Key signalling factors and pathways in the molecular determination of skeletal muscle phenotype

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The molecular basis and control of the biochemical and biophysical properties of skeletal muscle, regarded as muscle phenotype, are examined in terms of fibre number, fibre size and fibre types. A host of external factors or stimuli, such as ligand binding and contractile activity, are transduced in muscle into signalling pathways that lead to protein modifications and changes in gene expression which ultimately result in the establishment of the specified phenotype. In skeletal muscle, the key signalling cascades include the Ras-extracellular signal regulated kinase-mitogen activated protein kinase (Erk-MAPK), the phosphatidylinositol 3'-kinase (PI3K)-Akt1, p38 MAPK, and calcineurin pathways. The molecular effects of external factors on these pathways revealed complex interactions and functional overlap. A major challenge in the manipulation of muscle of farm animals lies in the identification of regulatory and target genes that could effect defined and desirable changes in muscle quality and quantity. To this end, recent advances in functional genomics that involve the use of micro-array technology and proteomics are increasingly breaking new ground in furthering our understanding of the molecular determinants of muscle phenotype.

Keywords: hyperplasia, hypertrophy, meat quality, muscle fibres, skeletal muscle

Introduction

Importance of muscle fibre phenotype

The key constituents of muscle are muscle fibres, and their associated extracellular matrix in which are located the vascular supply, collagenous component and adipose tissue (intramuscular fat) of muscle. Muscle phenotype conferred by fibres is central to the quantity and quality of meat production. Quantity is the outcome of muscle fibre hyperplasia and hypertrophy. Hyperplastic growth in utero is primarily dependent on myocyte proliferation and differentiation (Oka et al., 2002). Post-natal growth is mainly the result of hypertrophy or enlargement of existing and replaced muscle fibres. Quality is a more complex trait and is assessed by a variety of objective and subjective measurements, such as colour, pH, tenderness, odour and juiciness. It is well established that fibre type composition is a key determinant of both meat quantity and quality. In the pig, in particular, favourable meat traits such as tenderness and colour, have been found to closely associate with the greater abundance of oxidative fibres (Klont et al., 1998; Karlsson et al., 1999; Chang et al., 2003; Maltin et al., 2003; Wood et al., 2004). Red or highly oxidative muscles possess higher lipid concentration which is associated with more tender meat (Hocquette et al., 1998; Wood et al., 1999 and 2003). Fast-glycolytic fibres (see later for details), on the other hand, being relatively the largest fibre type are a major fibre type for muscle hypertrophy as well as in the predisposition of pale, soft and exudative (PSE) pork independent of ryanodine receptor 1 mutation.

In the living animal, muscle phenotype is a major factor in the functional integrity of mobility and movement, as well as of thermoregulation, which in turn governs its quality of life. A wide range of disease conditions often adversely affect muscle phenotype and lead to debilitating muscle wasting or atrophy. For instance, in human obesity, triglyceride accumulation in muscle (intramuscular fat) appears to be related to the development of insulin resistance and type 2 diabetes (Morio et al., 2001; Kelley et al., 2002; Wolfe, 2006). Hence, knowledge of the molecular events that affect muscle phenotype is of fundamental, agricultural, welfare and biomedical importance. By understanding the signalling processes and gene targets that effect muscle phenotype, strategic approaches, such as the use of marker-assisted selection (Beuzen et al., 2000), dietary manipulation (Casser-Malek et al., 2004) or even pharmacological targeting of novel effector genes, could be developed to improve muscle quality and quantity.

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This review endeavours to bring together recent advances in our understanding of the signalling pathways, and molecular mechanisms of several major growth promoting factors that affect muscle fibre phenotype relevant to farm animal production. Given that the subject area covered is wide, its purposes are to provide the reader with a general overview of the molecular aspects of muscle phenotype determination in the live animal and to highlight avenues for future research exploitation.

What is muscle fibre phenotype?

Post-natal muscle is a highly heterogeneous syncytial tissue, comprising muscle fibres and extracellular matrix, with the ability to rapidly undergo biochemical and physical fibre changes in response to appropriate external stimuli, such as nervous and hormonal stimulations, to adapt to the accompanying functional demands imposed on it (Caiozzo, 2004). The feature of functional plasticity indicates that skeletal muscle is highly amenable to changes in coordinated gene expression. Although the extracellular matrix of muscle, as exemplified by the orderly arrangement of endomycium, perimycium and epimycium, is a key determinant of meat quality (Fang et al., 1999; McCormick, 1999), this review is focussed on the regulation of muscle fibre phenotype. The physical and biochemical characteristics of muscle fibres can be conveniently defined under three distinct but overlapping categories: fibre number, fibre size and fibre type (Figure 1). Fibre types reflect differences in their biochemical (metabolic) and biophysical properties that arise from differences in coordinated expression of muscle gene isoforms (Schiaffino and Reggiani, 1996).

A feature of muscle fibre phenotype regulation that will be apparent is that the same signalling factor or pathway (such as IGF-1) can often have multiple phenotypic effects with regard to its influence on fibre number, size and type.

Muscle formation and regeneration

In vertebrates, most skeletal muscles are derived from muscle progenitor or precursor cells present in somites, which arise by segmentation of the paraxial mesoderm, located on either side of the notochord and neural tube in the early embryo. As it develops, each somite can be divided into the epaxial dermomyotome, which gives rise to the epaxial muscles, and into the hypaxial dermomyotome, from which derives the rest of the body and limb muscles. The first muscle mass to form, under the dermomyotome, is the myotome, which contributes to the trunk muscles. The early developmental events surrounding myogenesis are under intense molecular scrutiny (Buckingham, 2001). Myogenic cell fate specification is acquired from the activation of myogenic determination genes, namely the basic helix-loop-helix (bHLH) proteins encoded by Myf-5 and MyoD. The other two members of the myogenic bHLH gene family, myogenin and MRF4, are implicated, along with MyoD, in the subsequent activation of muscle-specific genes during myogenic differentiation. MEF-2 family, characterised by the presence of a MADS-box motif, also plays an important role in muscle differentiation (Buckingham et al., 2003). Prior to the activation of Myf-5 or MyoD, signalings by Wnts, produced by the dorsal neural tube and surface ectoderm, and hedgehog (Hh) proteins, from the notochord and floor plate of the neural tube, are needed to serve as early triggers or inductive signals of myogenesis (Tajbaksh and Buckingham, 2000). Hh signalling plays an additional role in committing progenitor cells to the slow-twitch lineage, a process specified by Blimp-1, originally identified as a factor that promotes B-cell maturation (Baxendale et al., 2004).

In limb muscle development, muscle progenitor cells opposite the limb buds must first delaminate from the hypaxial dermomyotome and migrate into the limb region. A number of homeobox proteins have been found to be essential for this migrational process (e.g. Pax3, c-met and its ligand hepatocyte growth factor (HGF)/scatter factor, and Lbx1) (Buckingham et al., 2003). Upon arriving at their destination, they begin to express MyoD and Myf-5 to mark the onset of myogenesis.

