ABSTRACT: Past efforts to improve animal performance have been mainly focused on genetic selections and a number of markers have been identified to be important for production traits. In addition to genetics, recent studies found that environmental factors affect animal development, especially during the embryonic and fetal stages, when all major organs and tissues are formed. Because all fetal nutrients are derived from their mothers, maternal nutrition is the major environmental factor affecting fetal development, which sets the developmental trajectory and has long-term impacts on livestock performance. Stem cells are sources of progenitor cells for the growth and development of adult animals, and epigenetic changes during fetal development shape the epigenome of fetal progenitor cells and their derived adult stem cells, which alter stem cell function, providing a key mechanism linking environmental factors during early development to the long-term performance of animals.

Keywords: beef cattle; epigenetics; fetus; marbling

Introduction

For economic reasons, livestock, especially pigs, have been extensively selected for rapid growth and high lean/fat ratio, while cattle are selected for high marbling. These fruitful efforts generate a number of superior breeds of livestock with great growth performance. In addition to the improvement in genetics, accumulating evidences point to the importance of environmental factors in regulating animal growth and development. Changes in these factors during the period when animals are actively developing profoundly affect their performance and the resulting product quality.

Animals are developed from a fertilized egg, which proliferate and further differentiate into tissues and organs. During development, each cell gains its unique epigenetic signature which allows the expression of a subset of genes to provide the cell with its identity. The formation of epigenetic pattern is partially encoded in the genomic DNA, and dramatically affected by the niche environment where cell locates. For example, the existence of morphogen gradients determines the pattern of cell differentiation during the embryonic stage (Plouhinec et al., 2011; Rogers and Schier, 2011). Thus, epigenetic modifications in cells during animal development are highly susceptible to the effects of environmental clues.

The major events of animal development occur during the embryonic and fetal stages. All nutrients available to fetuses are derived from maternal circulation and, thus, maternal nutrition and physiological conditions are major environmental factors affecting fetal development (Du et al., 2010b). Maternal under- or over-nutrition leads to epigenetic changes in fetal cells during development, which sets the developmental trajectory and has long-term effects on animal performance (Du et al., 2013). In this review, we discuss early animal development, focusing on muscle and fat development, two economically very important tissues. We further describe major epigenetic changes occur during early animal development, and their long-term impacts on the growth performance of animals.

Early skeletal muscle and adipose tissue development

Skeletal muscle development is roughly divided into 3 stages: embryonic, fetal and adult stages (Du et al., 2010a). Embryonic and fetal skeletal muscle development is collectively referred to as prenatal muscle development, which has dramatic impact on postnatal growth (Dauncey and Harrison, 1996). During the prenatal stage, skeletal muscle development mainly involves the formation of muscle fibers (i.e., myogenesis), but also the formation of intramuscular adipocytes (adipogenesis). In livestock, muscle fibers are formed during the prenatal stage and there is no net increase after birth. Intramuscular adipogenesis during the early developmental stage generates intramuscular preadipocytes and adipocytes, which accumulates lipid and forming marbling fat in later life.

Skeletal muscle development. During the prenatal stage, new muscle fiber formation ceases at late gestation in pigs (up to around 90 days, term 114 days) (Wigmore and Stickland, 1983), and mid-gestation in cattle (up to around 180 days, term 284 days) (Bonnet et al., 2010). Because muscle fibers result from the fusion of myogenic cells, higher abundance of myogenic cells due to active proliferation results in more muscle fiber formation during the fetal stage (Zhu et al., 2004). Consistently, mutation of myostatin, a negative regulator of myogenic cell proliferation, results in “double muscling” cattle (McPherron and Lee, 1997). In addition, the proliferation of myogenic precursor cells is highly sensitive to the availability of nutrients, and maternal nutrition affects the proliferation and abundance of myogenic cells and the subsequent formation of muscle fibers (Tong et al., 2009; Yan et al., 2010; Zhu et al., 2004; Zhu et al., 2008).

