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Systems analysis of biological networks in skeletal muscle function

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Abstract

Skeletal muscle function depends on the efficient coordination among subcellular systems. These systems are composed of proteins encoded by a subset of genes, all of which are tightly regulated. In the cases where regulation is altered because of disease or injury, dysfunction occurs. To enable objective analysis of muscle gene expression profiles, we have defined nine biological networks whose coordination is critical to muscle function. We begin by describing the expression of proteins necessary for optimal neuromuscular junction function that results in the muscle cell action potential. That action potential is transmitted to proteins involved in excitation–contraction coupling enabling Ca^{2+} release. Ca^{2+} then activates contractile proteins supporting actin and myosin cross-bridge cycling. Force generated by cross-bridges is transmitted via cytoskeletal proteins through the sarcolemma and out to critical proteins that support the muscle extracellular matrix. Muscle contraction is fueled through many proteins that regulate energy metabolism. Inflammation is a common response to injury that can result in alteration of many pathways within muscle. Muscle also has multiple pathways that regulate size through atrophy or hypertrophy. Finally, the isoforms associated with fast muscle fibers and their corresponding isoforms in slow muscle fibers are delineated. These nine networks represent important biological systems that affect skeletal muscle function. Combining high-throughput systems analysis with advanced networking software will allow researchers to use these networks to objectively study skeletal muscle systems.

INTRODUCTION

Skeletal muscle's primary function is to generate force and produce movement. This requires coordination among many physiological pathways and their associated components. Loss of skeletal muscle function because of disease results from altered transcriptional pathways that have responded to mechanical, biological, and chemical stimuli. Thus,

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FURTHER READING

The networks described in this article are available for analysis and manipulation using the tools of Cytoscape software. Available at www.cytoscape.org.

understanding components that regulate muscle function is a prerequisite to understanding mechanisms of muscle pathology. This review highlights the components that are most critical to muscle function and places them in a context of muscle physiology as a whole using current reviews in muscle physiology and muscle gene ontology.¹ We do not provide an exhaustive list of genes and proteins that regulate muscle function, but rather explore how various pathways are distorted in a variety of muscle pathologies and the downstream consequences of altered gene expression. The networks created here provide a foundation from which to build more detailed and specific networks. The networks have been created in a Cytoscape (Cytoscape 2.8)² for use in the interpretation of current high-throughput and system level technologies such as microarrays³ and protein arrays.⁴ This work will also be useful to provide a general reference for studying the interaction between transcription and muscle function.

SYSTEMS OVERVIEW

Proper muscle function requires coordination of many integrated biological networks. Muscle contraction (MC) is initiated at the specialized neuromuscular junction (NMJ) where acetylcholine (ACh) release from the nerve ending triggers an action potential. The action potential propagates across the sarcolemma and into the transverse tubules to initiate calcium release from the sarcoplasmic reticulum (SR) in the process known as excitation–contraction coupling (ECC). Calcium binding to regulatory proteins on the thin filament triggers the myosin cross-bridge cycle that creates MC. MC force is transmitted through specialized networks of proteins within the cell cytoskeleton (CYSK) to the costameres and out to the extracellular matrix (ECM). Myosin cross-bridge cycling requires ATP, and thus skeletal muscle function also requires metabolism (MET) and storage of carbohydrates and fatty acids. A variety of damage paradigms may cause an inflammatory (INF) response in muscle. With chronically altered use, muscle adapts by coordinating a change in muscle mass via synchronized muscle hypertrophy or atrophy (HA). Most aspects of these muscle networks are slightly tuned in the different kinds of muscle cells known as fiber types (FTs).

Understanding muscle diseases requires knowledge of the protein used for force production. Duchenne muscular dystrophy (DMD) is the most frequently studied muscle disease, and although it results from the loss of a single gene product, dystrophin, many muscle functions are compromised.⁵ Dystrophin is part of the costamere complex that links MC to the ECM and, when disrupted, allows mechanically induced membrane damage.⁶ This allows calcium influx that contributes ECC alterations and muscle degradation and damage⁷ and is associated with a large INF response^{8,9} as well as cycles of regeneration.¹⁰ As the HA pathway is exhausted, the muscle undergoes an increase in ECM fibrosis.¹¹ This response illustrates the interconnectivity among muscle subsystems and demonstrates how understanding a single protein's role is ultimately critical to understanding muscle pathology. This review provides a framework for those investigating muscle disease and adaptation to efficiently inspect muscle function as a system of related proteins, especially to take advantage of the high-throughput technologies currently available.

Neuromuscular Junction

At the NMJ, the motor neuron provides the initiation signal to the muscle and does so rapidly and transiently, necessitating an off switch. NMJ function requires coordination of many transcripts that are expressed primarily or exclusively local to the NMJ (Figure 1). MC is initiated by ACh release from the motor neuron, which crosses the synaptic basal lamina to bind to the nicotinic ACh receptor (CHRN), which consists of five subunits.¹² The γ subunit (CHRNA3) is expressed in immature muscle and replaced by the ϵ subunit upon maturity.¹³ CHRN is a ligand-gated channel that allows sodium influx upon binding to create an endplate potential. When sufficient ACh binding allows the endplate potential to reach threshold, muscle sodium channels (SCN4A) are activated to create an action potential, which propagates across the sarcolemma and throughout the muscle.

