

## REVIEW

# Molecular networks in skeletal muscle plasticity

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## ABSTRACT

The skeletal muscle phenotype is subject to considerable malleability depending on use as well as internal and external cues. In humans, low-load endurance-type exercise leads to qualitative changes of muscle tissue characterized by an increase in structures supporting oxygen delivery and consumption, such as capillaries and mitochondria. High-load strength-type exercise leads to growth of muscle fibers dominated by an increase in contractile proteins. In endurance exercise, stress-induced signaling leads to transcriptional upregulation of genes, with  $\text{Ca}^{2+}$  signaling and the energy status of the muscle cells sensed through AMPK being major input determinants. Several interrelated signaling pathways converge on the transcriptional co-activator PGC-1 $\alpha$ , perceived to be the coordinator of much of the transcriptional and post-transcriptional processes. Strength training is dominated by a translational upregulation controlled by mTORC1. mTORC1 is mainly regulated by an insulin- and/or growth-factor-dependent signaling cascade as well as mechanical and nutritional cues. Muscle growth is further supported by DNA recruitment through activation and incorporation of satellite cells. In addition, there are several negative regulators of muscle mass. We currently have a good descriptive understanding of the molecular mechanisms controlling the muscle phenotype. The topology of signaling networks seems highly conserved among species, with the signaling outcome being dependent on the particular way individual species make use of the options offered by the multi-nodal networks. As a consequence, muscle structural and functional modifications can be achieved by an almost unlimited combination of inputs and downstream signaling events.

**KEY WORDS:** Skeletal muscle, Molecular, Pathways, Myofibrils, Mitochondria, Capillary, Strength, Endurance

## Introduction: Skeletal muscle malleability

It must have been obvious for most of human history that physical performance capacity varies massively between individuals. The ancient Olympic games held in Greece between 776 BC and 394 AD are testimony to the fact that these differences were appreciated and held worthy of being exposed and celebrated. However, the underpinning of the large differences in individual functional performance capacity remained elusive. Astrand (1956) reviewed the physiological limits of human performance, noting that outstanding feats that could be achieved by athletes were the result of both natural endowment as well as specific exercise training. However, it was not until 1967 that Holloszy (1967) furnished the proof that respiratory enzymes in rat skeletal muscle as well as whole-body oxygen consumption could be increased with stringent endurance exercise training. While before Holloszy's seminal paper, most physiologists saw muscle tissue as inert and

somewhat boring, research into muscle plasticity burgeoned afterwards. Numerous scientists since have shown that skeletal muscle in all species studied is an extremely malleable tissue. It changes in a consistent fashion with many physiological stimuli such as strength and endurance training (Hoppeler, 1986), disuse (Bodine, 2013), hypoxia (Hoppeler et al., 2008), weightlessness (Desplanches, 1997), cold exposure (Buser et al., 1982; van den Berg et al., 2011) and nutritional modifications (Vogt et al., 2003). Muscle tissue's response to external stress can be rapid and extensive. With chronic electrical stimulation, mitochondrial content increased by as much as sevenfold in 28 days in superficial parts of rabbit tibialis anterior muscle (Reichmann et al., 1985). With human endurance training we have shown that the mitochondrial complement in the vastus lateralis muscle can be increased by more than 30% in 6 weeks of training (Hoppeler et al., 1985). With strength training over the same time period, fiber size and content in contractile proteins is increased by some 10% (Lüthi et al., 1986).

Many of the underlying molecular mechanisms that are responsible for the structural changes of muscle tissue have been unraveled over the last two decades. The purpose of the present review is to give a short overview of the currently known factors and their role in skeletal muscle plasticity. It is a focused update of a comprehensive review of the molecular mechanisms involved in exercise-induced phenotypic malleability of skeletal muscle tissue (Hoppeler et al., 2011).

## Sensing muscle stress

During physical activity, muscle experiences specific stressors that can challenge and disrupt homeostasis and occasionally muscle structural components. Muscle tissue structural and functional adaptations then occur as a consequence of repeated stimuli. Four major physiological stressors that are active during exercise have been identified: mechanical load, neuronal activation, hormonal adjustments and metabolic disturbances (Flück and Hoppeler, 2003). During exercise at a specific intensity and over a defined period of time, muscle tissue experiences a particular blend of these fundamental stressors. With strength training, the mechanical stress is dominant, while during endurance exercise, mechanical stress is low but metabolic disturbances can be severe and protracted. In any kind of muscle activity, neuronal activation and associated  $\text{Ca}^{2+}$  and other ion transients, as well as load- and duration-specific hormonal adjustments occur. These stresses experienced during and after physical activity are limited in time. However, they set in motion a range of molecular events within muscle cells that aim at preparing muscle towards similar future events. These skeletal muscle adaptations not only offer protection from future 'insults', they also generally improve skeletal muscle and whole-body performance with regard to the specific stress experienced or the exercise training undergone.

Much of our understanding of the molecular mechanisms of skeletal muscle plasticity has been obtained in experiments using cell cultures or model organisms with techniques such as

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**List of abbreviations**

ActRIIA/B	activin type II receptor A/B
Akt	protein kinase B
AMPK	AMP-activated protein kinase
CaMK	calmodulin-dependent kinase
CaN	calcineurin
COPD	chronic obstructive pulmonary disease
FAK	focal adhesion kinase
FoxO	forkhead box protein
GDF11	growth differentiation factor 11
HDAC	histone deacetylase
HIF-1	hypoxia inducible factor 1
IGF-1	insulin-like growth factor 1
LCFA	long-chain fatty acid
MEF	myocyte enhancer factor
mTORC1	mammalian target of rapamycin complex 1
NFAT	nuclear factor of activated T-cells
NF- $\kappa$ B	nuclear factor kappa of activated B-cells
NO	nitric oxide
PDK4	pyruvate dehydrogenase lipoamine kinase isoenzyme 4
PGC-1 $\alpha$	peroxisome-proliferator-activated receptor gamma, coactivator 1
PPAR	peroxisome-proliferator activated receptors
REDD1	regulated in development and DNA damage responses
RNS	reactive nitrogen species
ROS	reactive oxygen species
SIRT-1	sirtuin 1
Smad	homologs of the <i>Drosophila</i> protein mothers against decapentaplegic (MAD)
TLR2/4	toll-like receptor 2/4
UPS	ubiquitin-proteasome system
VEGF	vascular endothelial growth factor
Wnt	signaling protein (named after Wingless and Int-1 in <i>Drosophila</i> )

knock-outs or knock-ins, as well as pharmacological agents blocking or enhancing specific pathways. These techniques provide the information necessary to map the structure of signaling pathways and give information on how signaling networks modify downstream targets. This approach does not account for the transient nature of many of the physiological stressors and may lead to complex and compensatory processes that limit the interpretation of the importance of individual factors in physiological adaptations (Hawley and Holloszy, 2009).