The postnatal muscle growth is mainly due to the increase in muscle fiber size (Brameld et al., 2000), which relies on muscle satellite cells, a group of adult stem cells. Muscle satellite cells, originated from the embryonic myotome, lies between the sarcolemma of myofibers and surrounding basal lamina in adult skeletal muscle (Reznik, 1969). These cells proliferate and fuse with existing muscle fibers, resulting in muscle fiber hypertrophy. Indeed, the majority of nuclei in adult muscle fibers are derived from muscle satellite cells (Allen et al., 1979), showing the critical role of these cells for postnatal muscle growth.
Adipose tissue development. There are 4 major depots in livestock, which are visceral, subcutaneous, intermuscular and intramuscular depots. While the accumulation of adipose tissue intramuscularly is highly desirable, accumulation of adipose tissue elsewhere is a liability to livestock production. Adipocytes, especially intramuscular adipocytes, share immediately common progenitor cells with myogenic cells during early development; as a result, exploring mechanisms governing the early commitment of progenitor cells to either myogenic or adipogenic-fibrogenic lineages will shed a light to the understanding of the formation of intramuscular adipocytes and the resulting marbling (Du et al., 2013).

The formation of discernable adipocytes/adipose tissue begins before mid-gestation in beef cattle (Bonnet et al., 2010). In perinatal fat, adipocytes were detected as early as 80 days of gestation while adipocytes in the intramuscular fat are detectable at 180 days of gestation (Taga et al., 2011). Most adipocytes are formed during the fetal and early postnatal stages, and adipocyte hyperplasia largely ceases in perirenal fat after birth (Bonnet et al., 2010; Du et al., 2010a). In agreement, the total number of adipocytes is set when reaching adolescence (Goessling et al., 2009). Because livestock are slaughtered at a quite young age, adipocyte hyperplasia is ongoing lifelong but attenuates as animals become older due to the declining ability to generate new adipocytes (Du et al., 2010c). Therefore, nutritional and physiological conditions during the fetal, postnatal, and early postweaning stages affect adipogenesis and the total number of adipocytes in animals.

Adipogenesis is termed to describe the de novo generation of adipocytes, which is briefly separated into two stages: determination and differentiation (MacDougald and Mandrup, 2002). During the differentiation stage, peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding proteins (CEBP)s have critical regulatory roles (Avram et al., 2007). C/EBPβ/δ, which express at the very early stage of adipogenesis, triggers the expression of PPARγ (Fajas et al., 2001), an essential and indispensable transcription factor for adipogenic differentiation (Rosen and MacDougald, 2006; Spiegelman and Flier, 1996). Much less information is available regarding to adipogenic determination. Recently, Zinc-finger protein Zfp423 was identified as a transcriptional factor responsible for the adipogenic commitment of progenitor cells (Gupta et al., 2010). The expression of Zfp423 commits progenitor cells to pre-adipocytes, which further induces PPARγ expression and terminal adipogenic differentiation of adipocytes (Gupta et al., 2010; Gupta et al., 2012).

Fetal programming

Fetal programming, also named development programming or the Barker hypothesis, refers to the fetal origins of adult diseases. Based on epidemiological data, this hypothesis describes that low birth weight due to maternal malnutrition has lasting effects on adult health (Barker, 2002), and that the changes in maternal uterine environment as a result of nutritional stress at certain stages of fetal growth and development might permanently affect tissue structure and function in offspring (Drake and Walker, 2004).

Skeletal muscle has the lower priority of nutrition repartition, compared with brain and heart tissues, which makes the development of skeletal muscle vulnerable to nutritional change (Zhu et al., 2006). Studies on a sheep model indicated that both maternal under and over-nutrition affect skeletal muscle development and intramuscular adipogenesis (Quigley et al., 2005; Stannard and Johnson, 2004; Tong et al., 2008; Tong et al., 2009; Yan et al., 2010; Zhu et al., 2008), which has long lasting, irreversibly negative physiological consequences for offspring. This phenomenon has been proven in in-utero under-nourished lambs (Zhu et al., 2006), pigs (Dwyer et al., 1994) and guinea pigs (Ward and Stickland, 1991).

Compared to skeletal muscle, the nutrient partitioning priority for adipose tissue is even lower. Adipose tissue mainly develops between late gestation to 4 weeks of age in rodents (Greenwood and Hirsch, 1974; Knittle and Hirsch, 1968). Adipocyte number in epidymal fat increases little after weaning in mice (Johnson and Hirsch, 1972) and further development is primarily due to adipocyte hypertrophy (Bertrand et al., 1978; Greenwood and Hirsch, 1974). Thus, the major development of adipose tissue coincides with pregnancy and nursing, and maternal nutrition likely incurs permanent changes in properties of offspring adipose tissue. We have previously demonstrated that fetal stage is critical for the development of inter/intramuscular adipocytes (Yan et al., 2011; Yan et al., 2010). Consistently, a recent study found that maternal obesity correlates with adiposity in human neonates (Modi et al., 2011), consistent with adiposity in offspring of obese mice (Samuelsson et al., 2008; Tong et al., 2011). In short, existing data clearly show that maternal nutrition, including both under and over-nutrition, profoundly affects fetal development and the growth performance of offspring. But the question here is why? Very recent progress points to the epigenetic modifications in linking fetal development to offspring performance.

