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Molecular regulation of skeletal muscle tissue formation and development

M. Nesvadbova[1,2], G. Borilova[1,2]*

[1]Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
[2]CEITEC – Central European Institute of Technology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

*Corresponding author: gborilova@vfu.cz

ABSTRACT: This article provides a complex overview of the different stages of myogenesis with an emphasis on the molecular, genetic and cellular bases for skeletal muscle growth. Animals with higher number of medium-sized muscle fibres produce meat of higher quality and in higher quantity. The number of muscle fibres that are created in the body is largely decided during the process of myogenesis. This review describes the main stages of embryonic skeletal myogenesis and the myogenic factors that control myogenesis in epaxial and hypaxial somites, limbs, the head and neck as well as postnatal muscle fibre growth and regeneration. An understanding of the molecular and genetic factors influencing the prenatal and postnatal growth of skeletal muscle is essential for the development of the new strategies and practical approaches to meat production.

Keywords: myogenesis; muscle development; genetics; genetic regulation

Contents

1. Introduction

Meat can be defined as all parts of the carcasses of animals in a fresh or conditioned state that are suitable for human consumption and evaluated as safe by veterinarians. The majority of meat worldwide comes from the striated skeletal muscle of carcasses of domestic livestock species. Meat comprises several types of tissue, including nervous, fat and connective tissue. Skeletal muscle tissue generally constitutes 50–70% of the carcass weight of food animals and, consequently, most of their

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value. Muscle is a unique complex tissue in the body that assumes many differentiated forms in order to perform its various functions. Because muscle is transformed into meat *postmortem*, it is important to understand the mechanisms of myogenesis that regulate this tissue *antemortem* (Weaver 2012). Meat production and quality is considered as an important issue for the meat industry in the 21st century and consumers demand high-quality, tasty, safe and healthy meat. Therefore, understanding the mechanisms of skeletal muscle development could facilitate the identification of new growth-related genes and markers and factors that affect meat production (Shahjahan 2015).

2. Skeletal muscle tissue

From the functional and morphological point of view, we distinguish three types of muscle tissue in mammals: smooth, cardiac and skeletal. Smooth muscle tissue mostly forms the walls of hollow organs or blood vessels. Cardiac muscle, as is evident from its name, is the striated muscle of the heart wall. The active component of the musculoskeletal system consists of skeletal muscle tissue (striated), which is also extremely important from the point of view of human nutrition and meat processing. The main building block of skeletal muscle tissue is the muscle fibre, which is made up of long multinucleated syncytia formed during development by fusion of myoblasts. The surface of the fibres consists of the cytoplasmatic membrane (sarcolemma); myofibrils are located in the cytoplasm (sarcoplasm) of these fibres. Myofibrils are composed of individual myofilaments whose arrangement enables the striated nature of the muscle fibres which are constructed by molecules of actin, myosin, tropomyosin and troponin (Lullmann-Rauch 2012).

Although muscle fibres have a number of common features enabling their general description, muscle is a heterogeneous complex of fibres whose microscopic, histochemical and physiological properties differ (Schiaffino and Reggiani 2011). Determination of the characteristics of a skeletal muscle fibre type is performed on the basis of the activity of muscle enzymes, i.e. myosin ATPase, whose enzymatic activity is directly proportional to the speed of muscle contraction of the specific fibres, succinyl-dehydrogenase (SDH), an enzyme that is indicative of high levels of oxidative metabolism, and α-glycerol-phosphate dehydrogenase (αGPDH), whose expression determines whether the cell engages in glycolytic or anaerobic metabolism. Muscle fibres can also be distinguished by the proportions of different isoforms of contractile proteins, specifically myosin heavy chains (MyHC) (Hossner 2005).

It is generally accepted that there are eleven major MyHC isoforms in mammalian skeletal muscles. In the myocardium there are two predominant MyHCs; atrial or alpha and ventricular or beta, which are also expressed in skeletal muscles. The beta isoform corresponds to the type 1 isoform which is typical for slow skeletal muscle fibres, while the alpha isoform is also expressed in some specialised muscles, such as jaw muscles. The type 2 or fast isoforms include three adult, fast MyHC isoforms known as 2A, 2X and 2B, which are widely present in torso and limb muscles (Mascarello et al. 2016). The main difference between the various types of muscle fibres lies in their oxidative/glycolytic metabolism: slow oxidative (type 1), fast oxidative glycolytic (type 2A and 2X) and fast glycolytic (type 2B) types, which are characterised by different mitochondrial contents (Murgia et al. 2015). The critical factor determining the functional demands on skeletal muscles is the size of the organism. In small mammals (e.g. mice), the muscle tissue consists predominantly of 2X and 2B fibres with copious oxidative enzymes, while muscles from large mammals including humans show lower levels of oxidative enzymes and consist mainly of type 1 and 2A fibres (Schiaffino and Reggiani 2011).

In animals, both the size and number of muscle fibres are important factors influencing the growth potential, meat productivity and meat quality after slaughter. Although the pig is far from being the biggest mammal its muscle fibres are the largest (Rehfeldt et al. 2004). The muscle tissue of pigs contains fibres of different types, creating a typical pattern. Islands of slow fibres are surrounded by fast fibres of 2A and 2X types with fast 2B fibres on the edge (Lefaucheur et al. 2002). A high ratio of 2B fibres in pig muscle tissue may decrease meat quality, as it correlates with the presence of the recessive allele of the halothane gene and with lower pH values of meat *postmortem* (Depreux et al. 2002). Animals with higher numbers of medium-sized muscle fibres produce meat of higher quality and quantity than those with lower numbers of

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such fibres. The number of fibres that are created is usually determined during myogenesis, leading to the conclusion that the number of muscle fibres is determined primarily by genetic and environmental factors that are able to influence prenatal myogenesis. Achieving an optimum balance in both the number and size of muscle fibres and eliminating fibres of abnormal structure are important steps in the production of large quantities of high-quality meat. Recognition of environmental and genetic influences on prenatal and postnatal skeletal muscle growth is a prerequisite for the development of strategies and practical approaches in livestock production, based on selection or environmental modulation of prenatal myogenesis and postnatal muscle growth (Rehfeldt et al. 2004).