The question arises as to how the four generic physiological stressors mentioned above interact with the cellular proteome. How is it that muscle cells build mitochondria in response to metabolic stress experienced with endurance training? How is it that in strength training as few as 36 near-maximal contractions per week leads to accretion of myofibrillar proteins in those muscle fibers that were mechanically loaded? The generic answer to this question is that each of the fundamental stressors is associated with a number of signaling pathways within muscle cells that control gene expression on the transcriptional or translational level or interfere with protein degradation. Control of the protein pool of the muscle cell could in principle be exerted at these three canonical sites within muscle cells (see Favier et al., 2008). Transcriptional upregulation of gene expression could lead to an increase in the concentration of downstream mRNAs, which, at a constant translational rate, would result in an increase in coded proteins. Alternatively, with a constant rate of transcription and a constant mRNA pool, translational upregulation could be responsible for an increase in the protein content. Proteins must be directed to the correct location in the cell and must properly be assembled. Last but not least, modifications

of protein degradation could also affect the size of the muscle cell's protein complement. Previous research showed that the concentration of nuclear and mitochondrial coded mRNAs was almost double in highly trained endurance runners and in proportion to the mitochondrial content in the vastus lateralis muscle (Puntschart et al., 1995). Pilegaard et al. (2003) demonstrated that peroxisome-proliferator-activated receptor- $\gamma$  coactivator 1 (PGC-1 $\alpha$ ) transcription and mRNA was transiently elevated after a bout of endurance exercise in humans. This led to the generally accepted notion that each repeated bout of endurance exercise is followed by signaling and upregulation of mitochondrial gene transcripts resulting in a cumulated response – the endurance phenotype (Joseph et al., 2006) (Fig. 1). By contrast, the gain in muscle mass seen with strength training is mainly regulated by activating mammalian target of rapamycin complex 1 (mTORC1), a key modulator of mRNA translation (see Phillips, 2009), and is not immediately transcription dependent (Fig. 2).

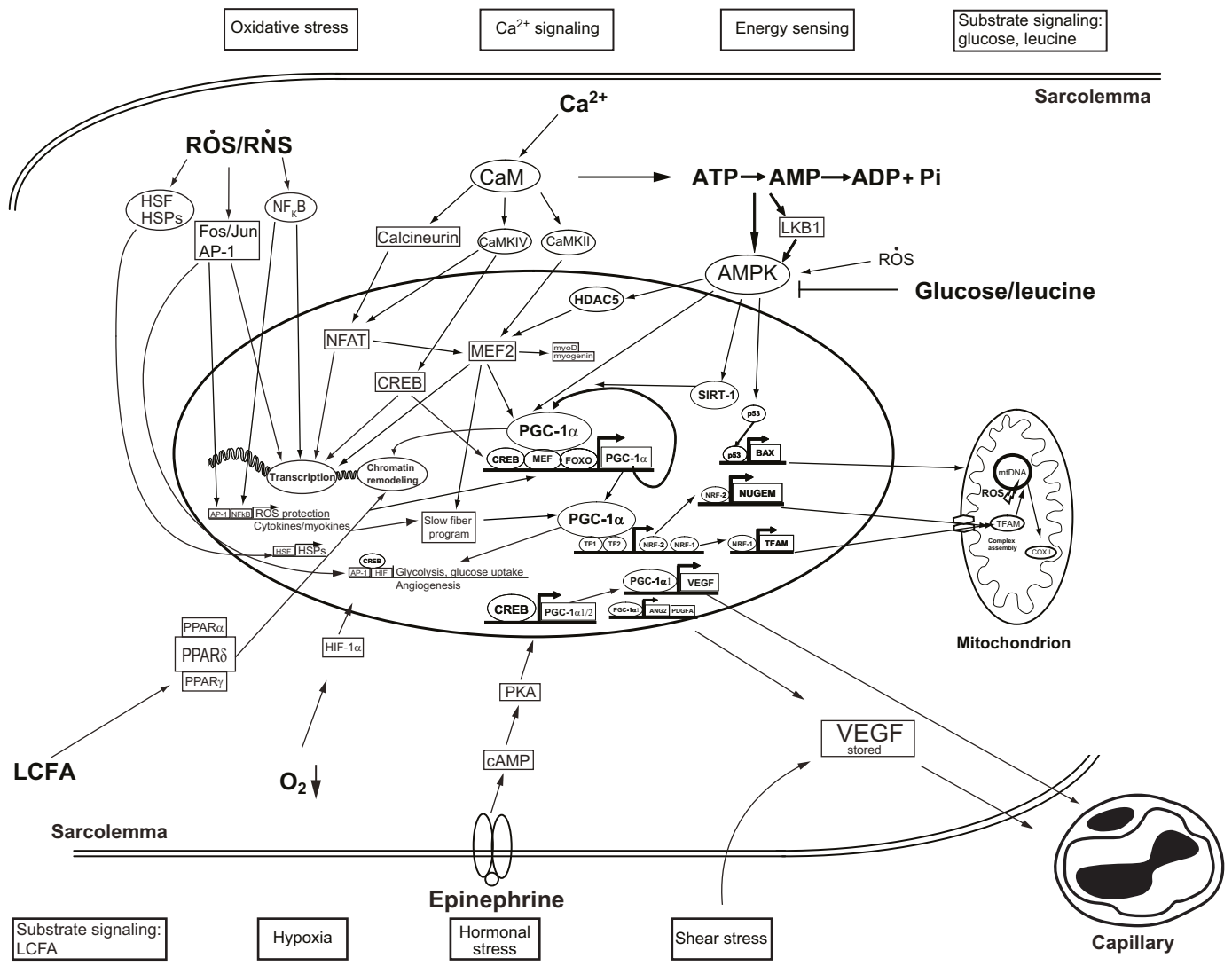
**Signaling in endurance exercise****PGC-1 $\alpha$** 

