing exosomes is derived from adipose tissue. Several hundred miRNA were significantly reduced in the exosomes of ADicer KO mice, which had impaired glucose tolerance and were insulin resistant. Adipose tissue transplantation from WT mice to ADicer KO mice increased the levels of the majority of exosomal miRNA and significantly improved glucose tolerance and insulin resistance in these mice (Thomou et al., 2017). Thus, it is likely that adipose-derived miRNA not only regulate adipose-specific metabolic processes but also regulate processes in other metabolically important tissues, such as the muscle and liver.

Table 1. Summary of miRNA profiling studies of metabolic tissues and cells in chickens.

Breed	Metabolic tissue/cell type	Number of differentially expressed miRNA	Downstream pathways and biological processes	Reference
D93 Beijing-You-Cobb-Vantress cross hens with high and low adipose weights	Adipose	Expression of 32 miRNA higher in hens with high adipose weight; expression of 30 miRNA lower	Adipose differentiation, lipid metabolism	Huang et al., 2015
6-wk-old and 30-wk-old Gushi hens	Adipose	Expression of 16 miRNA higher at 6 wk; expression of 12 miRNA higher at 30 wk	Adipocyte development, lipid metabolism, cell junctions	Chen et al., 2019b
Differentiated (in vitro) preadipocytes: 14-day-old Gushi chickens	Primary pre-adipocytes	Expression of 58 miRNA upregulated during differentiation; expression of 22 downregulated	MAPK signaling, insulin signaling, fatty acid metabolism	Ma et al., 2020
E10 Arbor Acres (broiler) and White Leghorn (layer)	Skeletal muscle	Expression of 10 miRNA higher in broilers; expression of 7 miRNA higher in layers	Myogenesis, TGF signaling	Li et al., 2011
7-wk-old hens, recessive White Rock (fast-growing) and Xinghua (slow-growing)	Breast muscle	Expression of 15 miRNA higher in recessive White Rock; expression of 7 miRNA higher in Xinghua	GHR signaling	Ouyang et al., 2015
≤8-wk-old commercial broilers (Cobb-Vantress) and foundational (slow-growing) breed (Barred Plymouth Rock)	Breast muscle	Expression of 8 miRNA higher in commercial broilers; expression of 1 miRNA higher in foundational breed	Calcium signaling, axonal guidance signaling, NRF2-mediated oxidative stress response	Khatri et al., 2018
10-wk-old Sichuan mountainous black-bone (slow-growing) and Dahen (fast-growing)	Pectoral muscle	Expression of 19 miRNA higher in Sichuan mountainous black-bone; expression of 13 miRNA higher in Dahen	Fatty acid metabolism, immunity, MAPK signaling	Zhang et al., 2020
E14 female sex-linked dwarf recessive White Rock and normal recessive White Rock	Leg muscle	Expression of 3 miRNA higher in dwarf chickens; expression of 2 higher in normal chickens	MAPK signaling, PI3k signaling, Akt signaling, Wnt signaling, insulin signaling	Luo et al., 2016
7-wk-old female sex-linked dwarf recessive White Rock and normal recessive White Rock	Leg muscle	Expression of 3 miRNA higher in dwarf chickens; expression of 4 higher in normal chickens	MAPK signaling, PI3k signaling, Akt signaling, Wnt signaling, insulin signaling	Luo et al., 2016
6-wk-old and 14-week-old Gushi hens	Breast muscle	Expression of 36 miRNA higher at 6 wk; expression of 30 miRNA higher at 14 wk	Focal adhesion, ECM–receptor interaction, FOXO signaling, cell cycle, p53 signaling	Li et al., 2019a
14-wk-old and 22-wk-old Gushi hens	Breast muscle	Expression of 29 miRNA higher at 14 wk; expression of 27 miRNA higher at 22 wk	Focal adhesion, ECM–receptor interaction, FOXO signaling, cell cycle, p53 signaling	Li et al., 2019a
22-wk-old and 30-wk-old Gushi hens	Breast muscle	Expression of 19 miRNA higher at 22 wk; expression of 42 miRNA higher at 30 wk	Focal adhesion, ECM–receptor interaction, FOXO signaling, cell cycle, p53 signaling	Li et al., 2019a
7-wk-old female sex-linked dwarf recessive White Rock and normal recessive White Rock	Liver	Expression of 5 miRNA higher in dwarf chickens; expression of 1 miRNA higher in normal chickens	mTOR signaling, Wnt signaling, MAPK signaling, TGF signaling	Ye et al., 2014
Growth hormone (GH)–treated primary hepatocytes obtained from 4-week-old Arbor Acres hens	Primary hepatocytes	Expression of 16 miRNA higher in GH-treated hepatocytes; expression of 1 miRNA higher in the control hepatocytes	Lipid metabolism	Wang et al., 2014
20-wk-old (pre–egg-laying) and 30-wk-old (egg-laying) Lushi hens	Liver	Expression of 67 miRNA higher at 20 wk; expression of 13 miRNA higher at 30 wk	Lipid-associated enzymes	Li et al., 2016
E18 and D3 White Leghorn	Liver	Expression of 27 miRNA higher at E18; expression of 13 miRNA higher at D3	Lipid metabolism, fatty acid synthesis, cholesterol synthesis	Hicks et al., 2017

Abbreviations: ECM, extracellular matix; GHR, growth hormone receptor; miRNA, microRNA; TGF, transforming growth factor.