After appropriate rounds of cell proliferation, myoblasts leave the cell cycle and fuse to form multinucleated myotubes. The homeobox proteins of Mox2 and Msx1, and several growth factors, including insulin-like growth factor (IGF)-1 and -2 (Moral et al., 2000), fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs) and platelet-derived growth factors, variously contribute to the regulation of proliferation and differentiation (Zorzano et al., 2003). The first muscle fibres that appear are known as primary fibres (around gestation day 35 in the pig and 12.5 days in the mouse), around which subsequent secondary fast fibres form at the time when innervation begins to be established (beginning at day 50 to day 87 in the pig and day 15 in the mouse) (Picard et al., 2002; Buckingham et al., 2003; Da Costa et al., 2003; Caiozzo, 2004).

As post-mitotic cells, skeletal muscle fibres are unable to undergo self-replication. Damaged fibres are replaced with newly synthesised fibres through the proliferation, differentiation and subsequent fusion of muscle satellite cells (myoblasts). Satellite cells constitute a reservoir of undifferentiated muscle precursor cells, located between the basal lamina and the sarcolemma, that are activated in response to muscle injury or growth stimuli to proliferate and ultimately fuse to generate new fibres. Muscle satellite cells are characterised by the expression of myostatin,

![Figure 1](https://doi.org/10.1017/S1751731107702070) Published online by Cambridge University Press
Fibre number: mediators of muscle hyperplasia

It is generally regarded that by birth, an animal, such as the pig, would have nearly the same number of muscle fibres as in adulthood (Wigmore and Stickland, 1983). Therefore, the extent of fibre number formation or hyperplasia during foetal development will have a major bearing on muscle growth potential. In post-natal muscle, fibre number may not necessarily be constant. Periodic repair and replacement of damaged fibres are necessary to maintain functional integrity, a process performed by satellite cells that can proliferate and fuse with damaged fibres or fuse to form new fibres (Goldring et al., 2002). The relative contribution of satellite cells to the formation of new fibres (hyperplastic growth) and to existing fibres (hypertrophic growth) is not clear, but is likely to be dependent on the nature of the inductive signals. Fibre number determination can be affected by any factor that plays a role in embryonic myogenesis through myoblast specification, proliferation and/or differentiation. The main signalling factors to be considered that affect fibre number are IGF-1 and -2, myostatin and nutrition.

IGF-1 and IGF-2 activate Erk-MAPK pathway of cell proliferation (hyperplasia)

Growth factors such as IGFs and FGF by virtue of their ability to stimulate cell proliferation are regarded as potent agents that can affect fibre number, pre- and post-natally (Bass et al., 1999). Indeed, daily injections of growth hormone (GH) in the sow during early pregnancy has been shown to enhance foetal fibre number (Rehfeldt et al., 2001), an effect likely to be mediated by IGF-1. IGF-1 and -2 stimulate both muscle cell proliferation and differentiation through the interaction with the type 1 IGF receptor (IGF1R), insulin receptor exon 11- (IR-A) and insulin receptor exon11 + (IR-B), all of which are transmembrane tyrosine kinase receptors (Denley et al., 2005). These activated receptors initiate two major signalling cascades (Figures 2 and 3). The type 2 IGF receptor (IGF-2R) (known also as mannose-6-phosphate receptor) and a family of high affinity IGF binding proteins (IGFBPs) 1 to 6 modulate the availability of IGF-1 and IGF2 to bind to receptors. IGF-2R has no intrinsic signalling transduction capability and serves to sequester IGF-2 from potential receptor interactions and to internalise and degrade IGF-2 (Denley et al., 2005). Liver is the main endocrine source of IGF-1 (IGF-1Ea), which is induced by GH. Additionally, damaged, stretched or load-bearing muscles are local sources IGF-1 and IGF-2 which induce proliferation and hypertrophy in an autocrine / paracrine manner (Adams, 2002). Besides the major IGF-1Ea isoform, a spliced variant with a carboxyl terminus different from IGF-1Ea, named MGF (mechano growth factor), is rapidly inducible in skeletal muscle and appears to be an early trigger for satellite cell proliferation (Yang and Goldspink, 2002). The autocrine / paracrine muscle production of IGF-2 during muscle differentiation and regeneration was found to participate in a positive feedback loop to further enhance muscle differentiation (Erbay et al., 2003; Wilson et al., 2003) and regeneration (Kirk et al., 2003). Although both IGF-1 and -2 are clearly important to muscle growth, their relative contribution to cell proliferation, differentiation and hypertrophy is uncertain.

IGF-1 (as well as insulin and IGF-2) binding activates the receptor tyrosine kinase (IGF1R or insulin receptor), which subsequently recruits insulin receptor substrate 1 (IRS-1), a non-enzymatic docking protein that propagates the signal to two crucial signalling pathways, the mitogen-activated protein kinase (Erk-MAPK) pathway, via Ras-Raf-MEK-Erk (Rommel et al., 1999; Zimmerman and Moelling, 1999; Glass, 2003a) and the phosphatidylinositol 3'-kinase (PI3K)-Akt1 pathway (Glass, 2003b; Vollenweider, 2003). The Ras-Erk MAP kinase (Erk-MAPK) pathway, a serine/threonine phosphorylation cascade, is responsible for cell proliferation and plays an important role in hyperplastic growth (Figure 2). The PI3K-Akt1 pathway is a major route to muscle differentiation and hypertrophy (see later section). Ras is a membrane bound GTPase, and cycles between an inactive Ras-GDP to an active Ras-GTP. Expression of oncogenic Ras inhibits myogenic differentiation (Mitin et al., 2001) in part by promoting cell proliferation via the MAP kinase pathway. Interestingly, activated Ras was also found to induce the expression of slow myosin heavy chain (MyHC), although the downstream mechanism that leads to this fibre type-specific effect is not understood (Murgia et al., 2000). Both Erk-MAPK and PI3K-Akt1 pathways are necessary and complement each other in mediating post-natal muscle growth (Haddad and Adams, 2004).

Myostatin inhibits cell proliferation

Myostatin (Mst), also known as growth and differentiation factor 8 (GDF-8), is a secreted negative regulator of muscle
mass that belongs to the transforming growth factor (TGF)-β superfamily (Kocamis and Killefer, 2002). Its biological significance is demonstrated in several breeds of double-muscling cattle (e.g. Belgian Blue and Piedmontese) and Mst-null mice as dramatic increase of muscularity through fibre hypertrophy and hyperplasia (Grobet et al., 1997; McPherron and Lee, 1997; Marchitelli et al., 2003). The Belgian Blue carries a naturally occurring homozygous 11-bp deletion in the coding region of Mst whereas the Piedmontese possesses a homozygous G → A point mutation that changes the cysteine residue in Mst to a tyrosine (Kambadur et al., 1997). The porcine Mst gene has been molecularly cloned and characterised, but a mutation that confers the enlarged muscle phenotype has not been described (Jie et al., 1998). Mst arrests muscle cells in the G1 and G2 phases of the cell cycle, through the up-regulation of cyclin-dependent kinase (cdk) inhibitor p21 and down-regulation of cdk-2, thereby inhibiting cell proliferation (Thomas et al., 2000). This inhibition is mediated, at least in part, through the p38 MAPK stress response pathway (Philip et al., 2005) (Figure 4). Mst also appears to directly inhibit muscle differentiation by interfering with the activity of MyoD (Langley et al., 2002). Muscle wasting conditions, such through disease or disuse atrophy, are associated with elevated Mst in affected muscles (McCroskery et al., 2003). A primary effect of Mst inactivation in post-natal growth is an increase of satellite cell proliferation and differentiation (McCroskery et al., 2003). GH, mediated through IGF-1, was shown to inhibit Mst expression in skeletal muscles and C2C12 myotubes (Liu et al., 2003). Conversely, Mst expression in porcine embryonic myogenic cells up-regulates the production of IGF binding protein-3 (IGFBP-3), which reduces the activities of IGF-1 and -2 (Kamanga-Sollo et al., 2003). These results suggest that the Mst and IGF signalling pathways are closely connected in an antagonistic manner.