PGC-1 $\alpha$  is considered to be the key downstream element of the signaling cascades activated by endurance exercise in muscle tissue (Chan and Arany, 2014) (Fig. 1). The PGC-1 $\alpha$  gene is controlled by several promoters, coupled to alternative splicing, involved in activating gene programs for adaptations to higher energy demands (see Martinez-Redondo et al., 2015). PGC-1 $\alpha$  is a member of a family of transcriptional coactivators (PGC-1 $\alpha$ , PGC-1 $\beta$  and PGC-1 related coactivator) that regulate mitochondrial biogenesis and capillarity in a wide variety of tissues. PGC-1 plays its role in the control of energy metabolism by serving as master regulator for coordinated programs of gene expression by a multitude of transcription factors. Specific variants of PGC-1 $\alpha$  are induced by a variety of physiological cues (discussed below) increasing muscle tissue mitochondrial content and capillarity (Rowe et al., 2014). This is not the case for PGC-1 $\beta$ , which is assumed to regulate basal mitochondrial function (see Villena, 2015). Both PGC-1 $\alpha$  and PGC-1 $\beta$  are highly expressed in skeletal muscle tissue with expression levels higher in oxidative than in glycolytic fibers. Although PGC-1 $\alpha$  is generally found to be increased with exercise, PGC-1 $\beta$  has been reported to actually decrease with exercise training (Mortensen et al., 2007). However, despite the acknowledged role of PGC-1 $\alpha$  in induced mitochondrial biogenesis, PGC-1 $\alpha$  does not seem to be indispensable for exercise-induced mitochondrial biogenesis (Rowe et al., 2012). It is thus assumed that other, as yet undefined factors can compensate for a lack of PGC-1 $\alpha$ .

The regulation of the PGC-1 isoforms is complex and not fully understood. A recently discovered splice form of PGC-1 $\alpha$ , PGC-1 $\alpha$ 4, has no effect on mitochondrial gene regulation. PGC-1 $\alpha$ 4 is increased with strength training protocols associated with muscle fiber hypertrophy (Ruas et al., 2012). These authors showed that muscle growth by PGC-1 $\alpha$ 4 is achieved by inducing insulin-like growth factor 1 (IGF-1) and by suppressing myostatin.

**AMP-activated protein kinase**

AMP-activated protein kinase (AMPK) senses the increase in energy turnover when muscle tissue is activated. AMPK activity is increased by binding AMP or ADP to its  $\gamma$  subunit. AMPK coordinates anabolic versus catabolic processes in cells and balances nutrient supply with energy demand (see Mounier et al., 2015). AMPK activation has short-term actions on key metabolic processes in skeletal muscle tissue by increasing glucose uptake and