3. Myogenesis of skeletal muscle tissue

The myogenesis of skeletal muscle tissue consists of four phases: embryonic, foetal, neonatal and adult (Murphy and Kardon 2011). Muscle tissue arises from the middle embryonic leaf, the mesoderm, which differentiates in the early embryonic period. The basis of most skeletal muscles in vertebrates is formed by somites arising from the paraxial mesoderm, which is located around the dorsal string (notochord) and the neural tube. During embryonic development, the somites differentiate into two sections, the dorsal dermomyotome, whose cells form the basis for the development of the dermis and muscle progenitor cells, and the ventral sclerotome, which is the basis of the axial skeleton and cartilage. Epaxial and hypaxial myotomes are formed from the edge of the dermomyotome. The epaxial myotome constitutes the basis for the development of deep back muscles; the hypaxial myotome is essential for the formation of limb muscles and abdominal muscles (Yokoyama and Asahara 2011; Eng et al. 2013). During the development of skeletal muscle tissue, myoblasts are first differentiated from myotomes, which arrange themselves behind one another in columns. The cell membranes of the myoblasts disappear at the point of contact and multinucleated myotubes are formed. During the development and differentiation of the muscle, they extend so that they fuse with other myoblasts and with what are known as satellite cells. Myofibrils are gradually formed in the sarcoplasm of such cells. Cell nuclei migrate to the surface of the myotube, myofibrils migrate to its centre, and the myotubes thus complete their development into muscle fibres (Wallace et al. 2016). Only muscle growth, fibre maturation and fibre regeneration take place in the postnatal phase of myogenesis (Hutcheson et al. 2009).

Two types of muscle fibres are formed during the course of myogenesis: primary and secondary. Cells of the myotome begin to fuse and create multinucleated muscle fibres, and this stage of embryonic myogenesis is necessary to create the basic muscle structure – primary muscle fibres (Messina and Cossu 2009). The number of muscle fibres formed is, however, very small and the influence of this stage on the future size and number of muscle fibres is therefore relatively negligible (Du et al. 2010). The critical phase for the development of the skeletal muscle tissue is the foetal stage, as most of the muscle fibres develop during this period. This second wave of myogenesis, which is also called secondary myogenesis, includes mutual fusion of foetal myoblasts or their fusion with primary muscle fibres and the creation of secondary fibres (Messina and Cossu 2009). The number of muscle fibres formed is conditional on the number of available myogenic precursor cells whose proliferation in turn depends on the availability of nutrients. This phase of myogenesis is therefore strongly influenced by the nutrition of the mother, as is evidenced by numerous studies (Yan et al. 2012). In the foetus of the pig, the formation of primary muscle fibres is completed between day 35 and day 65, and the formation of secondary fibres between day 54 and day 90 of pregnancy (Stickland et al. 2004). However, some additional studies have shown that porcine myogenesis is almost complete before 77 day of pregnancy and that the period between days 50 and 70 is a critical phase in foetal skeletal muscle development and in the formation of various muscle phenotypes (Zhao et al. 2015). In cattle, primary fibres are formed approximately in the first 125 days and secondary fibres between day 100 and the ninth month of pregnancy. In sheep foetuses, primary muscle fibres are formed between day 32 and day 38 and secondary muscle fibres between day 30 and day 62 of pregnancy. Finally, in the chicken, primary muscle fibres are formed around day 6, and secondary muscle fibres between day 12 and day 16 of incubation (Stickland et al. 2004).

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4. Molecular factors influencing myogenesis

4.1 Signalling pathways influencing myogenesis in epaxial and hypaxial somites

Sequence-specific transcription factors, which represent the main nodes of gene regulation networks, are involved in molecular specification, in the movement of cells and throughout the process of somitogenesis and myogenesis during the development of the muscle tissue of vertebrates. Signalling pathways defined by the expression of specific transcription factors are characterised by several peculiar features in epaxial and hypaxial somites, in the limbs, head, and neck and in the adult individual.

A key role in the induction of myogenesis is played by the proteins of the WNT signalling pathway from the dorsal part of the neural tube and Sonic hedgehog proteins (SHH) produced by the notochord and by the plate of the neural tube (Braun and Gautel 2011). The members of the WNT protein family play a vital role in the formation of the dermomyotome and myotome (Geetha-Loganathan et al. 2008) and, together with SHH proteins, are involved in the specification of muscle progenitor cells in somites. The products of the *SHH* gene also play a key role in the maturation of cells of the dermomyotome and in further specification of the myotome cells (Bentzinger et al. 2012). An important role in the activation of the myogenesis process is also played by signalling molecules such as Noggin and bone morphogenetic proteins (BMP) through the activation and inactivation of the receptors of members of the transforming growth factor beta family (TGF-β) (Braun and Gautel 2011). Ultimately, the signalling pathways that induce the differentiation of the cells, the formation of the muscle tissue and the regulation of the process of myogenesis activate what are known as myogenic regulatory factors (MRF). These MRFs include *MYF5* (myogenic factor 5), myoblastic differentiation factor (*MYOD*, *MYODI*), herculin (*MYF6*, *MRF4*) and myogenin (*MYOG*) (Brameld et al. 2010).