Neurons communicate their 'connection' to muscle cells and maintain proper clustering of the proteins required for transmission in the postsynaptic muscle. This is performed with motor neuron release of agrin (AGRN). AGRN binds to a receptor on the muscle transmembrane receptor MUSK along with its extracellular coreceptor LRP4.¹² MUSK binding eventually results in the activation of RAPSIN, which acts as an intracellular scaffold for CHRN. MUSK also interacts with 14-3-3 γ (YWHAG), a signal transduction protein involved in synaptic gene expression. Synaptic gene expression is also mediated through neuregulin (NRG1), a glycoprotein that binds to a family epidermal growth factor receptor (ERBB). ERBB binding to NRG1 results in the activation of GABP transcription factors for synaptic genes.¹³ This provides a method for nuclei in close proximity to the NMJ to act in coordination and turn on the genes required for the NMJ within a multinucleated muscle fiber.

The synaptic basal lamina plays an important role in organizing the NMJ by maintaining a short distance for ACh to diffuse across and to be quickly processed by maintaining a unique subset of proteins that are expressed preferentially in the region. The basal lamina is primarily composed of COL4 with the $\alpha 3-6$ subunits, instead of the $\alpha 1-2$ subunits found around the rest of muscle, and laminin subunits LAMA5 and LAMB2.¹² Collagen and laminin networks are linked by the glycoprotein NID1. Perlecan (HSPG2) is the major proteoglycan present at the synapse. The synaptic basal lamina also includes COLQ, which, along with HSPG2, binds and anchors acetylcholine esterase (ACHE) to the NMJ. The function of ACHE is to hydrolyze ACh that terminates muscle fiber activation.¹⁴ ACHE begins hydrolyzing ACh almost immediately upon release so as to limit transmission time and allow the neuron to take up the products necessary to resynthesize ACh efficiently.

Excitation–Contraction Coupling

The endplate potential generated at the NMJ activates muscle voltage-gated sodium channels (SCN4A) to transform the focal endplate potential into a global muscle cell action potential, which propagates throughout the muscle and to the interior of the muscle via the transverse-tubule (T-tubules) system (Figure 2). Translation of the action potential into an intracellular Ca^{2+} signal for contraction takes place at the junction between T-tubules and the SR where the terminal cisternae permit ECC.¹⁴ The SR is held in close approximation to the T-tubule system by the linking protein junctophilin (JPH1).¹⁵ The action potential

activates the voltage-gated Ca^{2+} channel DHPR, which consists of five subunits. The coupling of DHPR on T-tubules and the ryanodine receptor RYR1 on the SR ensures that Ca^{2+} entering the cell triggers opening of RYR1 to release Ca^{2+} from the SR store.^{16,17} A unique ryanodine receptor (RYR3) is expressed in immature muscle and is replaced by RYR1 during development.¹⁸ Ca^{2+} serves as the intracellular trigger for MC, but must be tightly regulated for efficient force production. To accomplish this RYR1 has multiple proteins that modulate Ca^{2+} release. FKBP1A is an SR protein that directly interacts with RYR1 and is required for full conductance¹⁶; S100A1 also binds to RYR1 to increase open probability,¹⁹ and SYLP2 acts from the T-tubules to increase open probability without increasing current amplitude.¹⁸ Aberrant regulation of RYR1 can occur as a reaction to common anesthetics producing life-threatening malignant hyperthermia in which muscle activity overwhelms the body. The standard treatment for malignant hyperthermia is dantrolene sodium, which inhibits RYR1 activity.²⁰

Coordinated movement requires controlled muscle relaxation as well, which can be the rate-limiting step and which requires a surprisingly large portion of the cellular energy supply. For muscle relaxation to occur, Ca^{2+} is pumped back into the SR via the ATP-dependent SERCA pumps (ATP2A), which have different isoforms for fast and slow muscle¹⁸ (Figure 2). Fast muscle fibers have faster relaxation times to permit higher frequency contraction. Additionally, fast fibers contain more abundant PVALB, which acts as an intracellular Ca^{2+} buffer by binding free Ca^{2+} .²¹ Conversely, muscle also has the capability to limit relaxation times through SR proteins SLN and PLN, which, when dephosphorylated, slow relaxation by inhibiting ATP2A reuptake of Ca^{2+} .^{18,22} The SERCA pumps must work against a higher concentration gradient of calcium, but are facilitated by Ca^{2+} -binding proteins within the SR, CASQ, which also has different isoforms in fast and slow muscle (Figure 9). CASQ is held within the SR and near RYR1 by luminal proteins triadin (TRDN) and junctin (ASPH).