Recent micro-array investigations further found that Mst-null mice exhibited raised Wnt4 expression which stimulated satellite cell proliferation (Steelman et al., 2006). The use of both micro-array and proteomic analyses showed a clear fibre phenotype switch in Mst-null muscles from slow to fast-twitch fibres (Bouley et al., 2006; Steelman et al., 2006) (see section IGF-1, β2-agonist and myostatin-null signal fast fibre phenotype). Mst has been recently found to promote adipogenesis in C3H 10T(1/2) cells which appears to be associated with adipocyte lineage commitment (Artaza et al., 2005). However, in committed bovine preadipocytes, Mst clearly suppresses differentiation of preadipocytes into adipocytes (Hirai et al., 2007).

**Nutrition on fibre number and characteristics**
Nutrition in animal growth is a complex subject of major importance, which due to its enormity cannot be fully

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**Figure 2** The IGF-induced Erk-MAPK proliferation signalling pathway of hyperplasia. IGF-1, IGF-2 or insulin binding to tyrosine kinase receptor triggers a phosphorylation cascade that leads to transcriptional and protein modifications, culminating in increase cell proliferation. Integrin can also activate the Erk-MAPK pathway independent of tyrosine kinase receptor binding. Note that activated Ras can also activate the PI3K pathway.

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[Diagram of Erk-MAPK proliferation signalling pathway]
addressed in the present review. Maternal nutrition is undoubtedly a key factor in foetal growth and its subsequent survival which manifests its effects through a complex signalling network that includes IGFs (Hornick et al., 2000; Maak et al., 2001; Wu et al., 2006). However, in the context of foetal fibre number, the precise role of maternal nutrition is not entirely clear. Earlier work suggests that doubling food intake during early sow pregnancy increases the number of secondary fibres in the newborn, which would confer greater potential for post-natal growth (Dwyer et al., 1994). More recent work, however, could not reproduce this finding (Nissen et al., 2003).

Connected to nutrition, mild dietary restriction on beef cattle has little apparent effect on muscle characteristics or meat quality (Casser-Malek et al., 2004). However, dietary changes during weaning in calves appear to have an appreciable effect on increasing the number of oxidative fibres (Picard et al., 1995). Under moderate protein and energy dietary restriction (20% less protein and 7% less energy) in young growing pigs, we recently found, with the use of a porcine cDNA muscle micro-array, significant increase in the accumulation of intramuscular fat, which could have production implications on meat quality (Da Costa et al., 2004). A similar dietary restriction study has been reported on Brahman cattle but no data on meat characteristics are provided (Byrne et al., 2005). Lamb and beef animals raised on grass showed greater flavour intensity in comparison with grain-fed animals because of a higher accumulation of 18:3 polyunsaturated fatty acids from a grass diet (Wood et al., 1999).

**Fibre size: mediators of muscle hypertrophy/atrophy**

Post-natal muscle growth is primarily a function of enlargement and elongation of existing fibres as a result of net protein synthesis, known as muscle hypertrophy (Figure 1) (Glass, 2003b). Conversely, loss of muscle mass as a consequence of disease or muscle inactivity is primarily due to a reduction in fibre size, described as muscle atrophy (Glass, 2003b). Muscle differentiation is a prerequisite to hypertrophy and, as detailed below, the same factors are often involved in both processes (differentiation and hypertrophy). In this section, we consider some well known signalling factors and pathways that mediate muscle hypertrophy or atrophy (IGF-PI3K pathway, contractile activity, p38 MAP kinase pathway, synthetic β2 adrenergic agonists, anabolic steroids and ski proto-oncogene).

**IGF activates PI3K-Akt1 pathway of hypertrophy**

The IGF-activated PI3K-Akt1 signalling pathway (Figure 3) is widely regarded as the primary route to skeletal muscle differentiation and hypertrophy (Coolican et al., 1997; Jiang et al., 1998; Glass, 2003b). Phosphatidylinositol 3'kinase
Figure 4 The p38 MAPK stress response pathway of cell arrest and atrophy. It is activated by a variety of stimuli, including exercise and activated Ras, and through different receptor families. It mediates a range of effects: cell arrest, differentiation, atrophy as well as mitochondrial biogenesis. Additionally, TGFβ receptors readily signal through Smad proteins which also lead to cell arrest. Ski functions to inhibit Smad signalling thus relieving cell arrest and promoting proliferation. Selected abbreviations: IL-1 = interleukin 1, TLRs = toll-like receptors, TNF α = tumour necrosis factor α, and TAK = TGFβ-activated kinase.

Glycogen synthase kinase 3β (GSK3β), a serine/threonine kinase, is another major substrate of activated Akt1 that modulates muscle hypertrophy (Figure 3). Like mTOR, GSK3β can also be phosphorylated independent of Akt1. Activation of protein kinase A or C is able to lead to the phosphorylation of GSK3β (Jope, 2003). Unstimulated (dephosphorylated) GSK3β is active and its phosphorylation activity appears to act as a general repressor on its substrates. Many substrates of GSK3β have been identified, including glycogen synthase, c-Jun, cyclin D1, β-catenin, nuclear factors of activated T-cells (NFATs), and Notch2 (Masuda et al., 1998; Espinosa et al., 2003). The inhibition of GSK3β by phosphorylation, leads to dephosphorylation activation of glycogen synthase, resulting in glycogen synthesis, and to activation of elf-2B, which promotes protein synthesis (Rommel et al., 2001). A primary effect of GSK3β inhibition on muscle is the promotion of hypertrophy (Rommel et al., 2001; Vyas et al., 2002). Clearly, with its wide range of targets, GSK3β can mediate a host of other effects. GSK3β inhibition, by lithium chloride, improves glucose uptake of muscle cells, which makes it a pharmacological target for the treatment of diabetes (MacAulay et al., 2003). Wnt signalling leads to the inhibition of GSK3β, an integral component of the linear cascade that results in the stabilisation of β-catenin, a co-activator necessary for cell proliferation (Novak and Dedhar, 1999; Giles et al., 2003) and myogenic differentiation (Martin et al., 2002; Petropoulos and Skerjanc, 2002; Shi et al., 2002). Hence the inhibition of GSK3β facilitates cell proliferation, muscle differentiation (via β-catenin) and, subsequently, hypertrophy.