Profiling studies have revealed a large and diverse group of miRNA is expressed in the adipose tissue of poultry. These studies have also demonstrated that adipose-specific miRNA expression is dynamic, with miRNA expression profiles differing across developmental stages and differing growth rates and physiological states. Next-generation sequencing profiling identified 222 miRNA expressed in the preadipocytes obtained from abdominal adipose tissue in Arbor Acre broilers (Yao et al., 2011). Although this study was limited in scope, with only 11-day-old males being used, it was one of the first studies to reveal the large repertoire of miRNA present in chicken adipose tissue. MicroRNA profile comparisons between hens (93-day-old) with either high or low abdominal fat weights identified 62 differentially expressed miRNA. Integration of these profiles with RNA-seq data indicated that these differentially expressed miRNA target genes were associated with adipocyte differentiation and lipid disposition (Huang et al., 2015). MicroRNA profiling of abdominal adipose tissue of Gushi (Chinese domestic breed) hens found the expression of 16 miRNA decreased and 12 miRNA increased from 6 wk to 30 wk of age and these miRNA target genes were associated with adipocyte development, lipid metabolism, and cell junctions (Chen et al., 2019b). Differentiation (in vitro) of primary chicken preadipocytes, obtained from abdominal adipose tissue of 14-day-old Gushi chickens, altered the expression of 80 miRNA, 58 upregulated and 22 downregulated (Ma et al., 2020). Target analysis and network mapping indicates these differentially expressed miRNA target a number of developmental and metabolic pathways, including MAPK signaling, insulin signaling, and fatty acid metabolism (Ma et al., 2020). As discussed previously, chickens, and poultry in general, undergo a major metabolic switch from oxidation of yolk lipids for energy (embryo) to glycolysis of carbohydrates obtained from feed (after hatch). We developed miRNA profiles of adipose-specific miRNA during this transitional period to better understand the contribution of miRNA to the metabolic switch. We found altered expression of 93 miRNA, 63 upregulated and 30 downregulated ones, in the adipose tissue of 3-day-old chicks compared with 18-day-old embryos (H.C. Liu, unpublished data). Integration of these miRNA profiles with target prediction and transcriptome profiling revealed that miRNA likely contribute to the metabolic switch by regulating adipogenic, lipolytic, developmental, and immunological processes in adipose tissue. A summary of miRNA profiling studies in chicken adipose tissue is provided in Table 1.

Muscle miRNA Repertoire

As one of the main energy-consuming tissues, muscle is a critical component of metabolic systems. In mammals, miRNA have been shown to regulate virtually all aspects of muscle biology, from development, to damage repair, to muscular pathologies. During the in vitro differentiation of primary human CD56+ myoblasts, 60 miRNA were found to be differentially expressed, with 43 upregulated and 17 downregulated (Dmitriev et al., 2013). MicroRNA expression profiling of the quadriceps muscle during the first 12 wk after birth of mice identified the expression of 353 miRNA (Lamon et al., 2017). Of these, 205 were differentially expressed, 55 upregulated and 150 downregulated, over these first 12 wk. Target prediction of these miRNA suggests they regulate a diverse set of cell cycle and cell differentiation networks (Lamon et al., 2017). These studies highlight the importance of miRNA-mediated regulation during muscle development. One the earliest studies to examine muscle-expressed miRNA, identified 3 muscle-specific miRNA, *miR-1*, *miR-133*, and *miR-206*, that were induced during the differentiation of both human and murine myoblasts (Rao et al., 2006). Chromatin immunoprecipitation analysis demonstrated that this induction is mediated by the myogenic regulators, myogenin and MyoD (Rao et al., 2006). That same year, it was discovered that the excessive muscle growth in Texel sheep is due to a mutation in the 3′UTR of myostatin, which introduced *miR-1* and *miR-206* target sites (Clop et al., 2006). This supports a conserved role for *miR-1* and *miR-206* in regulating myogenesis. Two other miRNA that have been identified as regulators of muscle development are *miR-143* and *miR-145*. The expression of *mmu-miR-143* and *mmu-miR-145* is enriched in murine cardiac progenitor cells and during cardiogenesis. It was found this enrichment is due to transcriptional regulation by SRF, myocardin, and Nkx2-5. It was further demonstrated that myocardin induction of *miR-145* expression was required for differentiation of progenitor cells into smooth muscle cells (Cordes et al., 2009). Other important miRNA regulating muscle development are *miR-208a*, *miR-208b*, and *miR-499*, which are located in introns of the myosin genes, *MYH6*, *MYH7*, and *MYH7B*, respectively (van Rooij et al., 2009). It was proposed that these miRNA participate in muscle fiber programming by inducing the expression of genes associated with slow myofiber formation and inhibiting the expression of genes involved with fast myofiber formation (van Rooij et al., 2009).

Similar to adipose tissue, muscle has also been found to release miRNA via exosomes (Coenen-Stass et al., 2016). Profiling studies revealed myoblasts, both primary human myoblasts and the murine mesenchymal cell line C2C12, increasingly release exosomal miRNA during differentiation. Increased exosomal miRNA release was also found in the earliest stages of muscle development and in response to exercise-induced muscle injury (Coenen-Stass et al., 2016). In a similar study, 171 exosomal miRNA were identified from C2C12 myoblasts, and 182 exosomal miRNA were found to be released by C2C12 cells that differentiated into myotubes (Forterre et al., 2014). This study further demonstrated that exosomal miRNA released from myotubes can target mRNAs in myoblasts, such as *SIRT1*, to influence myoblast differentiation.

Development of insulin resistance induced by a high-fat diet (**HFD**) altered the expression of several miRNA

in the skeletal muscle of mice. Mice exposed to a HFD for 8 wk had significantly less *mmu-miR-1a* expression in their muscles than mice at 4 wk, and *mmu-miR-1a* expression continued to decline through 12 wk; its expression was negatively correlated with glycemia (Frias et al., 2016). This suggests the *miR-1* regulates both key developmental and metabolic networks in muscle tissue. In a similar study in which male C57BL/6 mice were fed with a HFD for 12 wk, 30 muscle miRNA were differentially expressed, 8 upregulated and 22 downregulated (Chen et al., 2012). These miRNA may contribute to dietary-induced muscle insulin resistance by targeting genes involved in MAPK signaling (Chen et al., 2012). The expression of 4 miRNA in the quadriceps femoris muscle in C57B1/6J WT mice was altered by endurance exercise (Safdar et al., 2009). Among these is *mmu-miR-23*, with a reduction of 84% in the muscles of mice subjected to endurance exercise. Conversely, the *mmu-miR-23* target *PGC1A*, a transcriptional regulator of cellular energy metabolism, was upregulated in the muscle by endurance exercise. This suggests that miRNA may serve as a fast responding regulatory system in response to increased energy demands in the muscle.