The *PAX3* gene plays the main role in the initiation of MRF expression during the myogenesis of muscle tissue. This gene is first expressed in presomitic mesoderm immediately anterior to the first somite and then throughout the early epithelial somite. *PAX3* is expressed by muscle progenitor cells throughout the epithelium of the dermomyotome and its function lies in the segmentation and formation of the edge of the dermomyotome and it is induced by the products of the *SIX*, *EYA* and *DACH* gene families. Upon formation of the epaxial muscle tissue, *PAX3* initiates myogenic induction independently of other genes by activating the *MYOD* gene. On the other hand, in the hypaxial dermomyotome, *PAX3* induces the expression of the *MYF5* gene, which in turn activates the expression of the *MYOD* gene (Eng et al. 2013; Buckingham 2017). In addition to *PAX3*, progenitor muscle cells also express the *PAX7* gene in the central domain of the dermomyotome, from which progenitor cells of the myotome arise (Buckingham and Relaix 2007). *PAX* genes then together control expression of MRFs such as *MYF5* and *MYOD*, contribute to the proliferation and survival of myoblasts before their differentiation and control the survival and fate of myogenic progenitor cells during the myogenesis of vertebrates. Spontaneous mutation of the *PAX3* gene in mice (such mutants are known as *Splotch* mice) is the cause of the non-formation of the hypaxial somite domain and consequently of the muscles of the limbs and some muscles of the body, while the muscles derived from the epaxial part are less affected. *PAX7* is particularly important for muscle development in the postnatal period; further, the inactivation of the *PAX3* gene together with *PAX7* results in the inability of muscle progenitor cells to enter the process of myogenesis. These two factors are therefore critical for the formation of muscle tissue (Buckingham and Relaix 2015). The *SIX1* and *SIX4* genes are expressed in overlapping domains of the dermomyotome and myotome and in the limb buds. In epaxial somites, *SIX* genes control and regulate the expression of the *MRF4* gene, and their inactivation causes a delayed and lower level of expression of *MYOD* and *MYOG* genes in embryos, while the expression of the *MYF5* gene remains unchanged (Bismuth and Relaix 2010). In hypaxial somites, *SIX* control the expression of *PAX3*. The knockout of the *SIX1* gene in mice results in a variety of organ defects and in the death of individuals immediately after birth. Moreover, the knockout of both genes in mice leads to a complete absence of abdominal and limb muscles with simultaneous defects in craniofacial and back muscles (Grifone et al. 2005). In the same way, mutation of the *EYA1* and *EYA2* genes, which are expressed in the epaxial as

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well as the hypaxial portion of the dermomyotome, causes the non-formation of the muscle tissue of the limbs. This phenotype is the result of loss of the interaction between *SIX* and *EYA* proteins which is essential for the expression of *PAX3* in the dermomyotome (Grifone et al. 2007).

Myogenesis is induced in epaxial somites by products of the *WNT1* and *WNT3A* genes through activation of the myogenic factor *MYF5*. Products of the *MYF5* gene in epaxial somites subsequently activate the expression of the *MYOD* and *MYOG* genes. The *SHH* gene also influences myogenesis in the same way, i.e. by activating *MYF5* (Bentzinger et al. 2012). On the other hand, the expression of the *WNT7A* gene in the dorsal part of the neural tube directly enables the activation of the *MYOD* gene. Negative regulation of the expression of *MYOD* is maintained through the *BMP4* gene, which inhibits *Noggin*. In hypaxial somites, the *MYF5* gene, which triggers the expression of *MYOD* and *MYOG*, is activated by *PAX3*. The expression of *MYF5* is also positively regulated by *WNT7A* and *BMP* (Mok and Sweetman 2011). Numerous research studies show that epaxial progenitor cells are primarily dependent on the signals from the notochord and the plate of the neural tube, which express *SHH* and *WNT1* gene products, activating myogenesis by means of the induction of *MYF5*. On the other hand, hypaxial progenitor cells require signals from the dorsal ectoderm which express *WNT7A* gene products, activating myogenesis directly through *MYOD* (Rossi and Messina 2014).

4.2 Myogenic regulatory factors

Myogenic regulatory factors (MRFs) are important markers that specify the developmental lineage of the muscle fibre and ensure the activation of the differentiation programme. In the mouse embryo, the first MRF expressed is *MYF5* which is, at first, expressed on the dorsal-medial edge of the dermomyotome (E8.0) transitioning into the epaxial myotome. This gene is also expressed in the hypaxial myotome. The expression of *MYF5* is followed by that of *MYOG* (E8.5), *MRF4* (E9.0) and, finally, *MYOD* (E10.5). Surprisingly, knockout of the *MYOD, MYF5* or *MRF4* genes does not cause any serious developmental defects in mice. Seemingly normal development of the muscle tissue takes place in such animals, although delayed myogenesis of the limb muscles is observed in *MYOD* mutants and the expression of *MYF5* is enhanced and prolonged (*MYF5* and *MYOD* genes are able to substitute for each other to some extent during myoblast differentiation). Actual knockout of the *MYF5* gene causes delayed development of myotome cells, but skeletal muscle tissue does eventually develop properly. In these animals, however, there is no development of the distal portion of the ribs, and they are therefore unable to breathe (Francetic and Li 2011). The function of *MYOD* and *MYF5* can also be compensated for by *MRF4*, and in this way myogenesis can be independently initiated. However, complete failure of the formation of the skeletal muscle tissue occurs in mice with knockouts of *MYOD*, *MYF5* and *MRF4*, since there is no development of myoblasts. Similarly, mice lacking functional *MYOD* and *MRF4* gene experience severe muscle defects (a similar phenotype as in animals with mutation of *MYOG*) (Sweetman 2012). In mice lacking a functional *MYOG* gene, individuals do form myoblasts, but such myoblasts are unable to fuse into myotubes and the mutation leads to their death; after birth, only a small number of muscle fibres are observed to have formed (Bentzinger et al. 2012).