**Contractile activity and integrins**

Muscle hypertrophy can be an adaptive response to load-bearing exercise, which stimulates the local expression of...
IGF-1, a potent inducer of muscle hyperplasia and hypertrophy. There is also growing evidence that key signalling intermediates in the Erk-MAPK and PI3K-Akt1 pathways can be activated independent of growth factor stimulation (Figures 2 and 3). Integrins are heterodimeric (αβ) transmembrane adhesion proteins that link the actin cytoskeleton to the extracellular matrix, and participate in a vast array of signalling events including mechanical sensing, cell growth and cell migration. Stimulation of β3 integrin by a synthetic Arg-Gly-Asp ligand in feline cardiomyocytes led to activation of PI3K, mTOR, p70S6K and MEK/Erk (Balasubramanian and Kuppuswamy, 2003). It appears that in rats exposed to certain treadmill running regimes, and in certain isolated muscles subjected to passive stretch, Akt1 can be independently activated (Sakamoto et al., 2003). mTOR, but not Akt1, was also found to be activated in rat hindlimb muscles subjected to 6 hours of high frequency electrical stimulation (Parkington et al., 2003). Hence exercise or electrical stimulation can variably activate the Erk-MAPK and PI3K-Akt1 pathways as well as the p38 MAPK cascade (see next section) in the regulation of muscle development.

**p38 MAP kinase pathway of cell arrest and atrophy**

p38 MAP kinase represents another MAPK signal transduction pathway that is activated by cellular stress (e.g. heat shock, oxidative stress and certain cytokines) as well as insulin (Conejo et al., 2001; Lee et al., 2002). The p38 MAPK pathway is intriguing in that it appears to mediate a range of cellular processes in muscle besides fibre size (Figure 4). There are four members of the p38 MAP kinase family: p38 MAPK-α, -β, -γ and -δ. The dual specificity kinases, MKK3 and MKK6, are involved in p38 MAP kinase activation, with MKK3 activating p38 MAPKα and β, and MKK6 activating all four p38 MAPK isoforms (Lee et al., 2002) (Figure 4). A variety of receptor families (including interleukin-1 [IL-1], tumour necrosis factor α [TNFα] and TGFβ) signal through this cascade. Signalling of p38 MAPK has been reported to be essential for terminal differentiation (myoblasts fusion into myotubes), by enhancing the expression of MyoD, MEF2A, MEF2C, sarcomeric muscle genes and cdk inhibitor p21 (Cabane et al., 2003; Wu et al., 2000c). p38 MAPK has the added role of inhibiting the Erk-MAPK pathway thereby inducing cell cycle arrest during muscle differentiation (Lee et al., 2002). It is interesting to note that the Erk-MAPK pathway is also inhibited by activated Akt1 through the prevention of Raf and MEK phosphorylation (Rommel et al., 1999; Zimmermann and Moelling, 1999)(Figure 3). TNF-α stimulated p38 MAPK up-regulates MAFbx mRNA expression, which codes for a major E3 ubiquitin ligase responsible for muscle atrophy (Li et al., 2005) (see section on markers of atrophy and hypertrophy). Exercise induced p38 MAPK signalling has been shown to stimulate PGC-1α expression which leads to mitochondrial biogenesis hence promoting oxidative capacity (Akimoto et al., 2005). Furthermore, we recently found that the p38 MAPK pathway performs an important role in the activation of the fast oxidative-glycolytic MyHC 2x promoter in skeletal muscle (J.D. Meissner et al., unpublished data). Hence, depending on the nature of p38 MAPK stimulation, different phenotypic outcomes, such as cell arrest, atrophy, terminal differentiation or raised oxidative capacity, can result from this pathway (Figure 4).

**Synthetic β2 adrenergic agonists**

β2 adrenergic receptor agonists (β2-agonists), such as clenbuterol, cimaterol and fenoterol, are potent agents for muscle hypertrophy as well as fibre type switch from slow to fast fibres (Ryall et al., 2002). They are also important regulators of T-cells development in the thymus (Blanco et al., 2003), and were primarily developed for use as smooth muscle bronchodilators (Ryall et al., 2002). A feature of β adrenergic receptors signalling, through the binding of catecholamines, is triglyceride hydrolysis, a process that has been harnessed in animal production to produce leaner carcasses. Ractopamine (‘Paylean’, Elanco), a β1- and β2-agonist, is a commercial compound sold in the USA as a promoter of lean growing pigs (Mills et al., 2003). β2-adrenergic receptor but not β1 adrenergic receptor is responsible for mediating the hypertrophic effect of clenbuterol (Hinkle et al., 2002). The growth promoting efficacy of β2-agonists appears to show animal species variation; ruminants display the greatest and broiler chickens the least response, with pigs occupying an intermediate position (Mersmann, 1998). Although the effects of β-agonists on meat quality are somewhat equivocal, the overall picture, in particular in the pig, is that many β-agonists decrease intramuscular fat and increase shear force or toughness (Dunshea et al., 2005).

The signalling events of β2-agonists leading to muscle hypertrophy is poorly understood. There is evidence to suggest that clenbuterol induces local muscle production of IGF-1, which mediates hypertrophy (Awede et al., 2002). More recent work, however, could not detect sustained local production of IGF-1 in rat muscles treated with clenbuterol but a reduction in the expression of components of the ubiquitin-proteasome pathway was found (Yimlamai et al., 2005). The use of clenbuterol in meat production or in the treatment of muscle wasting conditions, however, has been impaired by reports of possible side effects. Pigs treated with anabolic doses of clenbuterol showed immunosuppressive effects, with raised T-cell apoptotic index (Blanco et al., 2003) and testicular degeneration (Blanco et al., 2002). In the pig, the hypertrophic effect of clenbuterol on muscle was short-lived after drug withdrawal, which could render its use ineffective in the growth promotion of food animals (Sillence et al., 2002). In rats, clenbuterol was found to cause significant cardiac and skeletal muscle necrosis (Burniston et al., 2002).

**Anabolic steroids**

Testosterone is an important male hormone whose effects on body composition are to increase muscle mass and
reduce fat, effects that are not dissimilar to $\beta_2$-agonists
(Kutscher et al., 2002). Testosterone administration is
associated with hypertrophy of type 1 and 2 fibres, and
increases in satellite cell number in humans and some ani-
mals (Sinha-Hikim et al., 2003; Chen et al., 2005). In por-
cine satellite cells, however, the effect of testosterone on
proliferation rate is equivocal (Doumit et al., 1996). Ana-
bolic androgenic steroids (stanozolol and nandrolone),
made notorious by their misuse in sports, are structural
synthetic derivatives of testosterone, designed to maximise
anabolic and reduce androgenic (male sexual) effects. Out-
side the European Union, where anabolic steroids are used
in beef production, meat quality can be adversely affected
by a small but significant rise in shear force (Dunshea
et al., 2005). Little is known about the anabolic steroid-
induced signalling mechanisms that regulate muscle mass.
One of the primary anabolic effects of androgens may be
their ability to stimulate localised IGF-1 production in skel-
etal muscle (Chen et al., 2005).