Aside from initiating contraction, Ca^{2+} may also play a signaling role in muscle. In some disease states, Ca^{2+} activates CAPN, a family of proteolytic enzymes important in muscle.²³ Through the activation of calmodulin (CALM1) Ca^{2+} can also activate many growth and metabolic responses that are discussed in subsequent sections through CAMK2 or calcineurin (PPP3CA) activation.²¹ Sarcolemmal channels such as TRPCs, which allow Ca^{2+} into the cell, also contribute to activation of these Ca^{2+} pathways.²⁴ These pathways can link muscle activity patterns to intracellular signaling mechanisms that control cell fate. The broad actions of Ca^{2+} as both contraction initiator and adaptation controller highlight the critical importance of maintaining proper Ca^{2+} levels and localization within a normal muscle cell.

Sarcomere Contraction

The basic functional unit of MC is the sarcomere, where force generation is produced by interaction between thick and thin filaments^{25,26} (Figure 3). The thick filament is made up primarily of type 2 myosin, which contains two myosin heavy chain (MYH) and four myosin light chain (MYL) subunits.²⁷ The myosin head interacts with the thin filament by binding to actin (ACTA) in a force-generating mechanism and is termed as the cross-bridge cycle. To control the binding of MYH to ACTA the thin filament has a set of regulatory

proteins. Tropomyosin (TPM) wraps around the thin filament and obscures the MYH-binding pocket of ACTA filaments. The position of TPM is regulated by the troponin (TN) complex, which consists of three subunits.²⁸ Regulation is mediated by Ca^{2+} binding to TNNC, which removes the inhibitory subunit TNNI from its position with TPM and both subunits are anchored to the thin filament by the third TNNT.²⁹ MYH is an ATPase that requires ATP for the release phase of the cross-bridge cycle. Deficits of ATP cause the muscle to stiffen into a state of rigor, where cross-bridges attach but do not cycle, such as occurs after death. Myosin-binding proteins (MYBPC) are important for thick filament formation and structural maintenance.

The functional components of the sarcomere require dynamic coordination. Thus, the distinct properties of muscle FTs require separate protein isoforms for optimal function. MYH is the major determinant of FTs with slow fibers characterized by myosin heavy chain I (MYH7) and is more oxidative and used for repetitive contractions. Fast fibers are generally larger, more glycolytic, and required for brief high force contractions.³⁰ Fast fibers in humans have two myosin heavy chain isoforms expressed in mature muscle, myosin heavy chain IIa (MYH2), from fast fibers with oxidative capacity, and myosin heavy chain IIx (MYH1), from the fastest most glycolytic fibers. Both MYL and MYBPC have different isoforms corresponding to FT that also effect cross-bridge cycling rates and force production. Differential regulation of MYH binding by the thin filament regulatory proteins also requires distinct isoforms of TPM and TN. MYH and MYL also have particular isoforms (MYH3 and MYH8) and (MYL4 and MYL5), respectively, that are expressed in immature muscle and also serve as a marker of muscle that undergoing regeneration.²⁷

Sarcomere structure is maintained by a variety of proteins. The largest protein in the body is titin (TTN), which spans a half sarcomere from myosin in the middle of the sarcomere near the m-line and interacts with myomesin (MYOM) at the Z-disc end of the sarcomere. Because it spans the thick and thin filaments, TTN plays an important role in conferring passive stiffness to muscle.³¹ The Z-disc is made up primarily of α -actinin (ACTN) with many interacting proteins that anchor muscle within the cytoskeleton. One of those is TCAP, which localizes titin to the Z-disc. It also interacts with CAPZ, which caps the barbed end of the actin thin filament. The large protein nebulin (NEB), which extends most of the length of the thin filament and contains many repeated actin-binding sites, maintains thin filament structure.²⁷ On the pointed end, NEB interacts with the actin-capping protein TMOD, which has different isoforms corresponding to muscle type.³² NEB is anchored to the Z-disc by MYPN, and MYOT also plays a role in thin filament stability.²⁷ The dependence of muscle on force production on sarcomere length is directly related to thin filament length.³³ Nemaline myopathies are congenital muscle disorders resulting in muscle weakness that originate from mutations in proteins responsible for thin filament stability, primarily NEB.³⁴