4.3 Signalling pathways influencing the formation and development of the muscle tissue of the limbs

During the formation of the muscle tissue of the limbs, thousands of progenitor cells from the hypaxial dermomyotome delaminate and migrate to the limb buds, where they multiply and express specific myogenic determination factors. A number of regulatory factors are involved in the process of the formation and development of the muscle tissue of the limbs. *PAX3* expression accompanies the migration of progenitor cells, which ensures the proliferating condition of migrating cells, maintains the active expression of the tyrosine kinase receptor *c-Met* and prevents the apoptosis of the cells of the hypaxial somite and MRF expression. As has already been mentioned, the presence of the products of the *PAX3* gene is indispensable for the development of limbs (*Splotch* mice). *HGF* (hepatocyte growth factor), which is produced by the surrounding cells of the mesenchyme, is the ligand for *c-Met*, expressed in progenitor muscle

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cells. *HGF* and *c-Met* together are crucial for the process of delamination and migration of progenitor cells. Their absence causes a similar phenotype as in *Splotch* mice – the muscle tissue of the limb is not created, as myogenic precursor cells do not migrate from the dermomyotome to their target sites (Bismuth and Relaix 2010). Precursor cells also express – together with *PAX3* – the transcription factor *LBX1*, whose inactivation causes the absence of the dorsal muscle of the forelimbs and all the muscles of the hind limbs, since this factor controls the finding of the correct path and the ingress of migrating cells into the limb bud (Dietrich et al. 1999).

In the presence of the transcription factors *MEOX2* and *PITX2,* migrating cells form two sections of the tissue, dorsal and ventral, which – after reaching their target site – subsequently differentiate into the muscle tissue of the forelimb and hind limb and express muscle-specific genes. *MYF5* is expressed first, followed several hours later by *MYOD* and *MYOG*, which together ensure generation of the ventral and dorsal muscles of the forelimbs and hind limbs (Bismuth and Relaix 2010). The expression of *MYF5* and *MYOD* can be induced by *DACH2*, *SIX1*, *SIX4*, *EYA2*, *PAX3*, *MOEX2* and *PITX2*. *MYOD* subsequently induces expression of *MYOG*, which triggers the process of myogenesis (Mok and Sweetman 2011).

4.4 Signalling pathways influencing the formation and development of the muscle tissue of the head and neck

Most of the muscle tissue of the head, known as the craniofacial muscle tissue, and which includes the muscles of the jaw, face and extraocular muscles, arises from the cranial paraxial mesoderm which does not, however, create segments, unlike the paraxial mesoderm of the trunk. The muscles of the tongue and larynx are derived from the cells of the occipital somites which migrate to their future targets. Not only are the genetic regulatory networks of the development of the muscles of the head and neck different from the networks that control the formation of the muscles of the body, but there are also differences between various groups of these muscles and even between various vertebrates (Sambasivan et al. 2011). The absence of the products of the *PAX3* and *PAX7* genes is typical of the muscles of the jaw and the eye. Instead, transcription factors such as *PITX2*, *TBX1*, *MYOR* (musculin) and *TCF21* (capsulin) are expressed (Mok and Sweetman 2011). Transcription factor *PITX2* plays a key role in the specification of the extraocular muscles by binding to the promoter of the *MYF5* and *MYOD* genes, and mutant mice without the functional gene do not form this group of muscles (Zacharias et al. 2011). This gene is, however, also an important regulator for the development of muscles that are derived from the branchial arches, i.e. the chewing and mimic muscles, and the muscles of the pharynx and larynx (Shih et al. 2007). The *TBX1* gene is a key factor for these muscles, performing a role which is equivalent to that *PAX3* in muscle progenitor cells and is responsible for initiating myogenesis of the muscles of the head and neck. Dysfunction of this gene causes defects in the craniofacial and cardiovascular structures, damage to the muscles of the head and neck and, ultimately, death of the individual at birth. Important roles in the specification of future masticatory muscles is also played by the *MYOR* and *TCF21* genes. Upon inactivation of these genes, neither the expression of MRF nor the development of muscles occurs in the first branchial arch, as myogenic cells undergo apoptosis (Bismuth and Relaix 2010).

The regulatory factor *MYF5* is crucial for the cranial myogenesis of mice (Sambasivan et al. 2009), while *MYOD* is critical in zebrafish (Hinits et al. 2009). *MYF5*, *MRF4* and *MYOD* act as determination factors of cell fate. In addition, *MRF4* and *MYOD* also regulate the differentiation of cells; *MYOG* serves as a differentiation factor of muscle tissue. Knockout of the *MYF5* and *MYOD* genes causes defects in the development of the muscle tissue of the head; development of the extraocular muscles does not take place upon knockout of the *MYF5* and *MRF4* genes (Sambasivan et al. 2011).