In one of the first studies of muscle-expressed miRNA in chickens, profiling of miRNA in the skeletal muscle of 28-day-old Arbor Acres broilers identified 115 miRNA (Wang et al., 2012). A comparison of miRNA profiles between broilers (Arbor Acres) and layers (White Leghorn) of muscle tissue in 10-day-old embryos identified 17 differentially expressed miRNA: 10 miRNA were highly expressed in broilers, and 7 were highly expressed in layers (Li et al., 2011). A comparison of miRNA expressed in the muscle between fast-growing broilers (recessive White Rock) and slow-growing broilers (Xinghua) identified 22 differentially expressed miRNA (Ouyang et al., 2015). These miRNA were predicted to target a number of genes associated with growth hormone receptor (**GHR**) signaling. In a similar study, miRNA profile comparisons in the breast muscle of a fast-growing modern commercial breed (Cobb Vantress, Inc.) and a foundational slow-growing breed (Barred Plymouth Rock) identified 8 upregulated miRNA and one downregulated miRNA in the modern fast-growing breed (Khatri et al., 2018). Target prediction and network mapping suggests these miRNA may regulate calcium signaling, axonal guidance signaling, and NRF2-mediated oxidative stress response pathways (Khatri et al., 2018). Profiling of the pectoral muscles of a small, slow-growing chicken breed (Sichuan mountainous black-bone) and a larger fast-growing chicken breed (Dahen) identified 32 differentially expressed miRNA between the 2 breeds at 10 wk of age (Zhang et al., 2020). Target prediction analysis of these miRNA suggests they regulate fatty acid metabolism, immunity, and MAPK activation (Zhang et al., 2020). At embryonic day 14, 5 miRNA were found to be differentially expressed in muscle between sex-linked dwarf chickens and normal chickens, and at 7 wk of age, 7 miRNA were differentially expressed (Luo et al., 2016). Target prediction analysis for these miRNA suggested that the reduced muscle mass of dwarf chickens could partially be due to disruption of miRNA regulation of MAPK, PI3k, Akt, Wnt, and insulin signaling pathways (Luo et al., 2016). MicroRNA profiles in the breast muscle of female Gushi chickens identified 96 differentially expressed miRNA, 30 upregulated and 36 downregulated, between 6 wk of age and 14 wk of age (Li et al., 2019a). In the same study, between 14 wk of age and 22 wk of age, 56 miRNA were found to be differentially expressed, 27 upregulated and 29 downregulated, and between 22 wk of age and 30 wk of age, 61 miRNA were found to be differentially expressed, with 42 upregulated and 19 downregulated (Li et al., 2019a). Integration of these profiles with transcriptome profiles identified 5 putative miRNA-regulated networks associated with breast muscle development in chickens: focal adhesion, extracellular matrix–receptor interaction, FoxO signaling, cell cycle, and p53 signaling (Li et al., 2019a). A summary of miRNA profiling studies in the chicken muscle is provided in Table 1.

Liver miRNA Repertoire

Numerous studies have also demonstrated that hepatically expressed miRNA also mediate metabolic function. Exposure of mice to a HFD was found to induce the hepatic expression of *mmu-miR-425-5p* (Min et al., 2018). This resulted in impaired insulin signaling and insulin resistance. These pathologies were linked to the reduced expression of the insulin receptor (*INSR*), a *mmu-miR-425-5p* target gene. In genetically obese mice (db/db), both *mmu-miR-143* and *mmu-miR-145* had increased hepatic expression (Jordan et al., 2011). The offspring of mice subjected to a HFD had altered expression of 23 hepatic miRNA (Zhang et al., 2009). These miRNA were predicted to target genes involved in early fetal growth and lipid metabolism (Zhang et al., 2009). This suggests that early programming of miRNA expression signatures has lifelong consequences for the organism. Profiling of hepatic miRNA during aging of mice found 4 miRNA that were upregulated in the livers of 33-mo-old mice compared with 4-mo-old and 10-mo-old mice (Maes et al., 2008). Three miRNA were significantly reduced in 33-mo-old mice compared with 10-mo-old mice. Combination of these miRNA expression profiles with matching proteomic data suggests the upregulated miRNA regulate genes involved in mitochondrial functions and oxidative defense (Maes et al., 2008).

A prevailing role for miRNA in regulating metabolic programs is via exosomal release. As discussed previously, both adipose and muscle cells have been shown to release miRNA via exosomes to execute both short- and long-range effects. Therefore, it is not surprising that liver cells have also been found to release exosomal miRNA. Exosomal release of hepatically expressed *mmu-miR-130a* can regulate lipid and glucose metabolism in adipose tissue (Wu et al., 2020). *mmu-miR-130a* KO mice were found to gain significantly more

weight on a HFD than WT mice. When *mmu-miR-130a* KO mice were treated with hepatic exosomes from transgenic mice overexpressing *mmu-miR-130a*, KO mice had improved glucose intolerance and decreased insulin resistance (Wu et al., 2020). In vitro treatment of hepatocytes with palmitic acid to induce cellular lipid accumulation significantly increased release of exosomes compared with control cells (Lee et al., 2017). Expressional profiling of exosomal miRNA identified 314 differentially expressed miRNA between palmitic acid–treated and control cells. Exosomes from palmitic acid–treated hepatocytes had significantly higher amounts of *hsa-miR-24*, *has-miR-19b*, *hsa-miR-34a*, *hsa-miR-122*, and *hsa-miR-192*, which have been previously associated with nonalcoholic liver diseases. Treatment of hepatic stellate cells with exosomes derived from palmitic acid–treated hepatocytes significantly upregulated genes associated with fibrosis (Lee et al., 2017). A related study using mouse models for NAFLD also found that liver cells exposed to excessive lipids released significantly higher numbers of exosomes and that these exosomes also contained higher amounts of *mmu-miR-122* and *mmu-miR-192* (Povero et al., 2014).

In one of the first chicken miRNA profiling studies and the first studies in the liver, we identified the expression of 114 miRNA at embryonic day 15 and 80 miRNA at embryonic day 20 (Hicks et al., 2010). Target prediction and validation of a select group of these miRNA suggested that miRNA target key lipid metabolism–associated genes including fatty acid metabolism and peroxisome proliferator-activated receptor signaling. MicroRNA microarray profiling of hepatically expressed miRNA in dwarf chickens vs. normal chickens found 5 miRNA were higher expressed in the livers of dwarf chickens compared to normal chickens and one miRNA was lower expressed (Ye et al., 2014). Treatment of primary hepatocytes obtained from 4-wk-old female Arbor Acres broilers, with growth hormone (**GH**), altered the expression of 17 miRNA. Among which, 16 miRNA were upregulated and one miRNA was downregulated (Wang et al., 2014). Integration with transcriptome profiles indicates that these miRNA target lipid metabolism–associated genes, including *ELOVL6*, *SERPINB1*, *ABCG5*, and *ACSL3* (Wang et al., 2014). Profiling of hepatic miRNA between 20-wk-old hens (pre–egg-laying period) and 30-wk-old hens (egg-laying period) identified 80 differentially expressed miRNA, 13 upregulated and 67 downregulated (Li et al., 2016). Integration with transcriptome profiles found that these miRNA target key lipid-associated enzymes, including *FADS1*, *FADS2*, *ELOVL6*, and *ACSL5* (Li et al., 2016). In profiling miRNA hepatic expressional changes during the metabolic switch, we found 30 miRNA, 13 upregulated and 27 downregulated, were differentially expressed between 18-day-old embryos and 3-day-old chicks (Hicks et al., 2017). Integration with transcriptome profiles revealed the differentially expressed miRNA target major metabolic genes, including *INSIG1*, *FASN*, *FADS2*, *ELOVL2*, and *HMGCS1* (Hicks et al., 2017). In a subsequent study in which male broiler (Ross 708) chicks were delayed fed for 48 h after hatching to retard the metabolic switch, a number of these switch-associated miRNA also had a delayed hepatic expressional pattern, providing additional evidence that miRNA are key regulators of hepatic metabolic processes in chickens (Hicks et al., 2019). A summary of miRNA profiling studies in the chicken liver is provided in Table 1.