Cytoskeletal Elements

Sarcomere force is transmitted throughout the cell to the surrounding tissue through various cytoskeleton proteins (Figure 4). The most widely studied in skeletal muscle is dystrophin (DMD), which provides a mechanical link from the sarcomere to the sarcolemma and ECM.³⁵ Utrophin (UTRN) provides a similar role as DMD, but also functions in the NMJ

and may be able to partially compensate in the absence of DMD. DMD is associated with many proteins that interact to form the dystrophin-associated glycoprotein (DAG) complex. Many muscular dystrophies originate from mutations in DAG complex genes, particularly DMD, the largest known gene, which, when not expressed, results in DMD. Dystroglycan (DAG1) serves as a link from the DAG to the ECM through its laminin-binding properties. DAG1 is glycosylated by membrane proteins LARGE and FCMD. Also associated with the DMD are sarcoglycans (SGC), transmembrane proteins that help stabilize the sarcolemma and also link to the cytoskeleton through interactions with filamin γ (FLNC). FLNC binds SGC at the sarcolemma and also filamen-associated protein myozenin (MYOZ2) in the Z-disc.²⁷ When the sarcolemma is mechanically disrupted (which probably occurs with normal activity and certainly with intense exercise), the damage can be repaired using dysferlin (DYSF) to cause membrane fusion.³⁶ Dystrobrevins (DTN, two isoforms) bind DMD and syntrophins (SNT) that localize nitric oxide synthase 1 (NOS1) near the sarcolemma. Nitric oxide production near the membrane provides regulation of blood flow to the muscle as well as playing a role in force generation, satellite cell activation, and glucose homeostasis.³⁷ Without a fully functional DAG complex, skeletal muscle is susceptible to increased membrane disruption and injury. The vital DAG complex also provides a scaffold for many signaling mechanisms to take place in skeletal muscle. Aside from the DAG complex, integrins also provide a physical link between the cell and the ECM. Integrins bind the actin cytoskeleton and also directly to laminins in the ECM. Integrins are dimers formed by α and β subunits, of which ITGA7 and ITGB1 are the most common forms in muscle.²⁷ Disruptions of integrins are responsible for a further class of muscular dystrophies, although there is some evidence that DAG complex and integrins may partially compensate for each other in providing links to the ECM.³⁸

The DAG complex is also linked to the intermediate filament system through DTN interactions with intermediate filaments syncoilin (SYNC) and synemin (SYNM). These intermediate filaments connect to desmin (DES), the primary intermediate of skeletal muscle. However, vimentin (VIM) predominates expression during muscle development, but is then lost at maturity. DES also links mitochondria and the nucleus across the cell. The nuclear membrane anchorage to the cytoskeleton is mediated by emerin (EMD), which also binds nuclear lamin (LMNA) with its role in nuclear stability and gene expression. The critical role of EMD and LMNA regulating nuclear envelope structure in muscle is emphasized by the fact that mutations in each cause Emery–Dreifuss muscular dystrophy. DES binds these organelles to the muscle structure at the Z-disc anchor. The Z-disc itself consists of the overlapping barbed-end actin filaments from adjacent sarcomeres with its principle component ACTN connecting actin filaments. The Z-disc also contains ACTN-binding protein cypher (LDB3), for the linkage of filaments through MYOZ2, and muscle LIM protein (CSRP3), which links to the ankryn (ANK) and spectrin (SPT) network within the muscle. ANK and SPT interact with actin and support membrane stability in prevention of muscle injury. They also interact with OBSCN, which plays a role in localizing the SR through interactions with both the SR and TTN in the sarcomere.²⁷ The numerous cytoskeletal proteins present within and without the muscle cell and their role in various skeletal muscle myopathies highlight the critical importance of the structured coordination of expression and function among these genes (Box 1).

Extracellular Matrix

The cytoskeleton provides a means to transmit force from the sarcomere to the ECM, which surrounds each muscle fiber with multiple layers of organization (Figure 5). The basal lamina ensheathes each fiber with a mesh-like network consisting primarily of COL4 (COL4A1 and COL4A2) and laminin (LAM; most commonly LAMA2, LAMB1/LAMB2, and LAMC1 in muscle).⁴³ HSPG2 is a proteoglycan in the basal lamina that binds both COL4 and LAM. Other basal laminar proteoglycans include syndecans (SDC), which play an important role in satellite cell differentiation, and biglycan (BGN), an important binding partner for UTRN.^{35,44} The glycoprotein fibronectin (FN1) is a multimeric protein that serves as a link to many proteins within the basal lamina.⁴⁵ The components of the basal lamina contain site of binding directly to the DCG complex or integrins within the muscle cell.

As opposed to the basal lamina, the fibrillar ECM has strong load-bearing capabilities that can limit the strain imposed on muscles. The fibrillar ECM is made up primarily of collagen I (COL1) and collagen III (COL3A1) and is contiguous within layers of ECM in muscle and to the tendon beyond the musculotendinous junction. Collagen VI (COL6) serves as an important link between the fibrillar and laminar ECM.⁴³ Decorin (DCN) is the major fibrillar proteoglycan in muscle.⁴⁴ FN1 also plays an important link in fibrillar ECM. It binds to glycoprotein tenascin C (TNC) and provides strength and elasticity to the ECM and is highly expressed in regenerating fibers and at the muscle–tendon junction.⁴⁵ Lysyl oxidase (LOX) is a copper enzyme primarily responsible for cross-linking collagen, which contributes to ECM stiffness.⁴⁶ Increased collagen cross-linking has been proposed as a mechanism of muscle stiffening with age.⁴⁷

The ECM also has a program for degradation and regulation through zinc-dependent matrix metalloproteinases (MMPs). MMP2 and MMP9 are abundant gelatinases in muscle that breakdown COL4 and are found in the basal lamina and further the breakdown of degraded fibrillar collagens. MMP2 is activated by membrane type MMP14 at the sarcolemma. MMP1 functions as a protease in the fibrillar ECM breaking down both COL1 and COL3A.⁴⁸ MMPs are expressed highly in periods of ECM remodeling such as during muscle regeneration.⁴⁹ To prevent excessive ECM breakdown and control MMP activity, muscles express tissue inhibitors of metalloproteinases (TIMPs), of which TIMP1 and TIMP2 and present in skeletal muscle.⁴⁸ The precise manner in which MMPs and TIMPs interact to regulate muscle ECM properties is not known.