Ski
Ski is a nuclear proto-oncogene that is essential for
embryonic development. It is expressed in most post-natal
tissues. Like IGF-1, it exhibits dual functions in promoting
cell proliferation and muscle differentiation. In addition to
transforming chicken embryo fibroblasts, Ski can induce
cells derived from quail embryonic body wall to undergo
myogenesis. Lines of transgenic mice carrying chicken Ski
cDNAs under the control of murine sarcoma virus (MSV)
long terminal repeat (LTR) preferentially express high levels
of Ski mRNA and protein in skeletal muscle, even though
MSV-LTR is usually active in other tissues (Sutrave et al.,
1990 and 2000). This near exclusive skeletal muscle
expression of Ski is associated with hypertrophy of fast gly-
colytic 2b fibres, without increase in fibre number or
nuclear number (Sutrave et al., 1990). The hypertrophic
effect of Ski on 2b fibres is associated with reduced protein
degradation rates (Costelli et al., 2003). It could be that
Ski over-expression is deleterious to cells, which would
account for its restricted distribution of expression in MSV-
LTR-Ski transgenic mice, and an absence of expression in
skeletal-{$\alpha$}-actin promoter-driven Ski transgenic mice
(Sutrave et al., 2000). Indeed, satellite cells isolated from
muscles of MSV-LTR-Ski transgenic mice showed accelerated
deterioration in termination differentiation with increasing age (Chage et al., 2002).

Unlike other oncogenes, the over-expression of the wild
type c-Ski is sufficient to cause transformation (Prunier
et al., 2003). The oncoproteins from Ski and the related
SnoN (ski-related novel gene) are able to interact with a
variety of transcription regulatory complexes, including his-
tone deacetylase complexes (HDACs) and tumour suppres-
sors (Ueki and Hayman, 2003). The TGF-{$\beta$} signalling
pathway has been identified as a key interacting site of
Ski. The regulation of cell growth and differentiation by
TGF-{$\beta$} is mediated by the Smad proteins, which are
important tumour suppressors. TGF-{$\beta$} signalling is initiated
when the bound ligand induces the formation of a hetero-
meric complex comprising type I and type II serine/threo-
nine receptors. Type II receptor transphosphorylates type I
receptor, which in turn phosphorylates Smad2 and Smad3.
Activated Smad2 and Smad3 form heterodimers with
Smad4 and translocate into the nucleus where they inter-
act with a host of complexes to bring about transcriptional
activation or repression of specific genes (Figure 4). Ski
was recently found to directly interact with Smad2, Smad3
and Smad4, and to block the phosphorylation of Smad2
and Smad3 by activated TGF-{$\beta$} type I receptor (Prunier
et al., 2003; Ueki and Hayman, 2003). Therefore, a mech-
anism of the transforming ability of Ski (and SnoN) is the
repression of Smad function, whose inactivation prevents
TGF-{$\beta$}-induced cell cycle arrest. The up-regulation of Ski in
proliferating satellite cells points to its possible role in
mediating cell proliferation in the regeneration of damaged
fibres (Soeta et al., 2001). The mechanisms behind the
effects of Ski on muscle differentiation and hypertrophy
are unknown. One speculation is that Ski inhibits the sig-
nalling of myostatin, a member of the TGF-{$\beta$} superfamily,
thereby enhancing cell proliferation and differentiation
(Costelli et al., 2003) (Figure 4). Ski might be developed as
a candidate marker for hypertrophic growth in marker-
assisted selection.

Fibre types: coordinated isoform-specific expression
The plasticity of muscle fibres is not confined to its ability
to undergo changes in fibre size. Physiological and bio-
chemical properties can show wide variations between
individual fibres, and such variations are further subjected
to modulations by external stimuli. Traditionally, classi-
fication of muscle fibre types is based on differences in a
number of biochemical parameters between fibres (Gil
et al., 2001; Zierath and Hawley, 2006). Succinate dehydro-
genase (SDH) histochemical staining, for example, is able
to differentiate fibres into two or three different types,
based on the relative amount of the enzyme present in
each fibre. As SDH is an integral component of the citric
acid cycle, strongly positive fibres are classified as oxida-
tive fibres. Another commonly cited histochemical stain-
ing method depends on the overall myosin adenosine
triphosphatase (ATPase) activity in each fibre (Brooke and
Kaiser, 1970; Bancroft and Gamble, 2002). Myosin ATPase
activity originates from myosin heavy chain (MyHC), the
principal sacromeric protein component of the thick myofi-
lament. Differential myosin ATPase staining is due to differ-
ces in susceptibility to pH between different MyHC
isoforms. Since myosin ATPase is highly sensitive to pH
change, this staining method is intrinsically prone to vari-
ability in results. Histochemical methods, such as SDH and
myosin ATPase stainings, are invaluable in describing the
biochemical profile of individual fibres. However, they are
less reliable in the objective determination of fibre types.
Different histochemical stains often provide slightly
different classification outcome of individual fibres, which in the past had made findings of association studies between fibre types and meat quality traits variable and even contradictory (Essén-Gustavsson, 1993; Klont et al., 1998; Lefaucheur et al., 2004).

**Definition of fibre types.** A recent major advance in farm animal muscle research has been the development of an objective approach to muscle fibre typing based on the identity of the primary MyHC isoform expressed in each fibre (Chang et al., 1993 and 1995; Chang and Fernandes, 1997). MyHCs are encoded by a highly conserved multigene family, of which eight isoforms are known in mammals (2a, 2x, 2b, embryonic, perinatal, slow/β, extraocular and α), each with its own myosin ATPase activity and each encoded by a distinct gene (Weiss and Leinwand, 1996). In pre-natal mammalian muscles, the embryonic, perinatal and slow/β/type I MyHC isoforms represent the three dominant skeletal muscle fibre types in the developing foetus. Shortly after birth, the post-natal MyHC isoforms (2a, 2x and 2b) replace the expression of embryonic and perinatal MyHC genes. Thus, in post-natal muscles of pigs, dogs and rodents, there are four major fibre types (Figure 5) characterised by the expression of the slow/β and fast 2b MyHC gene isoforms (Schiaffino and Reggiani, 1996; Wu et al., 2000b). In cattle and horses, MyHC 2b fibres are effectively absent (Chikuni et al., 2004; Maccatrozzo et al., 2004). Based on the MyHC approach, post-natal muscle fibres in animals can be resolved by immunocytochemistry or in situ hybridisation into three or four major types, depending on animal species. Metabolic, biochemical and biophysical characteristics, such as oxidative and glycolytic capacities, fibre size, colour, and glycogen and lipid contents, have been found to vary between MyHC fibre types (Schiaffino and Reggiani, 1996; Klont et al., 1998; Karlsson et al., 1999) (Table 1). The slow/β and fast 2b fibres, also known as slow oxidative (red) and fast glycolytic (white) respectively, represent two extreme metabolic profiles. Slow MyHC fibres are characterised by slow isoform contractile proteins, high levels of myoglobin, high volumes of mitochondria, high oxidative capacity, high lipid contents and high capillary density. Favourable meat traits such as colour and, in the pig in particular, tenderness have been found to closely associate with the greater abundance of red or highly oxidative fibres (Klont et al., 1998; Karlsson et al., 1999; Chang et al., 2003; Maltin et al., 2003; Wood et al., 2004). There is a general perception that leaner meat, especially pork, contains reduced intramuscular fat, resulting in increased toughness and reduced succulence (Dunshea et al., 2005). Red muscles possess higher lipid concentration (intra- and inter-fibre fat) which is associated with more tender / juicy meat (Hocquette et al., 1998; Wood et al., 1999 and 2003).