Metabolically Important Functions of miRNA in Poultry

Genetic variation at miRNA loci are contributory factors to variations in metabolic-related traits in vertebrates including poultry. For example, an SNP in the precursor sequence for chicken *gga-miR-1606* was found to be associated with both BW and breast muscle weight. It was suggested that this SNP may alter the processing of *pre-miR-1606*, thus affecting the levels of mature *gga-miR-1606* (Li et al., 2015). An SNP in the precursor sequence of *gga-miR-1666* was also found to be associated with muscle growth and body size in chickens (Wang et al., 2015). Several studies have linked the *miR-16* family, *gga-miR-15a*, *gga-miR-15b*, and *gga-miR-16*, to the regulation of numerous metabolic genes in chickens. *gga-miR-15a* was observed to be significantly less expressed in chickens with lower feed conversion ratios (Yuan et al., 2017). A 54-bp insertion mutation upstream of the *miR-16* family, which results in lower *gga-miR-16* expression, was found to be associated with increased BW and muscle mass in chickens. Furthermore, this mutation tended toward fixation in commercial broiler breeds (Jia et al., 2016). *gga-miR-15b* was expressed ∼2-fold higher in chicken hepatocytes treated with GH (Wang et al., 2014). Target prediction analysis in this study suggested that *gga-miR-15b* has a propensity to target genes associated with lipid metabolism. These studies together indicate that in chickens, the *miR-16* family miRNA may play multiple roles in the regulation of growth and lipid metabolism in chickens.

Another miRNA family that has been linked to the regulation of metabolic processes, not only in poultry but also in other vertebrates as well, is the *miR-30* family. This miRNA family consists of 5 members, *gga-miR-30a*, *gga-miR-30b*, *gga-miR-30c, gga-miR-30d*, and *gga-miR-30e*, which share a seed sequence, but differ at their 3′ ends, allowing for an overlapping yet slightly different repertoire of targets (Miranda et al., 2018). *gga-miR-30a* expression was found to be altered in the skeletal muscle during chick development, and functional studies suggest it can inhibit myoblast proliferation (Chen et al., 2019a). The expression of *aan-miR-30d* was significantly lower in the livers of overfed French Landes geese than in control birds (Chen et al., 2017). Reduced *hsa-miR-30b* expression in adipose tissue has been linked to insulin resistance in humans (Kirby et al., 2016). The dysregulation of adipose-specific expression of *hsa-miR-30e* was found in patients with type II diabetes (Klöting et al.,

Table 2. Top 5 metabolic canonical pathways with predicted target genes of chicken miRNA with known metabolic functions.

miRNA	Predicted target genes[1]
IGF-1 signaling	
gga-miR-223	*FOXO3*; *PRKACB*; *RASA1*; *RPS6KB1*; *RRAS2*
gga-miR-193a	*KRAS*; *SOS2*; *YWHAZ*
gga-miR-193b	*PRKAR2B*; *PTK2*; *RAP2A*; *SOCS2*
gga-miR-133a	*PIK3C2A*
gga-miR-33	*GRB10*; *IGF1*; *MAPK8*; *PDPK1*; *PIK3R3*; *RAP2A*
miR-30 family[2]	*FOXO3*; *GRB10*; *IRS1*; *IRS2*; *KRAS*; *MAPK8*; *NEDD4*; *PRKAR1A*; *RAP1B*; *RAP2A*; *RAP2B*; *RASA1*; *RASD1*; *SOCS3*; *SOCS6*; *SOS1*; *YWHAG*; *YWHAZ*
gga-miR-29b	*AKT3*; *FOS*; *FOXO3*; *IGF1*; *MAPK8*; *PIK3R1*; *PIK3R3*; *RRAS2*; *YWHAE*
gga-miR-22	*AKT3*; *CCN1*
gga-miR-18b	*CNN2*; *NEDD4*; *YWHAB*
gga-miR-34a	*RRAS2*; *YWHAG*
gga-miR-20b	*AKT3*; *MAPK1*; *PIK3R1*; *PRKACB*; *RASA1*; *RASD1*; *SOCS6*; *SOS1*
gga-miR-10b	*PIK3CA*; *RAP2A*
gga-miR-1/206	*IGF1*; *MAPK1*; *PRKACB*; *RAP1B*; *RASA1*; *YWHAB*; *YWHAQ*; *YWHAZ*
miR-16 family[2]	*AKT3*; *FOXO1*; *GRB10*; *IRS1*; *MAP2K1*; *MAPK8*; *NEDD4*; *PDPK1*; *PIK3R1*; *PRKAR2A*; *RAF1*; *SOCS5*; *SOCS6*; *SOS2*; *YWHAH*; *YWHAQ*
gga-miR-1606	*FOS*; *PRKAR1A*; *PRK2*; *PXN*; *SOCS2*; *YWHAG*
Insulin receptor signaling	
gga-miR-223	*FOXO3*; *PRKACB*; *RRS2*; *TSC1*
gga-miR-193a	*KRAS*; *SOS2*
gga-miR-193b	*ORK*; *PRKAR2B*; *RAP2A*; *SYNJ1*
gga-miR-133a	*ACLY*; *CRKL*; *PIK3C2A*; *SGK1*
gga-miR-33	*CRK*; *GRB10*; *MAPK8*; *PDPK1*; *PIK3R3*; *RAP2A*
miR-30 family[2]	*CRKL*; *FOXO3*; *FYN*; *GRB10*; *IRS1*; *IRS2*; *IRS4*; *KRAS*; *MAPK8*; *PPP1R12 A*; *PPP1R14 C*; *PRKAR1A*; *RAP1B*; *RAP2A*; *RAP2B*; *RASD1*; *SOCS3*; *SOS1*
gga-miR-29b	*AKT3*; *FOXO3*; *MAPK8*; *PIK3R1*; *PIK3R3*; *PTEN*; *RRAS2*; *SGK1*
gga-miR-22	*AKT3*; *PTEN*; *RAPGEF1*
gga-miR-18b	*PTEN*
gga-miR-34a	*ASIC2*; *RRAS2*
gga-miR-20b	*AKT3*; *CRK*; *FYN*; *GAB1*; *MAPK1*; *PDE3B*; *PIK3R1*; *PRKACB*; *PTEN*; *RASD1*; *SOS1*
gga-miR-10b	*CRK*; *PIK3CA*; *RAP2A*
gga-miR-1/206	*CBL*; *EIF4E*; *MAPK1*; *PRKACB*; *PTPN1*; *RAP1B*
miR-16 family[2]	*AKT3*; *CRK*; *CRKL*; *EIF4E*; *FOXO1*; *GRB10*; *GSK3B*; *INSR*; *IRS1*; *IRS4*; *MAP2K1*; *MAPK8*; *PDPK1*; *PIK3R1*; *PRKAR2A*; *RAF1*; *RPTOR*; *SGK1*; *SOS2*; *SYNJ1*
gga-miR-1606	*GSK3B*; *PRKAR1A*; *RPTOR*
PPARα/RXRα activation	
gga-miR-223	*ACVR2A*; *HSP90B1*; *PRKACB*; *RRAS2*
gga-miR-193a	*ABCA1*; *ADCY9*; *KRAS*; *PPARGC1A*; *SOS2*; *TGFB2*; *TGFBR3*
gga-miR-193b	*MAP2K6*; *MAP4K4*; *NCOA6*; *PLCD1*; *PLCH2*; *PRKAR2B*; *RAP2A*
gga-miR-133a	*CAND1*; *TGFBR1*
gga-miR-33	*ABCA1*; *MAP3K7*; *MAP4K4*; *MAPK8*; *PRKAA1*; *RAP2A*
miR-30 family[2]	*ACVR1*; *BMPR2*; *CAND1*; *CLOCK*; *GNAQ*; *IL1RAPL2*; *IRS1*; *KRAS*; *MAP4K4*; *MAPK8*; *PPARGC1A*; *PRKAR1A*; *RAP1B*; *RAP2A*; *RASD1*; *SMAD2*; *SOS1*