Production of ECM proteins is controlled by specific growth factors. The most well-studied inducer of ECM in skeletal muscle for its role in fibrosis is transforming growth factor, β 1 (TGFB1).⁵⁰ TGFB1 binds BGN in skeletal muscle and its activity is also inhibited through binding to LTBP4.^{51,52} Connective tissue growth factor (CTGF) is another critical component in ECM signaling that leads to expression of collagen genes.⁵⁰ After expression, collagen must be processed before becoming functional. Prolyl 4-hydroxylase (P4) catalyzes the formation of hydroxyproline, and PLOD3 catalyzes posttranslational modification and both proteins reflect the rate of collagen biosynthesis.⁴⁶ High levels of TGFB1 and CTGF are markers of muscle fibrosis, the build-up of excessive connective tissue that is present in most muscle diseases.⁵³ As a mechanical tissue, skeletal muscle is adversely affected by

stiff fibrous tissue in addition to the barrier it presents from typical cell interactions with the ECM. Once fibrosis is prevalent within skeletal muscle it prevents muscle regeneration making it predominately irreversible.⁵³

Energy Metabolism

As the motor for the body, muscle requires an extensive energy supply in the form of ATP. As mentioned above, ATP is used to power the cross-bridge cycle and maintain appropriate ion gradients by calcium transport in relaxation (Figure 6). Much of the metabolic machinery is similar to that found in other cells; however, many enzymes involved have muscle-specific isoforms expressed. Muscle can burn energy faster than can be produced within the cell necessitating a buffer system that uses creatine kinase (CKM) to transfer a high-energy phosphate from phosphocreatine stores to ADP to form ATP.⁵⁴ For short bursts of activity, skeletal muscle relies upon glycolysis for ATP production. This is the primary form of energy production within fast type II fibers because of the rapid ATP production relative to oxidative metabolism. To facilitate glycolysis, glucose is transported across the sarcolemma by GLUT4 (SLC2A4). SLC2A4 translocation to the sarcolemma is controlled by AMPK. AMPK is an energy-sensing enzyme that becomes activated in response to low energy levels.⁵⁵ Through an independent mechanism SLC2A4 also provides insulin sensitivity by increasing glucose uptake in response to elevated insulin levels.⁵⁶ Intracellular glucose can be stored in the form of glycogen via the enzyme glycogen synthase 1 (GYS1), which can then be broken down back into glucose via the enzyme glycogen phosphorylase (PYGM) when energy demands are high.⁵⁷ Glucose is broken down into pyruvate during glycolysis, which nets the required energy in the form of ATP. Glucose is prepared for glycolysis by the phosphorylating enzyme hexokinase (HK1); glycolysis is maintained by lactate dehydrogenase A (LDHA), and the rate-limiting step of glycolysis is controlled by the enzyme phosphofructokinase (PFKM).⁵⁸ Disruptions of the typical pathway from glycogen storage through glycolysis often have pathologic consequences in skeletal muscle characterized by the glycogen storage diseases. Glycogen storage diseases are often associated with exercise intolerance from limited energy supply and excessive build-up of glycogen within the cell.⁵⁹

When energy levels must be sustained over longer periods muscle must utilize the more efficient oxidative phosphorylation process. This is more active in slow type I fibers that experience repetitive low force contractions and to a lesser extent in type IIa fibers. Pyruvate conversion to acetyl-CoA by pyruvate dehydrogenase (PDH) allows progression through the tricarboxylic acid (TCA) cycle and subsequent oxidative phosphorylation.⁵⁸ Muscles also utilize more energy dense fatty acids during sustained activity in order to produce acetyl-CoA. Fatty acid uptake into the cell is also regulated through an AMPK-mediated transporter CD36.⁵⁵ Shuttling proteins fatty acid-binding protein 3 (FABP) and lipoprotein lipase (LPL) also mediate fatty acid transport.⁶⁰ Intracellular fatty acids may be stored as triglycerides for which the enzyme GPAM catalyzes the initial and committing step.⁵⁵ Hormone-sensitive lipase (LIPE) is responsible for triglyceride breakdown to free fatty acids in muscle along with ATGL (PNPLA2) for the initial step and also assisted by LPL for triglyceride hydrolysis.⁶⁰ To be used in energy metabolism fatty acids must be transported into the mitochondria via CPT1B.⁵⁸ Here, fatty acids can undergo β -oxidation to produce

acetyl-CoA and NADH, which is catalyzed by enzymes MYLCD and HADH.⁶⁰ Efficient processing of fatty acids is critical in skeletal muscle as a build-up of muscle lipid intermediates has been shown to result in decreased insulin sensitivity.⁶¹