4.5 The postnatal period of development and regeneration of muscle tissue

The postnatal period is associated with an increase in the length and volume of muscle fibres. The capacity for postnatal muscle growth is determined by the number of muscle fibres that are created during prenatal myogenesis. The growth rate is limited by physiological and genetic factors. As a rule, the greater the number of muscle fibres, the

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slower the postnatal speed of growth of the muscle fibres and the smaller the volume of fibres at the end of the intense period of growth and *vice versa*. This is due to the even distribution of energy from food which is shared among all fibres. However, this rule does not always apply in general, because some animals grow quickly even though they have a large number of muscle fibres (Rehfeldt et al. 2004). Satellite cells participate in the postnatal growth of the muscle fibres; they are the source for the new nuclei that are incorporated into already formed muscle fibre. The connection of the cells occurs at the end of the muscle fibre and this leads to its elongation. This process is mainly dependent on factors such as innervation, contractile activity, growth factors, hormones and nutrition. The number of muscle fibres in mammals and birds remains mostly unchanged after birth (Rehfeldt et al. 2000). During the early postnatal growth phase of the muscle tissue, its amount can double within a few days. In the mouse, pig or carp, the amount of muscle tissue doubles every 4–5 days in the first month after birth (Goldspink 2004). Once the mature muscle reaches a certain size, its satellite cells become mitotically quiet (they remain in the G0 phase of the cell cycle).

The size of the muscle tissue and the body can, however, also be affected by other signalling pathways, such as the overexpression of insulin-like growth factors (IGF) or repression of myostatin (MSTN), a member of the TGF-β family known for the role it plays in the inhibition of myogenesis (Rossi and Messina 2014). IGF factors play an important role in the development, differentiation and survival of the cells of the skeletal muscle tissue. They influence muscle precursor cells and are the only growth factors that are able to induce final differentiation of myoblasts and satellite cells. In the early stage of development, these growth factors stimulate proliferation. Continuous stimulation of cells leads to cell differentiation which is characterised by activation of the expression of *MYOG*, *MYF5* and *MYOD*. Moreover, the effect of the *IGF* gene is dependent on the concentration of these genes. Low concentrations stimulate differentiation, while higher concentrations inhibit the differentiation of myoblasts. During the growth of a mature skeletal muscle, these factors enable the proliferation of satellite cells and the differentiation and fusion of myotubes. Application of *IGF* to experimental animals induces the growth of muscle mass. On the other hand, knockout of the *IGF1* gene in mice causes a similar phenotype as the knockout of myogenin, which leads to the total failure of the development of skeletal muscle (Hossner 2005). Myostatin is a known negative regulator of muscle growth (Rodriguez et al. 2014) and functions as a suppressor of the precursors of proliferation in muscle cells (*MYOD* and *MYOG*) during embryonic development, consequently leading to the formation of muscle fibres with decreased numbers of myoblasts (Lee 2004). Knockout of the gene stimulates cell division in satellite cells, hypertrophy of skeletal muscle and up to a three-fold increase in muscle mass (McCroskery et al. 2003). Mutations of the *MSTN* gene that result in muscle mass growth occur in many animals, including humans. TGF-β functions, similarly to FGF, as a significant inhibitor of muscle differentiation. It is produced by a number of cells including myoblasts and regenerating muscle tissue. This gene inhibits differentiation of foetal, but not embryonic myoblasts (Yanagisawa et al. 2001) and the expression of *MYOD* and *MYOG* in myogenic cells (Brennan et al. 1991) and prevents premature differentiation of migrating myoblasts, thus enabling proper development of the future muscle tissue in the developing limb of the embryo (Olson et al. 1986).

During the regeneration of the muscle fibre, mitotically quiet satellite cells express the products of the *PAX7* and *c-Met* genes, whose HGF ligand stimulates the activity of satellite cells. The satellite cells undergo subsequent proliferation, differentiation and fusion with existing muscle fibres or myoblasts. In the phase of proliferation, satellite cells re-enter the cell cycle and begin to multiply and express the *PAX7* and *MYOD* genes. The expression of *PAX7* is reduced before the start of differentiation, but this rule does not apply to all satellite cells as some cells still maintain a high level of expression of *PAX7* while regulating the expression of gene *MYOD*, and return to their original quiescent state (Rossi and Messina 2014). Mutation of the *PAX7* gene in adult individuals is responsible for a significant reduction in the number of satellite cells or their apoptosis shortly after the birth of the individual (Bryson-Richardson and Currie 2008). The *MYF5* gene also plays an important role in the regeneration of the muscle tissue; the absence of its products causes an increased amount of hypertrophic fibres, a delay in differentiation and impaired muscle regeneration. *MYOD* is also equally

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important for the regeneration and differentiation of satellite cells, the growth of myoblasts and the growth of the individual (Yokoyama and Asahara 2011). When regenerating muscle tissue, satellite cells migrate to the site of damage and initiate their differentiation. At this stage, *MRF4* and *MYOG* are expressed together with transcription factors of the *MEF2* family. The differentiation phase is followed by fusion into multinucleated myotubes and the completion of muscle fibre regeneration. In mice, the whole process of muscle regeneration after an acute injury takes approximately three to four weeks (Rossi and Messina 2014).

4.6 A novel candidate gene for meat production traits in pig

Muscle fibres are composed of myocytes and adipocytes and their creation, development and differentiation are regarded as critical factors affecting meat quality (Mu 2012). The process of myogenesis influences the number, size and structure of muscle fibres and, consequently, the postnatal growth of muscle mass. The genes involved in this process, the expression of which is different among various breeds of pig, are candidate genes for meat productivity. Pigs with excellent growth potential and very good meat proportion have been shown to possess a higher number of myofibres and an increased share of fast twitch glycolytic fibres in comparison to their wild ancestors or primitive breeds of pigs (Murani et al. 2007).

Myogenesis in pigs is characterised by an increased formation of somites approximately between the 14th and 22nd days of gestation, followed by creation of primary myotubes between the 35th and 60th days. Secondary myotubes appears in the pig foetus around day 50 (45–54), and their number increases several times up to day 75. Subsequently, the number of muscle fibres increases only slightly and their production stops completely approximately between days 85 and 90. The whole process of maturation of the myotubes into myofibres is completed in the early postnatal period. In adult muscles, the ratio of primary to secondary myotubes is approx. 1 : 20 and both these populations influence considerably fibre number and muscle size in various breeds of pigs (Murani et al. 2007). Production of pigs with muscles of high quality is very important for the satisfaction of consumers and the competitiveness of the pork industry. The results of scientific studies show that different types of muscle fibres in different pig breeds affect the quality of pork. Therefore, it is very important to clarify the basic molecular mechanisms of muscle fibre formation (Sun et al. 2017).