(continued on next page)

Table 2. (*continued*)

miRNA	Predicted target genes[1]
gga-miR-29b	*MAP2K6*; *MAP4K4*; *MAPK8*; *RRAS2*; *TGFB2*
gga-miR-22	*EP300*; *PRKAB1*
gga-miR-18b	*GNAS*; *SMAD2*
gga-miR-34a	*ADCY5*; *BMPR2*; *GNAQ*; *MAP4K4*; *RRAS2*
gga-miR-20b	*ABCA1*; *ADCY2*; *BMPR2*; *CAND1*; *CHD5*; *CLOCK*; *IL1RAPL1*; *MAPK1*; *NR2C2*; *PLCB4*; *PRKACB*; *RASD1*; *SOS1*; *TGFBR2*
gga-miR-10b	*IL1RAPL1*; *NCOA6*; *NOCR2*; *NR2C2*; *RAP2A*
gga-miR-1/206	*ABCA1*; *CLOCK*; *GNAQ*; *HSP90B1*; *IL1RAPL1*; *MAPK1*; *PRKACB*; *RAP1B*; *TGFBR3*
miR-16 family[2]	*ACVR2A*; *ACVR2B*; *GHR*; *GNAQ*; *IL1RAPL1*; *INSR*; *IRS1*; *MAP2K1*; *MAPK8*; *PRKAR2A*; *RAF1*; *SOS2*; *TGFBR3*
gga-miR-1606	*IL1RAPL2*; *MAP2K3*; *NCOR1*; *PLCB1*; *PLCH2*; *PRKAR1A*; *RXRA*
HGF signaling	
gga-miR-223	*ETS1*; *PRKCE*; *RRAS2*
gga-miR-193a	*CCND1*; *ETS1*; *KRAS*; *SOS2*
gga-miR-133a	*CDC42*; *CRK1*; *ELF2*; *PIK3C2A*
gga-miR-33	*MAP3K7*; *MAPK8*; *PIK3R3*; *RAP2A*
miR-30 family[2]	*CRKL*; *KRAS*; *MAP3K1*; *MAP3K5*; *MAPK8*; *RAP1B*; *RAP2A*; *RAP2B*; *RASD1*; *SOS1*
gga-miR-29b	*AKT3*; *CDC42*; *ELF2*; *FOS*; *MAPK8*; *PIK3R1*; *PIK3R3*; *RASD1*; *SOS1*
gga-miR-22	*AKT3*; *ETS2*; *MAP3K1*; *RAPGEF1*
gga-miR-18b	*CDC42*; *MAP3K1*
gga-miR-34a	*CCND1*; *PRKD1*; *RRAS2*
gga-miR-20b	*AKT3*; *CCND1*; *ELK3*; *GAB1*; *MAP3K2*; *MAP3K3*; *MAP3K9*; *MAPK1*; *PIK3R1*; *RASD1*; *SOS1*
gga-miR-10b	*PIK3CA*; *RAP2A*
gga-miR-1/206	*CCND1*; *ETS1*; *MAPK1*; *RAP1B*
miR-16 family[2]	*AKT3*; *CCND1*; *CRKL*; *HGF*; *MAP2K1*; *MAP2K4*; *MAPL8*; *PIK3R1*; *RAF1*; *SOS2*
gga-miR-1606	*CCND1*; *FOS*; *PTK2*; *PXN*
Adipogenesis pathway	
gga-miR-223	*ATG7*; *FBXW7*; *FGFR2*; *RPS6KB1*
gga-miR-193a	*CTNNB1*; *RUNX1T1*; *TBL1XR1*
gga-miR-193b	*FZD4*; *FZD9*; *HDAC9*; *KAT2B*; *KAT6A*; *KAT7*; *SREBF1*
gga-miR-133a	*CTBP2*; *RUNX1T1*
gga-miR-33	*EBF1*; *RUNX1T1*; *SREBF1*; *XBP1*
miR-30 family[2]	*ATG5*; *BMPR2*; *CLOCK*; *DGKD*; *GTF2H1*; *HDAC9*; *NR2F2*; *SIRT1*; *SMAD1*; *SMAD5*
gga-miR-29b	*FBXW7*; *FZD8*; *HDAC4*; *RUNX1T1*; *WNT10 B*
gga-miR-22	*RBXW7*; *FZD8*; *HDAC4*; *KAT6A*; *KAT6B*; *RUNX1T1*; *SIRT1*
gga-miR-34a	*BMPR2*; *TBL1XR1*
gga-miR-20b	*ATG7*; *BMP2*; *BMPR2*; *CLOCK*; *KAT2B*; *KLF3*
gga-miR-10b	*GTF2H1*
gga-miR-1/206	*CLOCK*; *CTBP2*; *RBXW7*; *FGFR3*; *FXD7*; *HDAC4*; *KAT6A*; *KAT6B*
miR-16 family[2]	*BMPR1A*; *FBXW7*; *FGF2*; *FOXO1L*; *RUNX1T1*; *WNT10 B*; *ZNF423*
gga-miR-1606	*CDK5*; *CEBPB*; *FZD5*; *FXD8*; *SOX9*; *WNT5A*