Type I and type IIa muscle fibers have a high volume fraction of mitochondria, which are required to meet their oxidative metabolism demands. Within the mitochondria, acetyl-CoA enters the TCA cycle to produce NADH. Citrate synthase (CS) catalyzes the rate-limiting step within the TCA cycle, which also requires the enzyme succinate dehydrogenase (SDH).⁶² NADH is then used as an electron carrier in oxidative phosphorylation that uses oxygen as an oxidizing agent.⁶³ Oxygen is provided to the muscle through the vasculature, which is stimulated by VEGF, and transported within the muscle by MB.^{64,65} Oxidative phosphorylation is catalyzed by a set of four complexes in series (NDUF, SDH, CYC, and COX). The energy from the electron gradient produced is then converted to ATP by ATP synthase (ATP5).⁶³ Mutations in mitochondrial genes can lead to a set of severe mitochondrial myopathies in which compromised mitochondria signal for apoptosis and muscle necrosis.⁶⁶

Skeletal muscle is capable of adjusting the quantity of metabolic machinery present in order to meet changes in energy demands. The production of metabolic transcripts is largely controlled by PGC-1 α (PPARGC1A) within skeletal muscle in conjunction with many other transcription factors.⁶⁷ AMPK activates PPARGC1A when energy levels are low.⁵⁵ Ca²⁺ also plays a role through the activation of PPP3CA, CAMK4, and CAMK2. PPP3CA activates the transcription factor NFATC1 that produces muscle metabolic genes including myoglobin (MB).⁶⁸ PPP3CA and CAMK4 both activate CREB1, a transcription factor that is integral to PPARGC1A-mediated expression.⁶² CAMK2 activates p38 (MAPK14) that activates transcription factors MEF2 and ATF2, which participate in metabolic transcription through PPARGC1A.^{62,63} NRF1 together with PPARGC1A plays a large role in mitochondrial expression through transcription factor A, mitochondrial (TFAM). TFAM works in concert with transcription factor B1 and B2, mitochondrial (TFB), and nuclear respiratory factor 2 (NFE2L2) in the mitochondrial transcription complex, which is also maintained by TP53.⁶⁹ These proteins make up the refinery that allows muscle to convert a variety of energy sources into ATP used in force production.

Inflammation

Injury to skeletal muscle initiates a coordinated inflammatory response that is localized to the damage site (Figure 7). This is a critical step in the process of muscle repair and, if not properly regulated, leads to deterioration and fibrosis.^{50,70,71} In the early stages of the inflammatory process, proinflammatory cytokines such as interleukin 8 (IL8), interferon γ , (IFNG), and COX-2 (PTGS2) are released at the injury site attracting circulating neutrophils and classically activated macrophages, which then act to clear myofiber debris and promote myoblast proliferation.^{70,71} These monocytes secrete other proinflammatory cytokines such as tumor necrosis factor- α (TNF) and interleukin-1 β (IL1B), which stimulate phagocytosis.^{50,72}

The primary pathway for inflammation-mediated protein degradation is the nuclear factor κ B (NFKB)-dependent pathway. Activation of NFKB is controlled by the I κ B kinase

(IKBKE) complex, which phosphorylates I κ B targeting it for degradation and enabling the translocation of NF κ B to the nucleus.^{73,74} NF κ B affects protein turnover by increasing the expression of the ubiquitin ligase MuRF1 (TRIM63) and by binding to and activating interleukin-6 (IL6). IL6 is thought to act in a hormone-like manner to regulate glucose homeostasis possibly via AMPK and can also be produced by the muscle itself.^{74,77} IL6 may also be activated by heat shock factors 1 and 2 and by calcium via its activation of NF κ B.^{70,77} NF κ B is also activated by reactive oxygen and nitrogen species as well as SOCS3^{78,79}; however, inside the nucleus, reactive species inhibit NF κ B activity.⁷⁸ Once activated, NF κ B and IL6 inhibit muscle regeneration. Evidence suggests that the mitogen-activated protein kinase p38 (MAPK14) is also activated in response to TNF and IL1B. It then upregulates atrogin-1 (FBXO32), a transcript involved in muscle atrophy. Additionally, p38 has been shown to activate IL6.⁷⁶ TNF is also shown to increase circulating levels of interferon- γ (IFNG), which activates the JAK-STAT pathway and inhibits cell growth and proliferation.⁸⁰ TNF and IL1B inhibit the expression of IGF1, a key muscle growth factor.⁸¹