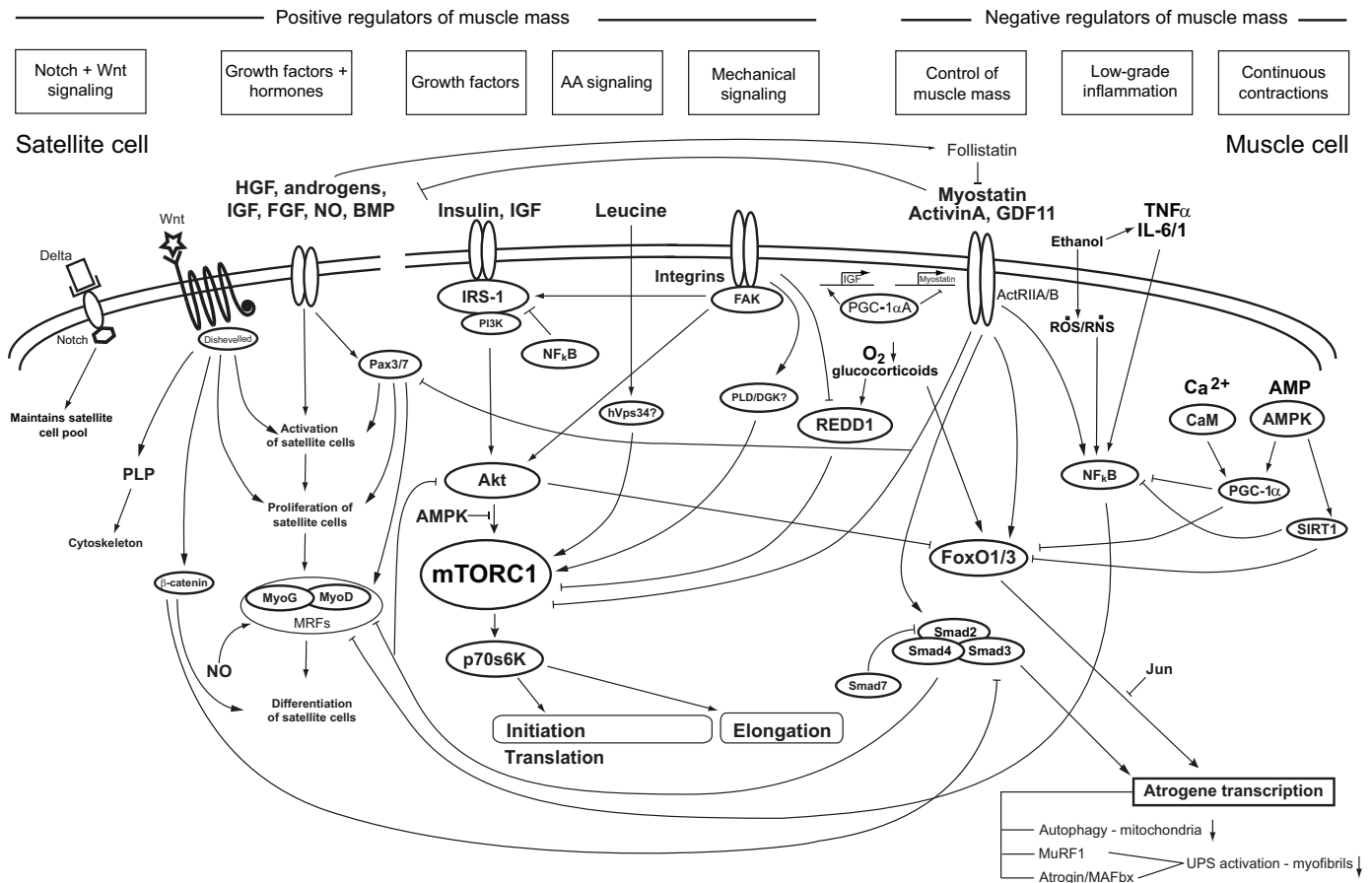
**Fig. 1. Simplified overview of signaling network controlling skeletal muscle adaptations with endurance type exercise (modified and updated from Hoppeler et al., 2011).** AMPK, AMP-activated protein kinase; AP-1, activator protein 1; Ang, angiotensin; BAX, apoptosis regulator Bax (regulated by tumor suppressor protein p53); CaM, calmodulin; CaMK, calmodulin-dependent kinase; cAMP, cyclic AMP; COX, cytochrome oxidase; CREB, cAMP response element-binding protein; fos/jun, transcription factors, proto-oncogenes; FoxO, forkhead box protein; HIF-1, hypoxia inducible factor 1; HDAC, histone deacetylase; HSF, heat shock factor; HSP, heat shock protein; LCFA, long chain fatty acids; LKB1, liver kinase B1; MEF, myocyte enhancer factor; MyoD, myogenic factor 3; mtDNA, mitochondrial DNA; NFAT, nuclear factor of activated T-cells; NF-κB, nuclear factor kappa; NRF1/2, nuclear respiratory factor 1/2; NUGEM, nuclear coded genes of mitochondria; PDGFA, platelet derived growth factor A; PGC-1α, peroxisome-proliferator-activated receptor gamma, coactivator 1; PKA, protein kinase A; PPAR, peroxisome-proliferator-activated receptors; ROS, reactive oxygen species; RNS, reactive nitrogen species; SIRT-1, sirtuin 1, a NAD-dependent deacetylase; TF1/2, transcription factor 1/2; TFAM, mitochondrial transcription factor A; VEGF, vascular endothelial growth factor.

fatty acid oxidation and by decreasing protein synthesis. Additionally, AMPK is involved in long-term metabolic changes involving mitochondrial biogenesis and promoting an oxidative muscle phenotype. While the short-term actions of AMPK are achieved by acute phosphorylation of key regulatory proteins, the long-term regulatory actions are mediated via direct phosphorylation of transcription factors and the transcriptional coactivator PGC-1α. PGC-1α is further activated by de-acetylation of sirtuin 1 (SIRT-1) while phosphorylating histone deacetylase 5 (HDAC5) leads to indirect activation of PGC-1α through the release of myocyte enhancer factor-2 (MEF2; see Fig. 1).

**Calcium-dependent signaling**

The muscle genetic programs that are activated during exercise training are importantly influenced by Ca<sup>2+</sup>-dependent signaling.

An initial step in Ca<sup>2+</sup> signaling is binding of Ca<sup>2+</sup> to calmodulin. Ca<sup>2+</sup>-activated calmodulin then interacts with downstream Ca<sup>2+</sup> decoders of which calcineurin (CaN) and calmodulin-dependent protein kinase II (CaMKII) have been characterized well in a functional context (see Tavi and Westerblad, 2011). Generally speaking, CaN and CaMKII are activated by tetanic stimulation, not single muscle twitches, whereby the response and decay of CaN is much more rapid than the response of CaMKII. As the decay of activity of the Ca<sup>2+</sup> decoders is up to 100 times slower than the Ca<sup>2+</sup> transients, it is suggested that they serve memory functions related to the prior activation events. Ca<sup>2+</sup> decoders modify gene transcription by activating transcription factors such as nuclear factor of activated T-cells (NFAT) and MEF2, and transcription modulators such as HDACs and PGC-1α (Norrbom et al., 2004). The available evidence indicates that the Ca<sup>2+</sup> decoders influence muscle fiber



**Fig. 2. Simplified overview of signaling network controlling skeletal muscle mass with hypertrophy and in muscle atrophy (modified and updated from Hoppeler et al., 2011).** ActRIIA/B, activin receptor protein II; Akt, protein kinase B; AMP, adenosine mono-phosphate; AMPK, AMP-activated protein kinase; BMP, bone morphogenic protein; DGK, diacylglycerol kinase; FA, focal adhesion kinase; FGF, fibroblast growth factor; FoxO, forkhead box protein; GDF11, growth differentiation factor 11; HGF, hepatocyte growth factor; hVps34, Class III PI 3-kinase; IGF, insulin like growth factor; IL, interleukin; IRS-1, insulin receptor substrate 1; MAFbx, muscle atrophy f-box; mTORC1, mammalian target of rapamycin complex 1; MuRF1, muscle RING finger 1; MRF, muscle regulatory factor; MyoD, myogenic factor 3; MyoG, myogenic factor 4 (myogenin); NF-κB, nuclear factor kappa; NO, nitric oxide; Pax, paired box; PGC-1, peroxisome proliferator-activated receptor gamma, coactivator 1; PI3k, phosphoinositide-3-kinase; PLD, phospholipase D; PLP, planar cell polarity; p70s6K, ribosomal protein S6 kinase; REDD1, regulated in development and DNA damage responses; ROS, reactive oxygen species; RNS, reactive nitrogen species; SIRT-1, sirtuin 1; Smad, Smad proteins homologs of mother against decapentaplegic (in *Drosophila*); TNF, tumor necrosis factor; UPS, ubiquitin-proteasome system; Wnt, signaling protein (named after Wingless and Int-1 in *Drosophila*).

type properties under drastic conditions of denervation or chronic electrical stimulation and also with CaN and CaMKII knock-outs or overexpression. Their role in more physiological low-intensity and high-intensity endurance type exercise remains to be established. An additional way in which Ca<sup>2+</sup> decoders could influence muscle phenotype is through activation by shifts of basal Ca<sup>2+</sup> concentrations during rest periods (Bellinger et al., 2008).