Abbreviations: miRNA, microRNA; PPARα, peroxisome proliferator-activated receptor alpha; RXRα, retinoid X receptor alpha.

[1]Predicted chicken target genes were downloaded from the TargetScan database (www.targetscan.org; version 7.2) and subjected to ingenuity pathway analysis (Qiagen, Germantown, MD).

[2]The *miR-30* family consists of *gga-miR-30a*, *gga-miR-30b*, *gga-miR-30c*, *gga-miR-30d*, and *gga-miR-30e*. The *miR-16* family consists of *gga-miR-15a*, *gga-miR-15b*, and *gga-miR-16*.

2009). Furthermore inhibition of *hsa-miR-30a* and *hsa-miR-30d* has been found to block adipogenesis in human adipocytes (Zaragosi et al., 2011). Taken together, these studies support a conserved role of the *miR-30* family in regulating lipid and glucose metabolism in both mammals and poultry.

The related miRNA *gga-miR-193a* and *gga-miR-193b* are both involved in regulating metabolic processes in chickens. Chicken hepatocytes treated with GH had significantly higher levels of *gga-miR-193a* than in untreated cells (Wang et al., 2014). *gga-miR-193b* hepatic expression was observed to be higher in selenium-deficient broilers, which was linked to the regulation of apoptosis-related genes (Liu et al., 2018). These studies suggest that *miR-193a/b* may function in the regulation of hepatocyte proliferation and apoptosis in response to developmental and nutritional cues.

A comparison of hepatic miRNA profiles between hens at 20 wk (pre–egg-laying period) and hens at 30 wk (egg-laying period) found that expression of *miR-22* significantly increased over this time (Li et al., 2016). *gga-miR-22* was predicted to target a number of lipid metabolic genes, including *ACSL5*, *ELVOL6*, and *PLIN2* (Li et al., 2016). Another study also linked the regulation of *ELOVL6* expression in the liver to *gga-miR-22* in egg-laying hens (Ma et al., 2017). These studies suggest that *gga-miR-22* may serve to regulate hepatic lipid production during egg production in chickens. Similar to *gga-miR-22*, *gga-miR-34a* exhibits significantly higher hepatic expression during the egg-laying period. It was further demonstrated that in chicken hepatocytes, *gga-miR-34a* could increase intracellular levels of both triglycerides and cholesterol by targeting *ACSL1* (Tian et al., 2019). *Apl-miR-34a* was also found to regulate *ACSL1* expression in duck adipocytes (Wang et al., 2018). Taken together, these studies reveal an important role of hepatic miRNA in regulating lipid production during the egg-laying period in poultry. This is likely to ensure sufficient hepatic lipogenesis for lipid disposition in yolks.

The miRNA *miR-33* is one of the most well-known metabolic miRNA. Primates and rodents encode 2 *miR-33* isoforms, *miR-33a* and *miR-33b*, located in an intron of *SREBF2* and *SREBF1*, respectively (Najafi-Shoushtari et al., 2010). Birds encode only a single form of *miR-33*, located in an intron of *SREBF2* (Horie et al., 2010). In chickens, *gga-miR-33* has been shown to target enzymes in fatty acid oxidation including *CROT* and *HADHB* (Shao et al., 2019) and *FTO* (Shao et al., 2014). The hepatic expression of *gga-miR-33* was found to increase from hatching through the first weeks (7 wk) of posthatch life (Shao et al., 2019). These studies indicate that in the chicken liver, *gga-miR-33* may serve as a negative regulator of fatty acid oxidation. We found that hepatic *gga-miR-33* expression is significantly lower in newly hatched chicks subjected to a 48-h delay in feeding than in their fed-from-hatch counterparts (Hicks et al., 2019). As delayed-fed chicks must exclusively rely on oxidation of residual yolk lipids for energy production, this reduced hepatic expression in delayed-fed chicks further supports a role of chicken *gga-miR-33* in the negative regulation of fatty acid oxidation. We also found that the hepatic expression of *gga-miR-20b* was downregulated after hatching (Hicks et al., 2017) and was significantly lower in fed-from-hatch chicks than in delayed-fed chicks (Hicks et al., 2019). We validated a number of lipid metabolism-associated target genes for *gga-miR-20b*, among which are *MSMO1* and *FADS1* (Hicks et al., 2017). *MSMO1* is involved in cholesterol biosynthesis, and *FADS1* is involved in the synthesis of fatty acids; both were significantly depressed in delayed-fed chicks (Hicks et al., 2019). These studies suggest the hepatic *miR-20b* may be regulated by nutritional cues to adjust lipid production. We further discovered that *gga-miR-20b* transcription is regulated by FOXO3, a major regulator of numerous oxidative stress and metabolic pathways (Hicks et al., 2017), and that *FOXO3* hepatic expression is significantly higher in delay-fed birds (Hicks et al., 2019). Interestingly, a number of miRNA associated with metabolic processes in chickens potentially regulate *FOXO3* expression (Table 2). This suggests that metabolic miRNA may participate in complex feedback loops to maintain the balance of metabolic homeostasis.