After initial invasion of neutrophils and classically activated macrophages, a second population of macrophages secrete cytokines such as interleukin 10 (IL10) and TGFB1 and reduce the inflammatory response.⁵⁰ IL10 and TGFB1 negatively regulate IFNG production and IL10 inhibits the proteolytic effects of IL1B.^{70,80} TGFB1 plays a role in both the initiation of fibrosis in skeletal muscle by stimulating fibroblast proliferation and inducing myogenic cells to differentiate into myofibroblastic cells. TGFB1 has been shown to inhibit regeneration via activation of Smad proteins.⁵⁰ While this inflammatory response is critical to muscle repair, persistent inflammation as seen in many myopathies leads to detrimental muscle fibrosis and an inability of muscle to regenerate.⁵³ It is thus probably not surprising that there are several reports in the literature that short-term inhibition of inflammation after injury leads to long-term muscle dysfunction.

Muscle Hypertrophy and Atrophy

Skeletal muscle hypertrophy and atrophy are required to maintain the appropriate skeletal muscle mass in response to altered levels of use and to recover from injury. These processes can be triggered at the cellular level by a variety of cues including growth factors, nutritional signals, and mechanical stress^{74,75} (Figure 8). Insulin and insulin-like growth factor 1 (IGF1) are potent inducers of hypertrophy via IGF1 receptor (IGF1R) and the PI3K/Akt pathway.⁷⁵ Activated phosphatidylinositol 3 kinase (PI3KR) creates a lipid-binding site on the cell membrane for AKT1, a serine/threonine kinase. AKT1 then results in the activation of the mammalian target of rapamycin (MTOR). Activation of MTOR then activates RPS6KB1, which activates proteins responsible for protein synthesis. In addition, PDK1 has also been shown to phosphorylate RPS6KB1 directly.⁷⁵ MTOR also participates in the growth process by phosphorylating EIF4EBP1, which results in the dissociation of the EIF4EBP1/EIF4E complex allowing EIF4E to initiate protein translation. A regulatory associated protein of MTOR (RPTOR) facilitates the phosphorylation of RPS6KB1 and 4EBP1 by MTOR and has been shown to bind both proteins. AKT1 may also influence translation by inhibiting the activity of glycogen synthase kinase 3 β (GSK3B) as GSK3B inhibits EIF2B, thereby blocking its promotion of protein translation.^{74,75} Along with the increased protein synthesis, IGF1 signaling is also able to limit active protein degradation.

AKT1 prevents the forkhead box proteins (FOXO) from entering the nucleus via phosphorylation.^{74,76} FOXO transcription factors are known to promote transcription of *TRIM63* and *FBXO32*, which are ubiquitin ligases involved in muscle degradation.⁸² These muscle atrophy proteins are critical for the active process of muscle atrophy that occurs during unloading or immobilization where muscle mass is removed.

Muscle hypertrophy requires increased protein, but multinucleated muscle fibers also require more nuclear content with growth that is provided by the local satellite cell population. Satellite cells are regenerating myogenic progenitor cells that reside next to muscle fiber beneath the basal lamina and are also activated by IGF1. Satellite cells undergo processes of proliferation, differentiation, migration, and fusion with muscle fibers in a process that is under transcriptional regulation. Satellite cell activation is centered around the four muscle regulatory transcription factors: muscle differentiation factor (MYOD1), myogenic factor 5 (MYF5), muscle regulatory factor 4 (MYF6), and myogenin (MYOG). MYOD1 and MYF5 are early myogenic factors important in differentiation.⁸³ MYOD1 activates p21 (CDKN1A) to arrest the cell cycle and promotes differentiation of muscle progenitors.⁸⁴ MYOD1 and MYF5 lead to the expression of *MYOG* and *MYF6* directly to control the transcription of many muscle-specific genes.⁸³ These transcription factors work in concert with NFATC1 and muscle enhancement factor 2 (MEF2) for muscle transcription.⁸⁵

The family of mitogen-activated protein kinases (MAPK) have also been implicated in growth and hypertrophy in response to exercise.⁷³ The MAPK family of proteins includes extracellular signal-related kinases 1 and 2 (MAPK1/3), MAPK14, and c-Jun NH2-terminal kinases (MAPK8), which are thought to couple cellular stress with an adaptive transcriptional response and are activated by MAP2K.⁸⁵ MAPK1/3 acts to activate downstream target (EIF4E) to initiate protein translation and also to activate MYOD1.^{85,86} MAPK1/3 can also be activated downstream of growth factors HGF and FGF2.⁸⁵ HGF and FGF2 are capable of enhancing satellite cell proliferation in muscle tissue.⁸⁷ MAPK14 has also been shown to activate MYOD1 as well as MYF5 and MEF2. The activation of MAPK8 is correlated with an increase in transcription of the early response genes c-jun and c-fos, which may enhance muscle regeneration.⁷³