**Reactive oxygen and nitrogen species in muscle signaling**

Muscular exercise increases reactive oxygen species (ROS). It has also been established that contracting muscle is also a source of nitric oxide (NO) and reactive nitrogen species (RNS) (Balon and Nadler, 1994). Initially it was widely believed that both ROS and RNS were damaging to muscle fibers, which led to widespread use of antioxidants in sport drinks. Over the last 15 years it was recognized that ROS/RNS play an important role in skeletal muscle plasticity with exercise and disuse. Superoxide in muscle is produced in the sarcoplasmic reticulum, transverse tubules, sarcolemma, cytosol and notably in mitochondria (see Powers et al., 2011). It has been estimated that less than 0.15% of oxygen

consumed in mitochondria leads to superoxide formation, with more superoxide formed at rest (St-Pierre et al., 2002). The source of exercise-induced superoxide remains debated. It is recognized that intracellular ROS is an important signal in muscle remodeling with exercise. However, it is also evident that inactivity-induced ROS production contributes to disuse atrophy. It is well established that endurance exercise training not only leads to an oxidative muscle phenotype but also to an improved antioxidant defense. The molecular pathways used by ROS/RNS to modulate gene expression to this effect, involve the transcriptional activator NF-κB (nuclear factor of activated B-cells) and the activation of PGC-1α. It has been noted that the suppression of ROS activity in active muscle by antioxidants leads to a blunted NF-κB response and abolishes the exercise induced increase in manganese superoxide dismutase, an important antioxidant enzyme (Gomez-Cabrera et al., 2005). PGC-1α seems to be sensitive to the redox status of the muscle cell through multiple upstream factors (among them cAMP response element-binding protein and MEF2). ROS have also been shown to be able to induce transcription of PGC-1α indirectly via AMPK. Moreover, the human PGC-1α promoter contains a binding site for

NF- $\kappa$ B (Irrcher et al., 2008). Overall, ROS/RNS play an important role in regulating their own defense by promoting cellular antioxidants. They also contribute to the endurance phenotype by activation of NF- $\kappa$ B and induction of PGC-1 $\alpha$ .

#### Long-chain fatty acids and the peroxisome-proliferator activated receptor system

Intracellular free long-chain fatty acids (LCFAs; saturated and unsaturated) regulate energy metabolism by acting as ligands to the nuclear receptor family of transcription factors PPAR (peroxisome-proliferator activated receptors; PPAR $\alpha$ , PPAR $\gamma$  and PPAR $\delta$ ; see Nakamura et al., 2014). PPAR $\alpha$  is mainly expressed in liver and is instrumental in adaptation to fasting, inducing ketogenesis and beta-oxidation in liver mitochondria. PPAR $\gamma$  is mainly active in adipocytes, sensing and storing non-esterified LCFAs. PPAR $\delta$  is expressed ubiquitously but at particularly high levels in skeletal muscle tissue. PPAR $\delta$  is more expressed in oxidative than in glycolytic fibers and its activation is crucial for the enhanced reliance of muscle on LCFAs during fasting and in long-term exercise. Starvation increases expression of PPAR $\delta$  in skeletal muscle, inducing its target genes forkhead box protein O1 (FOXO1) and pyruvate dehydrogenase lipoamine kinase isoenzyme 4 (PDK4), the latter suppressing glycogen utilization by inactivating pyruvate dehydrogenase. FOXO1 is a regulator of cell metabolism inhibited by insulin and capable of inducing PGC-1 $\alpha$ . Not surprisingly, long-term endurance exercise and the concomitant increase in LCFA also increase PPAR $\delta$  in active skeletal muscle fibers (Kannisto et al., 2006). This suggests that PPAR $\delta$  is involved in increasing lipid metabolism and mitochondrial biogenesis, the hallmark events of endurance exercise.

There is evidence that TLR2 and TLR4 (toll-like receptors), found in the sarcolemma of skeletal muscle fibers, can be activated by LCFAs that circulate in elevated amounts during long-term endurance exercise. Animal experiments using both endurance running and heparin to elevate LCFAs show that TLR2 and TLR4 signaling is able to specifically activate p38 MAPK (p38 mitogen-activated protein kinase) and JNK (c-Jun N-terminal kinase) but not NF- $\kappa$ B (Zbinden-Foncea et al., 2012). It is thus likely that TLR2 and TLR4 can contribute to endurance adaptations.

#### Hypoxia

Hypoxia is a major cellular stressor in all tissues, leading to stabilization of hypoxia inducible factor 1 (HIF-1), which, under normoxic conditions, is continuously degraded. HIF-1 is considered to be the master regulator of oxygen homeostasis. The main target genes of HIF-1 comprise erythropoietin, inducing erythropoiesis, vascular endothelial growth factor (VEGF), leading to angiogenesis, and various genes involved in promoting glucose metabolism and glycolysis (Semenza, 2012). Based on these systemic effects of HIF-1, various hypoxia exposure protocols in endurance exercise are in use. By varying levels and exposure times to hypoxia (or altitude) they aim at maximizing the benefit from HIF-1 adaptation (see Millet et al., 2010). While local hypoxia in working skeletal muscle was considered to be a prime candidate for skeletal muscle adaptations (Terrados et al., 1990), the role of HIF activation in long-term endurance training is currently unknown. Based on circumstantial evidence, Lindholm et al. (2014) suggest that endurance exercise training leads to suppression of the HIF-1 system by activating negative regulators of HIF-1 such as the major HIF-1 prolyl hydroxylase 2 (PHD2), marking HIF-1 for the ubiquitin-proteasome degradation pathway. The repression of HIF-1 could attenuate PDK1 (pyruvate dehydrogenase lipoamine

kinase 1) expression, which, through activation of the pyruvate dehydrogenase complex, would increase the capacity to utilize oxygen in long-term endurance exercise training. In line with this proposition is the finding that mice lacking skeletal muscle HIF-1 have an oxidative phenotype with elevated oxidative enzymes and an increased capillarity combined with a constitutively increased AMPK activity (Mason et al., 2007).