It has recently been proposed that circular RNA can be produced from protein-coding genes when an mRNA is "backspliced," resulting in the joining of 2 splice sites, and can act as sponges for miRNA to inhibit their activity (reviewed by Xiao et al., 2020). A circular RNA generated from the *FGFR2* gene can inhibit *gga-miR-133a* and *gga-miR-29b* activity in embryonic chick myoblasts (Chen et al., 2018). Overexpression of *circFGFR2* in chick myoblasts increased proliferation, and conversely, siRNA-mediated knockdown of *circFGFR2* inhibited proliferation (Chen et al., 2018). This indicates that *gga-miR-133a* and *gga-miR-29b* may function as negative regulators of myoblast proliferation in chickens. Another miRNA that has been associated with regulating myoblast proliferation in chickens is *gga-miR-223*. *gga-miR-223* expression in the breast muscle differs between fast-growing and slow-growing chickens (Ouyang et al., 2015) and is downregulated in intramuscular adipocytes during the egg-laying period in hens (Li et al., 2019b). Functional studies indicate that *miR-223* likely regulates both myoblast and adipocyte proliferation and differentiation by targeting a number of muscular and adipocytic regulators, including *FOXO3*, *ADAM17*, *MYH10*, *GPAM*, and *IGF2* (Ouyang et al., 2015; Li et al., 2017, 2019b). In ducks, *anl-miR-223* expression was correlated with adipogenesis (Wang et al., 2018). Taken together, these studies identify *miR-223* as an important mediator of both muscle and adipose development in poultry.

Intramuscular adipose tissue serves as a secondary lipid storage site when the adipose tissue has reached capacity (reviewed by Kitessa and Abeywardena, 2016). As discussed previously, *miR-223* was found to regulate intramuscular adipogenesis in chickens (Li et al., 2017). A small RNA profile screening during intramuscular

adipocyte differentiation in chickens found 117 miRNA were differentially expressed between intramuscular preadipocytes and adipocytes (Sun et al., 2019). Among these was *gga-miR-18b*, which was significantly downregulated during adipocyte differentiation. Functional analysis revealed that *gga-miR-18b* can serve as a negative regulator of intramuscular adipocyte differentiation by targeting *ACOT1B* (Sun et al., 2019). These miRNA studies highlight the importance of miRNA in regulating the proliferation and fates of precursor cells during adipose tissue development in birds.

Sex-linked dwarf chickens are significantly smaller than typical chicken breeds. This reduced size in dwarf chickens is most likely associated with a deletion mutation in *GHR*, comprising part of the last exon and 3′UTR (Agarwal et al., 1994). Profiles of miRNA expression in the muscles of dwarf chickens compared with normal-sized chickens identified 4 differentially expressed miRNA, *gga-miR-1623*, *gga-miR-181b*, *gga-miR-128*, and *gga-let-7b* (Lin et al., 2012). A binding site for *gga-let-7b* was identified in the 3′UTR of *GHR*, within the region of the deletion mutation of dwarf chickens. It was further suggested that inability of *gga-let-7b* to target the GHR contributes to the increased mRNA levels of *GHR* found in the muscles of dwarf chickens (Lin et al., 2012). A miRNA expression profile comparison in the adipose tissue of dwarf and normal chickens also revealed several differentially expressed miRNA (Ye et al., 2014). The miRNA *gga-miR-10b* and *gga-let-7k* were significantly upregulated in the adipose tissue of dwarf chickens, and *gga-miR-2188* was significantly downregulated. In ducks, *anl-miR-10b* expression was found to be significantly higher in preadipocytes and was reduced upon adipocyte differentiation (Wang et al., 2018). Several studies have reported that *hsa-miR-10b* expression also decreases during differentiation of human preadipocytes (Li et al., 2018; Tan et al., 2019). As *gga-miR-10b* levels are higher in the adipose tissue of dwarf chickens, this suggests that these birds have reduced amounts of adipogenesis compared with typical chickens, which, at least partially, is due to miRNA dysexpression, such as *miR-10b*. Furthermore, these studies suggest that *miR-10b* is an inhibitor of adipocyte differentiation, and its functions are conserved across vertebrates. A summary of experimentally verified metabolic functions of miRNA is provided in Table 3.

To further investigate the metabolic functions of the previously discussed miRNA, we obtained their predicted chicken target genes from the TargetScan database (www.targetscan.org; version 7.2) and explored the pathways associated with these targets using ingenuity pathway analysis (Qiagen, Germantown, MD). Interestingly, we discovered that these miRNA likely share numerous overlapping metabolic target genes and pathways (Figure 1; Table 2). This implies that in addition to their known metabolic functions discussed previously, they also participate in other, yet to be discovered, metabolic processes. Furthermore, it appears that, if a metabolic pathway is regulated by miRNA, then it is likely regulated by these miRNA at all levels, from the initial receptor engagement to the final outcome. Highlighted in Figure 1 are 2 such metabolic pathways: insulin-like growth factor 1 signaling and peroxisome proliferator-activated receptor alpha/retinoid X receptor alpha activation, which are both targeted by multiple miRNA with known metabolic functions in chickens and other poultry. Although not all of these predicted targets are or will be experimentally validated in poultry, the sheer number of metabolic genes with potential miRNA binding sites highlights the importance of taking into account the role of miRNA to fully understand the underlying molecular mechanisms governing metabolic development and function. Some miRNA, such as those in the *miR-16* family (*miR-15a*, *miR-15b*, and *miR-16*), appear to focus their regulation on the initial response to metabolic cues. For example, the *miR-16* family was predicted to target several metabolic receptors in chickens, including *INSR*, *GHR*, and *TGFBR* (Figure 1; Table 2). This may explain, at least in part, the link between this miRNA family and growth in poultry in several studies (Wang et al., 2014; Jia et al.,

Table 3. Experimentally verified metabolic functions of miRNA in chickens.

Breed and/or cell type	Metabolic trait	miRNA	Physiological effect	Reference
10-wk-old and 12-wk-old Gushi-Anka cross F_2	BW	*gga-miR-1606*	SNP(C>A) CA>AA>CC	Li et al., 2015
8-wk-old Gushi-Anka cross F_2	BW	*gga-miR-1666*	SNP (C>G) CC>GG>CG	Wang et al., 2015
7-wk-old Xinghua-recessive White cross F_2	BW	*gga-miR-16*	54-bp insertion reduces miR-16 expression, fixed in higher weight breeds	Jia et al., 2016
E10 Yuhe primary myoblasts	Myoblast differentiation	*gga-miR-30a*	Inhibits MEF2C-mediated myoblast proliferation	Chen et al., 2017
30-wk-old Lushi hen	Hepatocyte lipid content	*gga-miR-34a*	Increases intracellular triglyceride and cholesterol levels; targets ACSL1	Tian et al., 2019
E11 chicken primary myoblasts from leg muscles	Myoblast proliferation	*gga-miR-133a; gga-miR-29b*	Inhibit myoblast proliferation	Chen et al., 2018
2-wk-old Gushi primary intramuscular preadipocytes from the breast muscle	Adipocyte proliferation	*gga-miR-18b*	Inhibits preadipocyte differentiation; targets ACOT13	Sun et al., 2019

Abbreviation: miRNA, microRNA.