By contrast, fast MyHC 2b fibres are the largest of the four fibre types with fast isoform contractile proteins, low amounts of myoglobin and mitochondria, high glycolytic capacity (high glycogen store), low lipid contents and low capillary density. The fast MyHC 2a and 2x fibres are intermediate fast oxidative-glycolytic fibres. Fast 2a fibres are more closely related to slow/I fibres, and fast 2x are more similar to fast 2b fibres (Table 1). Fast glycolytic fibres, in particular 2b fibres, are major contributors of hypertrophic growth and of rapid fall in muscle pH post mortem, associated with the formation of PSE pork. Hence in the modern pig, hypertrophic growth potential (meat quantity) from an abundance of MyHC2b and 2x fibres comes at a cost to

![Figure 5 Typing of porcine muscle fibres.](https://doi.org/10.1017/S1751731107702070)
meat quality (Chang et al., 2003). On the other hand, the absence of MyHC 2b fibres in cattle is a likely explanation for the lack of a PSE problem in beef. Some beef studies have linked an abundance of fast-twitch fibres (defined by histochemical detection) to improved tenderness. The interpretation of fast-twitch fibres should be made with care as it is presently clear that in the absence of MyHC 2b fibres there are strictly speaking no fast-glycolytic fibres in bovine muscle (Geay et al., 2001; Maltin et al., 2003). Consequently, in a technical sense, bovine muscle may have higher oxidative potential than its porcine counterpart.

In addition to temporal regulation, each MyHC isoform is subjected to specific spatial control, such that the number and distribution of fibres expressing each isoform often vary between anatomical muscles, e.g. soleus and longissimus thoracis et lumborum (also referred by some as longissimus dorsi). Fibre type composition varies between muscles according to their functional adaptation. Postural muscles are under continual use and comprise a high proportion of oxidative fibres. Muscles that are periodically used for intensive activities like sprinting possess large numbers of fast fibres. A further degree of fibre type heterogeneity is the presence of a small number of hybrid MyHC fibres, usually presented as a mixture of two MyHC isoforms (slow/2a, 2a/2x, or 2x/2b) (Sant'ana Pereira et al., 1995; Pette and Staron, 2000). Thus fibre population in muscle is a continuum of pure and hybrid fibres that can be altered in the fast-to-slow or slow-to-fast direction under appropriate stimulatory conditions (Schiaffino and Reggiani, 1994).

Distinctive biochemical and biophysical differences between fibre types point to a coordinated programme of fibre-type or isoform specific expression (Hallauer and Hastings, 2002). However, compared with our knowledge of muscle hypertrophy, much less is known about the molecular mediators of fibre type specific expression. Coordinated fibre type specific expression requires the orchestrated regulation of a large number of gene isoforms consistent with the fibre phenotype. Gene family and differential splicing are features of muscle genes (Schiaffino and Reggiani, 1996). Understanding the complexities of fibre type specific expression, requires insights into the signalling pathways that coordinate the temporal and spatial distribution of expression of subsets of muscle gene isoforms. The emerging picture of fibre type specific regulation is that it is governed by multiple signalling pathways and factors rather than a single pathway (Spangenburg and Booth, 2003). These pathways and factors almost inevitably have additional cellular functions, such as proliferation, differentiation and hypertrophy, in addition to modulating fibre type specific expression.

<table>
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<th>Table 1 Metabolic profiles of MyHC fibre types</th>
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† Pale, soft exudative (PSE) meat quality, independent of ryanodine receptor mutation. The abundance of MyHC 2b fibres in a normal pig is a contributory factor to PSE. () indicates possibly variable levels or data not fully established in the pig.

IGF-1, β2-agonists and myostatin-null signal fast fibre phenotype

As highlighted earlier, the major phenotypic effects of IGF-1 and -2 are cell proliferation, muscle differentiation and hypertrophy. Such properties of IGF-1 have been shown to be beneficial to ageing, atrophic, and dystrophic muscles (Barton-Davis et al., 1998; Lynch et al., 2001; Musaró et al., 2001). IGF-1 has the additional effect of converting fibres to a fast glycolytic phenotype, as evidenced by raised expression of glycolytic enzymes in IGF-1-transfected C2C12 myotubes (Semsarian et al., 1999), by modest rise in fast 2b fibres in transgenic mice carrying muscle IGF-1 isoform driven by a rat myosin light chain (MLC)-1/3 promoter (Musaró et al., 2001) and by raised type 2a and 2b fibres at the expense of slow fibres in IGF-1-treated dystrophic mice (Lynch et al., 2001). As previously indicated, clenbuterol (a β2-agonist) treated rats (Ryall et al., 2002) as well as myostatin-null mice (Steelman et al., 2006) not only showed muscle hypertrophy but also slow-to-fast fibre conversion. The mechanism responsible for the slow-to-fast fibre type switch, however, remains elusive. In general, in the pig and ruminants, it appears that growth hormone (indirectly IGF-1) and β-agonists may negatively affect meat quality, as shown by increased shear force or sensory perception (Dunshea et al., 2005). Such effects may be connected to increased fast glycolytic (MyHC 2b or 2x) fibres but because fibre typing in meat quality association studies had been almost exclusively based on histochemical methods, the changes in MyHC 2b/2x fibres in the pig or MyHC 2x fibres in cattle have not been determined.
Calcineurin signals oxidative fibre type
Calcineurin (protein phosphatase 2B/PP2B) is an enzyme complex that comprises calcineurin A (CnA) catalytic subunit, calcineurin B (CnB) regulatory subunit and calcium-binding protein calmodulin (Schulz and Yutzey, 2004). It is a calcium dependent serine-threonine phosphatase that is widely distributed throughout the body. Calcineurin has been implicated in a wide variety of biological processes, including T-lymphocyte activation, vascular, neuronal and cardiac development and growth, and, more recently, skeletal muscle development (Crabtree, 2001; Bueno et al., 2002; Horsley and Pavlath, 2002). In cardiac muscle, calcineurin signalling is necessary for cardiomyocyte maturation, heart chamber formation and cardiac hypertrophy (Schulz and Yutzey, 2004). In skeletal muscle, calcineurin appears to be needed in a number of key developmental processes, namely enhanced muscle cell differentiation, and in the fibre type context, conversion to slow (oxidative) muscle phenotype (Musarò et al., 1999; Sensmian et al., 1999; Bigard et al., 2000; Delling et al., 2000). It has been reported that activated calcineurin mediates the hypertrophic effect of IGF-1 (Musarò et al., 1999; Senssman et al., 1999). However, there is compelling evidence, including transgenic and knock-out data, to show that calcineurin has no effect on muscle hypertrophy but that the hypertrophic effect of IGF-1 is mediated by the PI3K pathway as detailed earlier (Naya et al., 2000; Bodine et al., 2001b; Rommel et al., 2001; Pallafacchina et al., 2002). Calcineurin is activated by raised intracellular calcium, triggered by extracellular signals like nervous impulses, or hormonal input, such as IGF-1 stimulation. To date, few substrates, namely NFATs, MEF2s and PGC-1α, of calcineurin action in skeletal muscle are known.