#### Stressors involved in angiogenesis

By necessity there is an extremely tight correlation between mitochondrial content and capillary supply in muscle tissues across species (Hoppeler and Kayar, 1988). It has long been held that shear stress on endothelial cells induced by increased blood flow as well as by active and passive stretch of muscle tissue is key to the angiogenic response (Egginton et al., 1998; Hellsten and Hoier, 2014). A pivotal role in angiogenesis is played by VEGF. VEGF is involved in both shear-stress- and stretch-induced angiogenesis. It is expressed in endothelial cells, pericytes and muscle cells. Muscle cells have been shown to store VEGF in vesicles that translocate to the sarcolemma during contraction to release VEGF into the extracellular space (Hoier et al., 2013). Active or passive exercise can increase interstitial VEGF levels several-fold, with endothelial nitric oxide synthase having a role in shear-stress-induced angiogenesis. Beta-adrenergic signaling associated with exercise has been shown to induce VEGF expression in muscle tissue via PGC-1 $\alpha$  independent of the HIF system (Chan and Arany, 2014). This further stresses the central role played by PGC-1 $\alpha$  in exercise-induced muscle plasticity.

#### Signaling in strength training and muscle maintenance

Muscle tissue is the main reservoir of proteins in all vertebrates. Muscle mass is thus critically controlled in terms of protein synthesis and degradation. mTORC1 is considered to be the key regulator of translation initiation and elongation of protein synthesis in muscle tissue. mTORC1 can be activated or depressed depending on upstream signaling events whereby muscle mass seems to be more regulated through modification of protein synthesis than through changes in protein degradation (Phillips, 2009) (Fig. 2). The mechanisms of mTORC1 activation through membrane-bound receptors activated by insulin and growth factors are well described and critical for all types of cells (see Dodd and Tee, 2012). mTORC1 activation further depends on the complex regulation of the intracellular amino acid pools. It is less well understood how mechanical stimuli and leucine affect mTORC1 activation in muscle cells. The presence of amino acids is necessary for growth-factor-mediated activation of mTORC; however, leucine and in particular mechanical stress are also capable of activating mTORC1 in the absence of growth factors.

#### Essential amino acids

Muscle myofibrillar protein synthesis is strongly dependent on nutrient status, in particular the availability of essential amino acids. The anabolic effect of ingested food depends mainly of the presence of sufficient quantities of essential amino acids, in particular leucine. The anabolic effect of essential amino acids seems to level off at a total dose of 20–25 g protein day<sup>-1</sup> (Tipton and Phillips, 2013). An oral bolus of whey protein has been shown to increase muscle protein synthesis by 300% while muscle protein breakdown is repressed by 50% in young men (Atherton et al., 2010). The increase in muscle proteins synthesis rate is mirrored by mTORC1 activation at least during the first 90 min of the response. The fact that the muscle protein synthesis rate decreases despite the

continued presence of precursors in the blood stream has been construed as a ‘muscle-full’ signal (Phillips, 2009). This author further hypothesizes that muscle wasting syndromes, such as sarcopenia in old age, cancer or other diseases, may be due to an early ‘muscle full’ signal.

### Mechanical signaling

As mentioned above, skeletal muscle mass depends strongly on mechanical stress. High load exercise (>60% one repetition maximum) can activate mTORC1 via several pathways. Mechanical stress generated in sarcomeres is transferred to the extracellular matrix via focal adhesions, where the actin cytoskeleton is connected via linker proteins to the extracellular-matrix-bound trans-membranous integrins. Changes in mechanical stress are responsible for changes in the multi protein complexes (called costameres in skeletal muscle) involved in mechano-transduction and mechano-sensing, notably focal adhesion kinase (FAK) (Goldmann, 2014; Janošíak et al., 2014). A critical role in the costamere complex is attributed to FAK, as it has been shown that FAK is required for IGF-1-mediated muscle growth (Crossland et al., 2013). FAK has also been implicated in load-dependent turnover of costameres, with both decreased activity (bedrest) and increased activity (resistance training; Li et al., 2013). In this context, FAK has been identified as a protein kinase B (Akt)-independent mechano-sensing pathway, capable of directly activating ribosomal protein S6 kinase (p70S6K; Klossner et al., 2009).

### Activation of satellite cells

Satellite cells located beneath the basal lamina of muscle cells are instrumental for growth and repair of muscle tissue. Satellite cells are a heterogeneous population of cells with the majority of cells being committed, satellite myogenic cells, which, upon stimulation, undergo symmetric division and differentiation. A smaller number of satellite cells (satellite stem cells) undergo asymmetric division, repopulate the satellite cell niche and maintain long-term muscle regenerative potential. The behavior of satellite cells is controlled by a complex interplay of the molecular constitution of satellite cells themselves as well as extrinsic local and systemic factors affecting the satellite cells in their niche (see Yin et al., 2013). The satellite cell niche harbors a number of growth factors in precursor form, such as hepatocyte growth factor, fibroblast growth factor, epidermal growth factor and insulin-like growth factors (IGF-I and IGF-II) that can rapidly be activated in particular after muscle injury. Satellite cells are under neural influence, as nerve growth factor and brain-derived neurotrophic factor seem to play a role in muscle development and satellite cell maintenance. There is evidence for cross-talk between satellite cells and the microvasculature with both VEGF and NO synthase signaling reported in muscle regeneration.