Research into genes expressed in the *longissimus dorsi* in the prenatal and postnatal period in different breeds of Lantang and Landrace pigs explains the mechanism of muscle development and provides valuable information for improving the quality of pig meat. The study of Zhao et al. (2011) showed that myogenesis in pigs is almost finished in foetuses of 77 days of age. In the Lantang breed, the process of myogenesis starts earlier but progresses more slowly than in the Landrace breed. The period between the 49th and 77th days post fertilisation is critical for formation of different muscle phenotypes. Genes belonging to the MRF and MEF2 families play an important role in the formation of different phenotypes among these breeds. The *GSK3B*, *IKBKB*, *ACVR1*, *ITGA* and *STMN1* genes promote a later onset of myogenesis and a greater number of muscle fibres in the Landrace breed. The balance between intramuscular adipogenesis and myogenesis is controlled by the *MYOD* and *MEF2A* genes. The *MYF5* and *MEF2C* genes play an indispensable role throughout the whole process of myogenensis, whereas the *MEF2D* gene is specifically involved in muscle growth and maturation.

Liu et al. (2018) investigated the key phases of myogenesis during the five developmental stages (pig foetuses aged 40, 55, 63, 70 and 90 days) in Tongcheng and Yorkshire pigs using RNA-seq. Although the process of myogenesis has very similar features in all breeds of pigs, a total of 1677 genes showed significantly different expression in *longissimus dorsi* muscle among these breeds of pigs, especially in 55-day-old foetuses. On the other hand, at the foetal age of 70 days, the lowest number of differentially expressed genes was detected. These results suggest that the development of secondary muscle fibres causes dynamic changes in gene expression and is the cause of developmental differences between breeds of pigs. The *PTEN*, *EP300*, *MYO9A*, *CDK14*, *IRS1* and *PPP1CC* genes together with some ribosomal proteins have been proposed as key candidate genes to elucidate the developmental differences between the analysed breeds.

The *Akirin2* gene is considered as a potential functional candidate gene for meat quality. The results of

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Chen et al. (2017) show that the *Akirin2* gene stimulates proliferation and differentiation of porcine skeletal muscle satellite cells and further plays a key role in proliferation and differentiation of porcine skeletal muscle satellite cells through extracellular-signal regulated kinase-1/2 (ERK1/2) and NFATc1 signalling pathways. Reis et al. (2016) identified the *LEF1* gene as a candidate gene whose differential expression determines differences in muscle mass between pig breeds. Higher gene expression in 21- and 40-day-old foetuses of the three-way Duroc, Landrace and Large-White cross in comparison with Piau pigs suggests that this gene influences the formation and proliferation of somites and myoblast fusion. The *LEF1* gene is involved in the WNT signalling pathway, which plays a key role in the development of muscle fibres by controlling the expression of MRFs such as *MYF5* and *MYOD*.

The Notch signalling pathway plays a key role in regulating the activation, proliferation and differentiation of porcine satellite cells. The mammalian genome harbours four Notch genes (*Notch1–4*) whose expression differs during prenatal and postnatal development of tissues. Unlike the *Notch2* and *Notch3* genes, the *Notch1* gene has an indispensable function in regulating porcine satellite cells. Notch1 signalling controls the expression of the *HES5* gene, which regulates the expression of the myogenic regulatory factors *MYOD* and *MYOG*. In addition, Notch1 also regulates the expression of the *GSK3β-3* gene, which plays a key role in the Wnt signalling pathway (Qin et al. 2013).

5. Conclusions

The size and number of muscle fibres is an important factor that affects growth potential, animal meat performance and meat quality after slaughter. Achieving the optimum balance in the size and number of muscle fibres, including the elimination of fibres of abnormal structure, is a key step in the production of meat of high quality and quantity. This requires knowledge of factors affecting prenatal and postnatal growth of the skeletal muscle tissue. This knowledge serves as a basis for the development of strategies and practices in animal production using the principle of selecting or environmentally modulating prenatal myogenesis and postnatal muscle growth (Rehfeldt et al. 2004). The number of muscle fibres created in the body is decided largely during the process of myogenesis. Extensive research in recent decades using model organisms of molecular biology, such as the mouse (*Mus musculus*), chicken (*Gallus gallus*) and zebrafish (*Danio rerio*), has led to the identification of a number of genes and regulatory pathways that are involved in the process of creation and development of the muscle tissue of vertebrates (Brameld et al. 2010).

6. References

Bentzinger CF, Wang YX, Rudnicki MA (2012): Building muscle: molecular regulation of myogenesis. Cold Spring Harbor Perspectives in Biology 4, doi: 10.1101/cshperspect.a008342.

Bismuth K, Relaix F (2010): Genetic regulation of skeletal muscle development. Experimental Cell Research 316, 3081–3086.

Brameld JM, Greenwood PL, Bell AW (2010): Biological mechanisms of fetal development relating to postnatal growth, efficiency and carcass characteristics in ruminants. In: Greenwood PL, Bell AW, Vercoe PE, Viljoen GJ (eds): Managing the Prenatal Environment to Enhance Livestock Productivity. Springer. 93–120.

Braun T, Gautel M (2011): Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. Nature Review Molecular Cell Biology 12, 349–361.