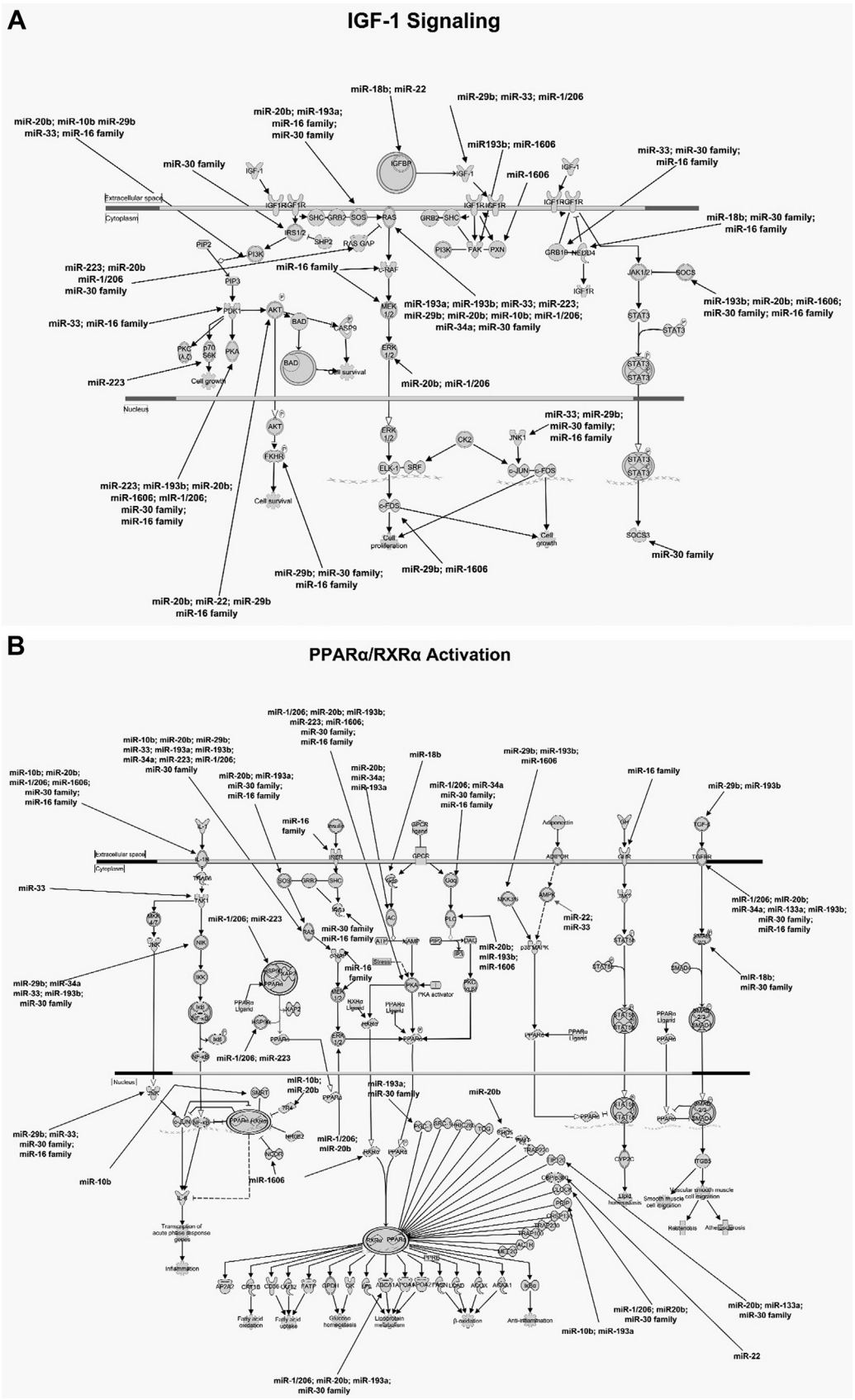

Figure 1. Examples of potential miRNA-mediated regulation of metabolic pathways in chickens. The predicted target genes of chicken miRNA with known involvement in metabolic processes were downloaded from the TargetScan database (www.targetscan.org; version 7.2) and subjected to ingenuity pathway analysis (IPA; Qiagen, Germantown, MD). Highlighted are 2 major metabolic pathways, (A) insulin-like growth factor 1 (IGF-1) signaling and (B) PPARα/RXRα activation, which are potentially regulated at all levels by chicken metabolic miRNA. Abbreviations: miRNA, microRNA; PPARα, peroxisome proliferator-activated receptor alpha; RXRα, retinoid X receptor alpha.

2016; Yuan et al., 2017). Altered expression of the *miR-16* family could lead to altered expression of these receptors, all of which have been associated with myogenesis and/or muscle growth in chickens (Masseagué et al., 1986; Agarwal et al., 1994; Cai et al., 2017). Other miRNA appear to exert their effects on further downstream targets. For example, the *miR-30* family, *gga-miR-33*, and *gga-miR-20b* are predicted to target a number of intermediary enzymes in metabolic signaling cascades and also target a number of effector enzymes (Figure 1; Table 2).

When all of the studies discussed here are taken together, a complex picture emerges in which miRNA can tip the scales to alter the outcome of virtually all metabolic processes in poultry. With the advent and wide adoption of next-generation sequencing technologies, a large and diverse inventory of miRNA expression signatures across a variety of developmental and metabolic states in poultry has been and continued to be cataloged. However, elucidation of the functional roles of these miRNA has only just begun. Future efforts must focus on developing a better understanding of how the effects of miRNA targeting of specific metabolic genes and pathways manifest in both normal and disrupted metabolic states in poultry.

CONCLUSION

It has become increasingly evident that many diverse transcriptional and post-transcriptional regulatory systems must coordinate to maintain proper homeostatic operations of an organism and disruption of this finely balanced system has dire consequences. Metabolism is a complex physiological machine with many cogs that are constantly in flux owing to ever-changing nutrient sources and energy demands. As miRNA are thought to have evolved as a rapid response system to "finely tune" gene expression in reaction to physiological and environmental stimuli, it is not surprising they elicit broad effects on metabolic development and function. Although much remains unknown regarding miRNA-mediated regulation of metabolic pathways in poultry, we and others have discovered that miRNA are expressed early in chicken metabolic development and play lifelong metabolic functions (Tables 1 and 2). In the years to come, many more metabolic functions of miRNA and other regulatory RNAs will be discovered in poultry.

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DISCLOSURES

The authors declare no conflicts of interest.

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