In humans, strength training resulting in an increase of muscle fiber cross-sectional area also leads to an increase in satellite cell number (Kadi et al., 2004), whereby modest gains in muscle fiber size seem to be possible without the integration of satellite cells into the muscle fibers. In mice, the increase in satellite cell number and fiber size has been shown to be related to Wnt signaling activated by Wnt binding to Frizzled receptors. Wnt signaling involves a Wnt/ $\beta$ -catenin and a Wnt/planar cell polarity pathway and is further linked to the Akt/mTOR axis (Bentzinger et al., 2014). The second major pathway involved in satellite cell proliferation and differentiation is the Notch pathway activated by the ligand Delta. This pathway seems to be particularly important for muscle

regeneration after injury (Yin et al., 2013). Androgens promote satellite cell activation and proliferation possibly through Notch signaling and further support muscle hypertrophy by elevating IGF-1 levels and suppressing myostatin via follistatin (see Sinha-Hikim et al., 2006).

### Negative regulators of muscle mass

Muscle atrophy occurs in isolated muscles with inactivity and denervation. The loss of muscle mass as a systemic response occurs in severe disease states such as sepsis, AIDS, cardiac failure, chronic obstructive pulmonary disease (COPD), glucocorticoids (Cushing syndrome), diabetes, burns and trauma. Muscle wasting is a prominent symptom in most patients with cancer (cachexia) and happens inevitably in old age (sarcopenia; see Cohen et al., 2015). Common to these conditions is the activation of a number of catabolic stimuli that increase the protein degradation by the ubiquitin-proteasome system (UPS) and by autophagy, as well as through suppression of anabolic pathways. Myofibrillar components are mostly attacked by the UPS (loss of contractile force), while mitochondria and soluble proteins are preferentially degraded by autophagy (loss of endurance capacity). In humans, muscle wasting conditions contribute to mortality and to the loss of quality of life; research into the mechanisms of muscle atrophy has therefore received much recent attention (Dutt et al., 2015).

### Myostatin, activin A and growth differentiation factor 11

All three proteins bind to ActRIIA/B (activin type two receptors), which in turn phosphorylate and activate the Smad complex, particularly Smad2 and Smad3, considered to be key players in muscle atrophy. An exception is Smad7, which functions as a negative feedback inhibiting myostatin signaling (see Rodriguez et al., 2014). The best researched of the proteins binding to ActRIIA/B is myostatin. It is a potent player in muscle mass homeostasis, negatively influencing protein synthesis and activating catabolic pathways. It is suggested that myostatin negatively influences the Akt/mTOR axis directly as well as through the Smad protein complex. Myostatin also suppresses bone morphogenic protein signaling, this protein being a potent promoter of muscle hypertrophy, at least in mice (Sartori et al., 2013). Circumstantial evidence strongly suggests a role of myostatin in muscle protein breakdown through both the ubiquitin-proteasome and the autophagy pathways. However, the role of myostatin in muscle atrophy is less well understood. The direct activation of FoxO and increased FoxO activity through suppression of the Akt/mTOR axis has been proposed to explain the role of myostatin in muscle protein degradation (Rodriguez et al., 2014). Myostatin and growth differentiation factor 11 (GDF11) seem to be important in humans as both increase in disease states and with age. GDF11 has been shown to inhibit muscle regeneration and to decrease satellite cell expansion (Egerman et al., 2015).

### Chronic inflammation

The presence of elevated levels of pro-inflammatory cytokines such as tumor necrosis factor  $\alpha$ , IL-1, IL-6 and others is the hallmark of tumor cachexia but is also observed in other chronic diseases that lead to muscle wastage, not least in old-age-associated sarcopenia (Cohen et al., 2015). Chronically elevated levels of pro-inflammatory cytokines activate NF- $\kappa$ B, which leads to increased transcription of atrogens. Moreover, chronic inflammation is also known to increase myostatin levels, thus activating the FoxO and Smad complexes. In contrast to the damage done by chronically elevated levels of pro-inflammatory cytokines, the intermittent

increase of these factors with acute exercise plays a role in promoting favorable muscle adaptations with exercise training (Beiter et al., 2015).

### Glucocorticoids

A number of disease conditions (among them sepsis, cachexis, starvation, stress and insulinopenia) are associated with high levels of circulating glucocorticoids. Additionally, glucocorticoids are used to treat many chronic inflammatory diseases and are used as immunosuppressants after organ transplantations. Muscle wastage is observed as an important consequence of continuously elevated levels of glucocorticoids. Glucocorticoids generally suppress mTORC1 by activating REDD1 (regulated in development and DNA damage response 1), which explains their inhibitory action on protein synthesis. Glucocorticoids have been observed to directly stimulate the transcription of FoxO, thus leading to an increased transcription of atrogens, in particular muscle RING finger 1 and Atrogin1. Glucocorticoids have also been shown to increase levels of myostatin both by increasing myostatin transcription and by post-transcriptional modifications (see Schakman et al., 2013).

### Hypoxia

Systemic hypoxia has long been established as a negative regulator of muscle mass. In COPD, hypoxia is believed to be a major factor for the debilitating muscle wastage of this disease. Likewise, environmental hypoxia is held responsible in muscle atrophy observed with permanent sojourn at high altitude >5000 m (Howald and Hoppeler, 2003). Hypoxia and other stress signals influence a REDD1–mTORC1 feedback loop. Active mTORC1 inhibits the UPP-dependent degradation of REDD1. Hypoxia transcriptionally induces REDD1, leading to mTORC1 inhibition, leading to a concomitant increase in REDD1 degradation. The REDD1–mTORC1 feedback loop thus limits the inhibitory action of REDD1 on mTORC1 (Tan and Hagen, 2013).

There is recent evidence that muscle hypertrophic responses can be obtained by low-intensity resistance exercise (20–50% one repetition maximum) combined with blood flow restriction to the working muscle. The molecular mechanisms responsible in this context have not yet been established but may be related to the hypoxia stimulus being intermittent and localized (Scott et al., 2014).