Brennan TJ, Edmondson DG, Li L, Olson EN (1991): Transforming growth factor beta represses the actions of myogenin through a mechanism independent of DNA binding. Proceedings of the National Academy of Sciences of the United States of America 88, 3822–3826.

Bryson-Richardson RJ, Currie PD (2008): The genetics of vertebrate myogenesis. Nature Reviews Genetics 9, 632–646.

Buckingham M (2017): Gene regulatory networks and cell lineages that underlie the formation of skeletal muscle. Proceedings of the National Academy of Sciences 114, 5830–5837.

Buckingham M, Relaix F (2007): The role of Pax genes in the development of tissues and organs: Pax3 and Pax7 regulate muscle progenitor cell functions. Annual Reviews of Cell and Developmental Biology 23, 645–673.

Buckingham M, Relaix F (2015): PAX3 and PAX7 as upstream regulators of myogenesis. Seminars in Cell & Developmental Biology 44, 115–125.

Chen X, Luo Y, Huang Z, Jia G, Liu G, Zhao H (2017): Akirin2 regulates proliferation and differentiation of porcine skeletal muscle satellite cells via ERK1/2 and NFATc1 signaling pathways. Scientific Reports 7, doi: 10.1038/srep45156.

https://doi.org/10.17221/7/2018-VETMED

Depreux FFS, Grant AL, Gerrard DE (2002): Influence of halothane genotype and body-weight on myosin heavy chain composition in pig muscle as related to meat quality. Livestock Production Science 73, 265–273.

Dietrich S, Abou-Rebyeh F, Brohmann H, Bladt F, Sonnenberg-Riethmacher E, Yamaai T, Birchmeier C (1999): The role of SF/HGF and c-Met in the development of skeletal muscle. Development 126, 1621–1629.

Du M, Yan X, Tong JF, Zhao J, Zhu MJ (2010): Maternal obesity, inflammation, and fetal skeletal muscle development. Biology of Reproduction 82, 4–12.

Eng D, Ma HY, Gross MK, Kioussi C (2013): Gene networks during skeletal myogenesis. ISRN Developmental Biology, doi: 10.1155/2013/348704.

Francetic T, Li Q (2011): Skeletal myogenesis and Myf5 activation. Transcription 2, 109–114.

Geetha-Loganathan P, Nimmagadda S, Scaal M, Huang R, Christ B (2008): Wnt signaling in somite development. Annals of Anatomy 190, 208–222.

Goldspink G (2004): Local and systemic regulation of muscle growth. In: Pas MFW, Everts ME, Haagsman HP (eds): Muscle Development of Livestock Animals: Physiology, Genetics, and Meat Quality. CABI Publishing, Wallingford. 157–172.

Grifone R, Demignon J, Houbron C, Souil E, Niro C, Seller MJ, Maire P (2005): Six1 and Six4 homeoproteins are required for Pax3 and Mrf expression during myogenesis in the mouse embryo. Development 132, 2235–2249.

Grifone R, Demignon J, Giordani J, Niro C, Souil E, Bertin F, Maire P (2007): Eya1 and Eya2 proteins are required for hypaxial somitic myogenesis in the mouse embryo. Developmental Biology 302, 602–616.

Hinits Y, Osborn DP, Hughes SM (2009): Differential requirements for myogenic regulatory factors distinguish medial and lateral somitic, cranial and fin muscle fibre populations. Development 136, 403–414.

Hossner KL (2005): 4. Developmnet of muscle, skeletal system and adipose tissue. In: Hossner KL (ed.): Hormonal Regulation of Farm Animal Growth. 1st edn. CABI Publishing, Wallingford. 55–93.

Hutcheson DA, Zhao J, Merrell A, Haldar M, Kardon G (2009): Embryonic and fetal limb myogenic cells are derived from developmentally distinct progenitors and have different requirements for β-catenin. Genes & Development 23, 997–1013.

Lee SJ (2004): Regulation of muscle mass by myostatin. Annual Reviews of Cell and Developmental Biology 20, 61–86.

Lefaucheur L, Ecolan P, Plantard L, Gueguen N (2002): New insights into muscle fiber types in the pig. Journal of Histochemistry & Cytochemistry 50, 719–730.

Liu H, Xi Y, Liu G, Zhao Y, Li J, Lei M (2018): Comparative transcriptomic analysis of skeletal muscle tissue during prenatal stages in Tongcheng and Yorkshire pig using RNA-seq. Functional & Integrative Genomics 18, 195–209.

Lullmann-Rauch R (2012): 10. Muscle tissue. In: Lullmann-Rauch R (ed.): Histology (in Czech). 1st edn. Grada Publishing, Prague. 186–210.

Mascarello F, Toniolo L, Cancellara P, Reggiani C, Maccatrozzo L (2016): Expression and identification of 10 sarcomeric MyHC isoforms in human skeletal muscles of different embryological origin. Diversity and similarity in mammalian species. Annals of Anatomy – Anatomischer Anzeiger 207, 9–20.

McCroskery S, Thomas M, Maxwell L, Sharma M, Kambadur R (2003): Myostatin negatively regulates satellite cell activation and self-renewal. Journal of Cell Biology 162, 1135–1147.

Messina G, Cossu G (2009): The origin of embryonic and fetal myoblasts: a role of Pax3 and Pax7. Genes & Development 29, 902–905.

Mok GF, Sweetman D (2011): Many routes to the same destination: lessons from skeletal muscle development. Reproduction 141, 301–312.

Mu C (2012): The mRNA expression pattern of skeletal muscle regulatory factors in divergent phenotype swine breeds. Kafkas Universitesi Veteriner Fakultesi Dergisi 18, 685–690.