NFATs. The best characterised calcineurin substrates are members of the phosphorylated NFAT (nuclear factor of activated T-cells) family of transcription factors. Five NFAT genes, each with a distinct cellular role, have been identified: NFATc1 (NFAT2/NFATc), NFATc2 (NFATp/NFAT1), NFATc3 (NFAT4/NFATx), NFATc4 (NFAT3), and NFAT5 (Delling et al., 2000; Horsley and Pavlath, 2002). Several NFAT isoforms are expressed in skeletal muscle, each of which undergoes activation at specific stages of myogenesis. For instance, NFATc2 is activated only in new myotubes and plays a crucial role via IL-4 in mediating myoblast fusion (Horsley et al., 2003). Dephosphorylation of NFATs unmasks their nuclear localisation signal, resulting in nuclear translocation, where they bind to NFAT-binding sites on their own or as cooperative complexes with other factors, such as calcineurin, AP1, MEF2 and GATA2/4, to activate gene transcription (Musarò et al., 1999; Sugiyama et al., 2001; Schulz and Yutzey, 2004). Cultured muscle fibres showed NFAT nuclear translocation only when electrically stimulated at a slow muscle pattern, which results in high intracellular calcium (100 to 300 nM) but not at a fast muscle pattern that results in low intracellular calcium (<50 nM) (Liu et al., 2001; Frayssé et al., 2003). Nuclear NFATs are deactivated and re-located back to the cytoplasm through phosphorylation by several protein kinases, including GSK-3β (Figure 3), protein kinase A, p38 MAP kinase and casein kinase (Schulz and Yutzey, 2004). In T-cells, simultaneous activation of AP-1 (Jun/Fos) and NFAT is absolutely essential for cytokine transcriptional induction (Rao et al., 1997). In skeletal muscle, however, only GATA2 has been shown to be a co-operative binding partner of NFATs (Paul and Rosenthal, 2002).

MEF2s. Members of the MEF2 (myocyte enhancer-binding factor 2) family belong to a class of transcription factors that are responsible for the activation of many muscle-specific genes with a conserved A/T-rich cis-acting regulatory element (Naya and Olson, 1999) and play a crucial role in p38 MAP kinase-mediated terminal muscle differentiation (discussed above). Like NFATs, MEF2 proteins form cooperative complexes with other factors, like members of the MyoD family, to regulate transcription. Calcineurin up-regulates the transcription of MEF2 genes (Wu et al., 2000a). It can also dephosphorylate MEF2 directly and enhance its transcriptional activity (Dunn et al., 2001; Wu et al., 2001). Hence members of the MEF2 family are transcriptional targets and protein substrates of calcineurin.

PGC-1α. Peroxisome proliferator-activated receptor-γ co-activator-1 (PPAR-γ co-activator 1α, PGC-1α), a ubiquitous transcriptional co-factor for nuclear receptors, is a potent inducer of mitochondrial biogenesis (Lin et al., 2002). It was recently shown to be able to convert fast fibres to the slow phenotype when over-expressed in transgenic mice. Part of this effect is thought to be mediated through the action of calcineurin on PGC-1α as a substrate (see PPARs below) (Lin et al., 2002). As a co-factor, PGC-1α is likely to exert its effects indirectly by modulating the expression of a specific group of downstream genes. Although all three substrates (NFATs, MEF2s and PGC-1α) are important mediators of calcineurin activation, none is exclusively expressed in skeletal muscle. Additional substrates not yet identified may well be involved in the signalling pathway. It is not known which regulatory target genes activated by NFAT, MEF-2 or PGC-1α are responsible for the phenotypic effects of calcineurin. Further work is needed to identify the genes regulated by the known and, possibly, other unidentified substrates of calcineurin.

Several endogenous calcineurin-specific inhibitors have been discovered. AKAP79, cain/cabin 1, and CBHP are ubiquitous factors and were found to inhibit NFAT function or translocation to the nucleus (Crabtree, 2001). More recently, two additional endogenous calcineurin-specific inhibitors (DSCR1/MCIP1 and ZAKI-4/DSCR1L1/MCIP2), highly expressed in striated muscles and brain, were found (Yang et al., 2000; Rothermel et al., 2001). DSCR1 expression is induced by calcineurin and hence forms a negative feedback loop to limit calcineurin activation.
Peroxisome proliferator-activated receptors (PPAR) α, γ and δ/β belong to an important family of nuclear hormone receptors (transcription factors) that regulate genes that are involved in lipid metabolism (Muio et al., 2002). PPARα, initially identified as the mediator of a class of compounds that induces peroxisomal proliferation in rodent liver, is most abundantly expressed in striated muscle (Muio et al., 2002). Its activation increases fatty acid β-oxidation in muscle and reduces body weight (Koh et al., 2003). PPARγ is more highly expressed in adipose tissues (Koh et al., 2003) and, in contrast to PPARα, stimulates adipocyte differentiation (Obekofler et al., 2002; Yu et al., 2006). PPARδ is highly expressed in adipose tissue, heart and skeletal muscle. Recently, it was shown that activated PPARδ increases fatty acid oxidation in adipose tissue and muscle (Wang et al., 2003) and that during starvation its expression is up-regulated in skeletal muscle (Holst et al., 2003). PPARα, PPARγ and PPARδ bind to the same consensus sequence, suggesting that mechanisms must exist that regulate PPAR type specificity (Obekofler et al., 2002).

PPARγ co-activator 1α (PGC-1α), cloned from a brown fat cDNA library for its interaction with PPARγ, is a potent transcriptional activator that interacts with several nuclear hormone receptors, which include PPARs, steroid hormone receptors (glucocorticoid, oestrogen and mineralocorticoid), retinoic X receptor, thyroid hormone receptor (Oberkofler et al., 2002), as well as MEF2 (Handschin et al., 2003). PGC-1α stimulates mitochondrial function and number (biogenesis), and fatty acid oxidation in cardiac and skeletal muscles by the induction of mitochondrial and nuclear genes involved in energy production pathways, including PPARα and nuclear respiratory factor-1 (NRF-1) (Miura et al., 2003). Therefore, the phenotypic effect of PGC-1α activation, in partnership with nuclear hormone receptors, including PPARs, in skeletal muscle is increased oxidative capacity.

**Markers of atrophy and hypertrophy**

Markers of atrophy and hypertrophy are relevant to farm animal production from the perspective of disease and welfare. Animals suffer from a wide range of infectious and non-infectious diseases that are often manifested as reduced weight gain or net weight loss. Markers that can readily measure muscle atrophy or hypertrophy could have veterinary applications.