### Ethanol

Acute alcohol intoxication and chronic alcohol abuse affect many organ systems partly due to a state of increase in pro-inflammatory cytokines and damage related to elevated levels of ROS. It has been suggested that the cytokines and ROS are produced by activated Kupffer cells exposed to intestinal bacteria that reach the liver in abundance due to an ethanol-induced increase in gut permeability (González-Reimers et al., 2014). As a consequence, muscle wastage associated with alcohol abuse follows the pattern described for chronically elevated cytokine levels in the preceding paragraphs. In addition, alcohol abuse is also shown to lead to a direct reduction of mTORC1 activity via poorly understood mechanisms (Steiner and Lang, 2015).

### Summary and comparative perspective of muscle plasticity

The present synopsis gives a brief, incomplete and tentative overview of the currently known molecular mechanisms involved in skeletal muscle plasticity and their interdependence. In addition to the transcriptional and translational signaling networks involved with skeletal muscle plasticity described in this review, there are

additional levels of control, not discussed, such as microRNAs that are capable of targeted post-transcriptional silencing of genes (Kirby et al., 2015) and epigenetic modifications of DNA that are involved in acute, long-term and transgenerational tuning of skeletal muscle gene expression (Moresi et al., 2015).

It is noted that two key characteristics of muscle tissue, namely endurance and strength, are governed by controls at different levels of the gene to protein axis. Endurance capacity, closely related to the capacity of skeletal muscle tissue to use oxygen for regeneration of ATP, is dominantly controlled on the level of gene transcription. The central activator of mitochondrial biogenesis and angiogenesis, the transcriptional coactivator PGC-1 $\alpha$ , is orchestrating the transcriptional activation of hundreds of genes. As indicated in Fig. 1, at least six independent but interrelated signaling pathways are responsible for activating PGC-1 $\alpha$ . Strength is related to muscle bulk, which is dominantly controlled by the activity of the translational machinery, with mTORC1 considered to be the key governor of muscle protein synthesis (Fig. 2). Several activating pathways are impinging on mTORC1, of which growth factors, mechanical signaling and amino acid availability are the most prominent. Satellite cell activation is also part of an interrelated signaling network controlling muscle mass in development and in the adult state. However, mTORC1 has also a number of potent negative regulators responsible for muscle wastage in various diseases and in old age. Additionally, there is cross-talk between strength and endurance signaling in muscle tissue as a number of factors are common to both pathways.

From an evolutionary perspective we can surmise that the pathways outlined in Figs 1 and 2 involved in skeletal muscle plasticity, as incomplete and premature as they may be, are highly conserved among animal species (Croce and McClay, 2009). Much of the research on muscle signaling has been done in genetically engineered mice and in C2C12 muscle cell cultures (derived from mice). It has been suggested that the topology of the muscle signaling networks are very similar in mammals, probably also in vertebrates, and thus, in a general sense, are applicable to humans (Baldwin et al., 2013). The massive research effort to unravel the molecular mechanisms of muscle plasticity in animal model systems and cell culture experiments implies the expectation that similar exercise stress on muscle tissue should lead to similar changes in muscle function and structure at least across mammalian species. However, this is not what is observed. Endurance exercise training in rats leads to an increase of myoglobin in the trained muscles (Pattengale and Holloszy, 1967). In humans, normoxic endurance training has no effect on muscle myoglobin content (Masuda et al., 2001); to increase myoglobin concentration in human muscle an additional hypoxic stimulus is necessary (Desplanches et al., 2014). In humans, mitochondrial content in the vastus lateralis muscle and endurance capacity are completely unaffected by 4 weeks of a high fat diet (Vogt et al., 2003). By contrast, high fat diets increase muscle oxidative capacity and endurance in exercising and sedentary rodents (Lee et al., 2001). Even more striking examples of skeletal muscle plasticity are presented by migratory birds. For the semipalmated sandpiper (*Calidris pusilla*), Maillet and Weber (2007) observed a massive increase in muscle oxidative enzyme activity immediately preceding the sandpipers' migratory flight from Canada to South America. The authors relate this change to the consumption of an amphipod, containing high amounts of n-3 polyunsaturated fatty acids. In later laboratory experiments it was shown that unsaturated dietary fatty acids could increase oxidative enzymes by up to 90% in sedentary quail muscles, whereby observed functional changes

were attributed to changes in membrane fluidity and PPAR expression (Nagahuedi et al., 2009). Observations on migratory birds further indicate that flight muscle mass changes seasonally by up to 40% in red knots, *Calidris canutus*, kept in captivity, under constant photoperiod and without evidence of exercise training (Dietz et al., 1999; Pierce and McWilliams, 2014). Similar seasonal variation (unrelated to exercise) in muscle mass and composition of fat and muscle tissue have also been observed in migratory bats (McGuire et al., 2013).

The comparative examples indicate that the enormous malleability of skeletal muscle mass and composition essentially depends on the specific (seasonal) requirements of a species. Muscle bulk and composition can be changed without invoking endurance or strength training paradigms, depending on the needs of a species. Available signaling networks can be invoked by any adequate internal or external cue. Conceptually speaking, signaling controlling muscle composition (Fig. 1) and signaling controlling muscle mass (Fig. 2) are set up in a similar fashion. Several signaling cascades depend on ‘sensor’ systems that inform muscle cells about changes in the muscle external or internal milieu impinging on the master regulators PCC-1 $\alpha$  and mTORC1, respectively. The signaling cascades are interrelated arranged in a network with multiple nodes including feedback and feedforward at various levels, making these networks resilient to perturbations. As the general topology of the signaling network is evolutionary conserved, it can be speculated that different species have tuned these networks according to their specific needs using different cues to modify muscle mass and composition. A consequence of this would be that there is considerable species specificity in phenotypic malleability of muscle tissue. Model organisms may therefore be mainly useful for establishing the general qualitative layout of the relevant signaling networks that enable this plasticity but are unable to provide answers as to how individual species make use of the available pathways.

#### Acknowledgements

I thank Glenn Lurman and Barbara Krieger for substantial help in preparing the manuscript and figures.

#### Competing interests

The author declares no competing or financial interests.

#### Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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