Murani E, Muraniova M, Ponsuksili S, Schellander K, Wimmers K (2007): Identification of genes differentially expressed during prenatal development of skeletal muscle in two pig breeds differing in muscularity. BMC Developmental Biology 7, 109.

Murgia M, Nagaraj N, Deshmukh AS, Zeiler M, Cancellara P, Moretti I, Mann M (2015): Single muscle fiber proteomics reveals unexpected mitochondrial specialization. EMBO Reports 16, 387–395.

Murphy M, Kardon G (2011): Origin of vertebrate limb muscle: the role of progenitor and myoblast populations. Current Topics in Developmental Biology 96, 1–32.

Olson EN, Sternberg E, Hu JS, Spizz G, Wilcox C (1986): Regulation of myogenic differentiation by type beta transforming growth factor. The Journal of Cell Biology 103, 1799–1805.

Qin L, Xu J, Wu Z, Zhang Z, Li J, Wang C, Long Q (2013): Notch1-mediated signaling regulates proliferation of porcine satellite cells (PSCs). Cellular Signalling 25, 561–569.

Rehfeldt C, Fiedler I, Dietl G, Ender K (2000): Myogenesis and postnatal skeletal muscle cell growth as influenced by selection. Livestock Production Science 66, 177–188.

Rehfeldt C, Fiedler I, Stickland NC (2004): Number and size of muscle fibres in relation to meat. In: te Pas MFW,

https://doi.org/10.17221/7/2018-VETMED

Everts ME, Haagsman HP (eds): Muscle Development of Livestock Animals: Physiology, Genetics, and Meat Quality. CABI Publishing, Wallingford. 1–38.

Reis EP, Paixao DM, Brustolini OJ, Silva FF, Silva W, Araujo FM, Salim AC, Oliveira G, Guimaraes SE (2016): Expression of myogenes in longissimus dorsi muscle during prenatal development in commercial and local Piau pigs. Genetics and Molecular Biology 39, 589–599.

Rodriguez J, Vernus B, Chelh I, Cassar-Malek I, Gabillard JC, Sassi AH, Seiliez I, Picard B, Bonnieu A (2014): Myostatin and the skeletal muscle atrophy and hypertrophy signaling pathways. Cellular and Molecular Life Sciences 71, 4361–4371.

Rossi G, Messina G (2014): Comparative myogenesis in teleosts and mammals. Cellular and Molecular Life Sciences 71, 1–19.

Sambasivan R, Gayraud-Morel B, Dumas G, Cimper C, Paisant S, Kelly RG, Tajbakhsh S (2009): Distinct regulatory cascades govern extraocular and pharyngeal arch muscle progenitor cell fates. Developmental Cell 16, 810–821.

Sambasivan R, Kuratani S, Tajbakhsh S (2011): An eye on the head: the development and evolution of craniofacial muscles. Development 138, 2401–2415.

Schiaffino S, Reggiani C (2011): Fiber types in mammalian skeletal muscles. Physiological Reviews 91, 1447–1531.

Shahjahan M (2015): Skeletal muscle development in vertebrate animals. Asian Journal of Medical and Biological Research 1, 139–148.

Shih HP, Gross MK, Kioussi C (2007): Cranial muscle defects of Pitx2 mutants result from specification defects in the first branchial arch. Proceedings of the National Academy of Sciences of the United States of America 104, 5907–5912.

Stickland NC, Bayol S, Ashton C, Rehfeldt C (2004): Manipulation of muscle fibre number during prenatal development. In: Pas MFW, Everts ME, Haagsman HP (eds): Muscle Development of Livestock Animals: Physiology, Genetics, and Meat Quality. CABI Publishing, Wallingford. 69–82.

Sun J, Xie M, Huang Z, Li H, Chen T, Sun R, Wang J, Xi Q, Wu T, Zhang Y (2017): Integrated analysis of non-coding RNA and mRNA expression profiles of 2 pig breeds differing in muscle traits. Journal of Animal Science 95, 1092–1103.

Sweetman D (2012): The myogenic regulatory factors: critical determinants of muscle identity in development, growth and regeneration. In: Cseri J (ed.): Skeletal Muscle – From Myogenesis to Clinical Relations. InTech, Rijeka. 31–48.

Wallace MA, Hughes DC, Baar K (2016): 3. mTORC1 in the control of myogenesis and adult skeletal muscle mass. In: Maiese K (ed.): Molecules to Medicine with mTOR: Translating Critical Pathways into Novel Therapeutic Strategies. Academic Press. 37–56.

Weaver AD (2012): Muscle biology. In: Hui YH (ed.): Handbook of Meat and Meat Processing. CRC Press, New York. 35–45.

Yan X, Zhu MJ, Dodson MV, Du M (2012): Developmental programming of fetal skeletal muscle and adipose tissue development. Journal of Genomics 1, 29–38.

Yanagisawa M, Nakashima K, Takeda K, Ochiai W, Takizawa T, Ueno M, Takizawa M, Shibuya H, Taga T (2001): Inhibition of BMP2-induced, TAK1 kinase-mediated neurite outgrowth by Smad6 and Smad7. Genes to Cells 6, 1091–1099.

Yokoyama S, Asahara H (2011): The myogenic transcriptional network. Cellular and Molecular Life Sciences 68, 1843–1849.

Zacharias AL, Lewandoski M, Rudnicki MA, Gage PJ (2011): Pitx2 is an upstream activator of extraocular myogenesis and survival. Developmental Biology 349, 395–405.

Zhao W, Mu Y, Ma L, Wang C, Tang Z, Yang S, Zhou R, Hu X, Li K (2015): Systematic identification and characterization of long intergenic non-coding RNAs in fetal porcine skeletal muscle development. Scientific Reports 5, 8957.

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