Epigenetic modifications

Stem cells and progenitor cells maintain their plurip- or multi-potency through reversible inhibition of lineage-specific genes while allowing genes for self-renewal to express. Conversely, during differentiation, lineage-specific genes are expressed while plurip-or multi-potency genes are inhibited (Meissner et al., 2008; Mohn et al., 2008). Polycomb repression complexes (PRCs) are mainly responsible for reversible inhibition of genes. There are two well-characterized PRCs, namely PRC1 and PRC2. The core of PRC2 has four components, which are EZH2 (Enhancer of Zeste 2), SUZ12 (Suppressor of Zeste 12), EED (Embryonic ectoderm development), and RbAp48 (Retinoblastoma-associated protein 48) (Margueron and Reinberg, 2011). EzH2 mediates histone 3 lysine 27 trimethylation (H3K27me3) (McCabe et al., 2012; Qi et al., 2012), a marker for gene silencing (Bernstein et al., 2006). Until now, no specific DNA binding element for PRC2 has been identified, though PRC2 preferably binds to promoters...
with rich CpG sites, which attracts PRC1 binding (Mendenhall et al., 2010; Mohn et al., 2008). In the absence of stimulation to release PRCs, these promoters frequently become DNA methylated later (Ko et al., 2008; Lorente et al., 2006; Mohn et al., 2008), converting to permanent silencing (Mohn et al., 2008).

Trithorax group (trxG) catalyzes H3K4 trimethylation (H3K4me3), which activates gene transcription. It appears that H3K4me3 is transient, and only induced when gene expression is needed to counter the inhibitory effect of Polycomb group (Eissenberg and Shilatifard, 2010; Schuettengruber et al., 2011). Interestingly, H3K4me3 and H3K27me3 co-exist, forming a ‘bivalent state’ (Meissner et al., 2008; Mikkelsen et al., 2007), which poise genes for activation or inhibition. During differentiation, non-induced bivalent genes lost active H3K4me3 but kept repressive H3K27me3 mark (Schuettengruber and Cavalli, 2009), leading to largely permanent inhibition of gene expression by inducing DNA methylation (Mohn et al., 2008).

The dynamics of these epigenetic regulatory systems are affected by both genetic and environmental factors. Gene polymorphisms in the promoters of key developmental genes affect the binding of complexes involved in epigenetic modifications, altering the lineage commitment of progenitor cells during development. Similarly, environmental factors and clues alter cell signaling pathways which regulate epigenetic modifications to alter animal development (Figure 1). Because there are vast information and rapid progress in this field, in the following, we will only discuss the regulation of one key developmental gene committing progenitor cells to adipogenic lineage, the Zfp423.

![Figure 1. Genetic and environmental factors converge on the epigenome of progenitor cells to regulate cell lineage commitment during the early stage of animal development.](image)

**Zfp423 epigenetic modifications and adipogenic commitment**

In our previous studies, we observed that adipogenic differentiation was enhanced in fetuses of obese dams (Yan et al., 2010; Zhu et al., 2008), and increased intramuscular fat content in offspring (Yan et al., 2011), as well as overall adiposity (Samuelsson et al., 2008; Tong et al., 2011). To define the mechanisms leading to the enhanced adipogenic differentiation in fetal tissue, we first used gain and loss of function studies to demonstrate that Zfp423 regulates adipogenic commitment and adipogenesis in the developing fetal tissue (Yang et al., 2013). The remaining question becomes how maternal obesity and over-nutrition regulates Zfp423 expression during fetal development. Our bioinformatics analyses show that the Zfp423 promoter presents exceptionally rich CpG sites and islands, meeting the characteristics of “key developmental gene” with high CpG density promoters (Meissner et al., 2008). These rich CpG sites recruit PRC2 which catalyzes H3K27me3 in mouse embryonic fibroblasts (Goren et al., 2009). To test whether there is a change in epigenetic modifications, we designed primers corresponding to the CpG islands overlaying H3K27me3 peak in the Zfp423 promoter. Indeed, we observed that maternal obesity reduced DNA methylation in the Zfp423 promoter by about 50% (Yang et al., 2013), which aligned with the higher expression of Zfp423 in obese fetal tissue. Our data has been independently confirmed by another study in rats (Borengasser et al., 2013).