Biological markers that can predict the functional state of muscle are useful indicators in the diagnosis and monitoring of muscle conditions. Clearly, muscles that have undergone gross hypertrophy or atrophy would be apparent during examination. Muscles in the transitional process of remodelling are more difficult to recognise. Thus the use of predictive biological markers would be valuable in identifying the underlying changes that are taking place in muscle.

**E3 ubiquitin ligases as atrophic markers.** Muscle atrophy is a consequence of a variety of conditions: denervation, injury, limb immobilisation, inactivity, glucocorticoid treatment, infection, diabetes, renal failure, cancer and ageing. In muscle atrophy, muscle loss is often the result of raised protein degradation and turn-over rather than simply due to reduced protein synthesis. Thus, atrophy is as much an active degradation process as a passive one from reduced stimulation of the anabolic (hyperplastic and hypertrophic) pathways. Of the different protein degradation pathways (Costelli et al., 2003), the ATP-dependent ubiquitin-proteasome proteolytic system is thought to be of major importance in skeletal muscle (Dehoux et al., 2003; Glass, 2003a). In this process, ubiquitin (Ub) is activated by an ubiquitin-activating enzyme (E1 family) and conjugated to a substrate protein by ubiquitin-conjugating enzyme (E2 family) in conjunction with ubiquitin protein ligase (E3 ligase family). E3 ligases, of which hundreds have been identified, confer substrate specificity (Glass, 2003a). During muscle atrophy, many of the genes encoding the Ub-proteasome pathway are up-regulated (Lecker et al., 2004), including E2 (such as E214k and UbcH2) and E3 (such as E3α / UB1) genes (Li et al., 2003; Lecker et al., 2004). In particular, two E3 ubiquitin
ligases, MuRF1 (muscle ring finger 1) and MAFbx (muscle atrophy F-box or atrogin-1) have been identified as mediators of muscle atrophy (Bodine et al., 2001a; Dehoux et al., 2003). MuRF1 and MAFbx are selectively induced in skeletal muscle and heart when subjected to a variety of atrophic conditions, such as denervation and glucocorticoid treatment. Their absence in null knock-out mice led to the preservation of muscle mass under atrophying conditions (Bodine et al., 2001a). TNF-α stimulated proteolysis may be mediated through the increased expression of MAFbx and MuRF1 (Dehoux et al., 2003; Glass 2005). MuRF1 contains three domains: a RING-finger domain, required for ubiquitin ligase activity, a B-box of unclear function, and a coil-coil domain, which may be required for heterodimerisation with a related protein MuRF2. Activation, by cachectic factors such as TNFα, of the NF-κB transcription pathway induces skeletal muscle atrophy, which is mediated in part by NF-κB on the up-regulation of MuRF1 (Glass, 2005). MuRF1 physically interacts with titin, which suggests a possible role for MuRF1 in titin turnover (Centner et al., 2001). MAFbx contains an F-box domain, characteristic of SCF family of E3 ubiquitin ligases. F-box E3 ligases usually bind post-translationally modified substrates, such as phosphorylation, and may target proteins involved in cell signalling. We recently found that in porcine congenital splayleg, a condition characterised with severe muscle weakness and fibre atrophy, MAFbx is abnormally up-regulated in relation to normal littermates (Ooi et al., 2006).

FOXO proteins, a subgroup of the Forkhead family of transcription factors, have been recently identified as mediators that link the signalling pathways of muscle hypertrophy and atrophy. FOXO transcription factors are important for the induction of cell quiescence and apoptosis. FOXO factors are targets of activated Akt (Figure 3). Direct and multiple phosphorylation by Akt on FOXO factors leads to their displacement from the nucleus to the cytoplasm and to the inhibition of their transcriptional activities (Burgering and Medema, 2004). Under atrophic conditions, FOXO1 (Stitt et al., 2004) and FOXO3 (Sandri et al., 2004) have been shown to up-regulate the expression of MAFbx and MuRF1. Inhibition of FOXO activity that leads to the down-regulation of MAFbx and MuRF1 is a necessary anti-atrophy step mediated by the PI3K-Akt1 pathway (Sandri et al., 2004; Stitt et al., 2004; Tesseraud et al., 2007). This anti-atrophy effect can be triggered by insulin as ligand which has the dual roles of enhancing protein synthesis (Figure 3) and decreasing proteolysis via FOXO inhibition (Tesseraud et al., 2007).

**GATA-2 as a hypertrophic marker.** Factors involved in signalling, muscle metabolism or sarcomeric structure that are associated with the process of muscle hypertrophy could potentially be used as hypertrophic markers. GATA-2 has been recently identified as a potentially useful candidate marker gene of muscle hypertrophy (Musaró et al., 1999; Paul and Rosenthal, 2002). In mammals, the GATA zinc-finger transcription factor family comprises six genes members that can be divided into 2 sub-groups, based on structure and function (LaVoie, 2003). GATA-1/-2/-3 members are often associated with haematopoiesis and neural development. GATA-4/-5/-6 members are commonly associated with organ development, including heart, gut, blood vessels and parts of the genito-urinary system. With the exception of GATA-5, gene ablation of

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**Figure 6** Summary actions of signalling factors and pathways on fibre size, fibre number and fibre types. p38 MAPK activation can result in a range of phenotypic outcome depending on the nature of activation. The proposed inhibition of myostatin by Ski has not been formally demonstrated.
the GATA family members results in embryonic lethality. GATA-2 is necessary for mast cell development, and maintenance and expansion of multipotential progenitors and haematopoietic stem cells (Fujiwara et al., 2004). GATA-2 is usually absent or lowly expressed in skeletal muscle at all stages of development, but is induced in muscle cell culture and in vivo by IGF-1, during muscle regeneration after bupivacaine injection, and by exercise (Paul and Rosenthal, 2002; Sakuma et al., 2003). GATA-2 co-precipitates with calcineurin and NFATc1 (see below), suggesting it mediates its effect on muscle gene expression in conjunction with components of the calcineurin signalling pathway as a protein complex (Musaro et al., 1999; Sakuma et al., 2003). There is no direct evidence to show that GATA-2 is a hypertrophic factor, but its expression is closely associated with factors (e.g. IGF-1) and conditions (e.g. exercise, regeneration) that are involved in the process of hypertrophy and regeneration.

Conclusion

The determination of muscle phenotype (fibre number, size and fibre type) is highly complex and coordinated that requires the integration of several major signalling cascades (e.g. Erk-MAPK, p38 MAPK, PKB-Akt and calcineurin signalling pathways), intracellular factors (such as NFATs, and co-factors, such as PGC-1α and extracellular factors (e.g. ligands, like myostatin and IGFs, and nutrition) (Figure 6). Enhanced hypertrophic growth, as exemplified in the pig, for greater lean meat production is exemplified in the pig, for greater lean meat production is achieved by anabolic treatments with the β2-adrenergic agonist clenbuterol. Veterinary Immunology and Immunopathology 96, 111-115.


Signalling and pathways in molecular determination of skeletal muscle phenotype


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