Because the Zfp423 promoter has exceptionally rich GC sites, PRC2 is positioned as a key mediator of Zfp423 expression and adipogenic commitment, which induces H3K27me3 (Bernstein et al., 2006). Our data show that the H3K27me3 and EZH2 levels in the Zfp423 promoter were lower in obese compared to control fetal tissue, consistent with the lower DNA methylation and the high expression of Zfp423. Furthermore, the level of H3K4me3 in the Zfp423 promoter was slightly higher in OB fetal tissue, which indicates that trxG was also involved in the control of Zfp423 expression due to maternal obesity. In brief, we observed that maternal obesity and over-nutrition reduced DNA methylation in the Zfp423 promoter, which was correlated with higher Zfp423 expression and enhanced adipogenesis of progenitor cells. Because DNA methylation is stable, the reduced DNA methylation in the Zfp423 promoter and enhanced Zfp423 expression in fetal progenitor cells enhances the adipogenic capacity of their derived cells in offspring adipose tissue, altering adipose tissue development.

In an related study, we analyzed the role of Zfp423 in intramuscular adipogenesis and marbling in beef cattle. We sampled beef muscle for separation of stromal vascular cells. These cells were immortalized with pCI neo-hEST2 and individual clones were selected by G418. A total of 288 clones (3 x 96 well plates) were isolated and induced to adipogenesis. The presence of adipocytes was assessed by Oil-Red-O staining. Three clones with high and low adipogenic potential respectively were selected for further analyses. In addition, fibro/adipogenic progenitor cells were selected using a surface marker, platelet derived growth factor receptor (PDGFR)α (Joe et al., 2010; Uezumi et al., 2010; Uezumi et al., 2011). The expression of Zfp423 was much higher (307.4 ± 61.9%, P < 0.05) in high adipogenic cells. Following adipogenic differentiation, the expression of PPARγ and C/EBPα were much higher (239.4 ± 84.1% and 310.7 ± 138.4%, respectively, P < 0.05) in high adipogenic cells. Over-expression of Zfp423 dramatically increased their adipogenic differentiation, while knockdown by shRNA prevented adipogenic differentiation of progenitor cells. The differential regulation of Zfp423...
between low and high adipogenic cells is associated with the DNA methylation in the Zfp423 promoter (Huang et al., 2012). In conclusion, data show that Zfp423 is a critical regulator of adipogenesis in stromal vascular cells of bovine muscle, and Zfp423 may provide a molecular target for enhancing intramuscular adipogenesis and marbling in beef cattle.

Besides the impact of maternal nutrition on the epigenetic modifications of genes determining adipogenic commitment, there are also reports about the impact of maternal nutrition on density and myogenic differentiation of satellite cells, critical for postnatal skeletal muscle development. Prenatal malnutrition in mice results in a reduction in satellite cell density in offspring muscle, which reduces the muscle regeneration capacity during injury, a process highly dependent on the myogenic differentiation of satellite cells (Woo et al., 2011). This study clearly shows that the function of satellite cells is affected by early maternal nutrition, strongly supporting our notion that environmental factors during early development exert long-term changes in the properties of adult stem cells via epigenetic modifications, which provides a major mechanism linking early development to later animal performance.

Conclusions

Early determination of progenitor cells to either myogenic or adipogenic lineage has dramatic impacts of the lean/fat ratio of animals, thus the growth efficiency of animals and the quality of meat. The lineage commitment is affected by genetics, maternal nutrition and physiological status. Because all fetal nutrients are derived from their mothers, maternal nutrition is the major environmental factor affecting fetal development, which sets the developmental trajectory and has long-term effects on livestock performance. Rapid progress in epigenome research propels epigenetic regulation to the frontline in the field of animal growth and development. Both genetic and environmental factors interact to affect epigenetic changes, altering progenitor commitment during early animal development, which exerts long-term effects on animal growth performance and the quality of animal products.

Literature Cited