Ca²⁺ can also signal muscle hypertrophy through activation of CALM1 and PPP3CA. These proteins prevent the translocation of NFATC1 to the nucleus where it acts as part of the muscle transcription machinery.⁶⁸ Calcium/calmodulin-dependent protein kinase II (CAMK2) is also activated by CALM1 and prevents the binding of histone deacetylase complexes (HDAC). HDAC blocks the binding of important muscle transcription factors such as MEF2.⁸⁸ The role of Ca²⁺-induced signal is thought to be critical for slow type I fiber formation and growth as the more frequent activation generates higher cytoplasmic-free Ca²⁺ concentrations.⁸⁹

Skeletal muscle also contains a key autocrine signal to limit muscle growth. Myostatin (MSTN), a member of the transforming growth factor β family, has been shown to play a significant role in muscle growth as an inhibitor of hypertrophy. MSTN signaling is mediated through its receptor activin IIB (ACVR2B), which conducts a signal to the nucleus through the SMAD pathway. Expression of follistatin (FSTN) has been shown to increase

muscle mass through action as a MSTN antagonist.^{50,74} This presents MSTN as a potent and muscle-specific potential drug target for therapies aimed at increasing muscle mass. Indeed, various methods of therapy using FSTN, soluble ACVR2B, and anti-MSTN antibodies have been attempted in order to inhibit MSTN action and are currently under research.⁹⁰

Muscle Fiber Types

Skeletal muscles have different FTs that play a role in muscle function (Figure 9) with myosin heavy chain as the major differentiator among FT in skeletal muscle.³⁰ In fact, muscle FTs are often referred to in terms of their dominant myosin heavy chain as type I, type IIa, type IIx, and type IIb (not expressed in humans). Rather than define the details of each muscle FT in this section, we have integrated this discussion into the processes described above. It is clear that many transcripts related to muscle function are differentially regulated according to FT. A full description of the proteins related to muscle FT determination has been provided elsewhere.^{91–95} In addition to the various MYH genes expressed, several of the complexes listed in the sections above have distinct isoforms that are activated specifically in either fast or slow muscle fibers. Figure 9 helps in identifying the individual genes for each FT-specific protein. This provides a useful description of muscle FT beyond just MYH and highlights the fact that ‘FT’ simply refers to a general physiological function (e.g., fast or slow contracting, high or low endurance) that is created by coordinated expression of scores of genes (Box 2).

CONCLUSION

For skeletal muscle to function properly, coordination among multiple biological networks is required. Each network has critical components responsible for its function, most of which have been established in the literature. We delineated the critical components of these nine networks and their relationship to muscle function.

A systems approach such as that described here is critical to define the appropriate regulatory systems that enable normal muscle function and to understand muscle pathological states. Importantly, these regulatory networks are likely to involve multiple genes so that a systematic, quantitative analytical approach, using these nine networks, is critical to dissecting the disease process. For example, if the typical network includes 20 genes and 3 genes interact to produce a disease state, it would require 120 combinations of 3 gene sets to sort out this network. The systems approach defined here produces topological network models which in turn can be cast into equation-driven mathematical models. These models lend themselves to quantitative studies of normal and diseased function. For instance, reduced concentration or absence of a species can be incorporated into the model to assess alterations in the phenotypes.

The models can also serve the function of assessing the effect of pharmacological intervention. In fact, a large-scale screening of therapeutic compounds can be assessed quantitatively. Muscle cells grow well in culture and can be used as a screening tool when combined with a systems approach to analyzing experimental results. Drugs can be titrated with regard to beneficial and potential detrimental side effects, and complete cocktails of drugs can be developed by linear combination of experimental results.

One specific application of the use of muscle microarray data is in patients with DMD. One can imagine that expression data can be plugged into the pathways to quickly investigate the transcriptional effects on skeletal muscle. These muscle-specific pathways would allow rapid analysis of how associated cytoskeletal transcripts are expressed. Beyond the expected network adaptations in glucose metabolism, inflammation, or cytoskeletal remodeling, unexpected adaptations of muscle systems could also be defined. Certainly, some of these details are already available scattered across the literature; however, this review provides a framework to simplify analysis of high-throughput technologies by placing gene networks in a physiological context.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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BOX 1**DESMIN IN SKELETAL MUSCLE**

Desmin represents the muscle-specific intermediate filament protein that interconnects sarcomeres at their Z-discs and permits efficient force transmission throughout the sarcomere.³⁹ Interestingly, mice lacking desmin generate lower stress because of this 'impaired' mechanical force transmission, but also show less overt signs of injury.⁴⁰ Recent studies showed that the loss of desmin also results in a chronic inflammatory effect that ultimately leads to skeletal muscle fibrosis of the ECM.⁴¹ As the desmin knockout muscle fibers are more compliant compared to wild type,⁴² but the ECM of knockouts is stiffer compared to wild type, this suggests that the knockout may mount a 'compensatory' response to loss of desmin and increased fiber compliance. The precise mechanism for this compensation is not known.

BOX 2**FIXED MUSCLE CONTRACTURES**

Skeletal muscle is an extremely adaptable tissue with motor neuron firing being a major determinant of muscle characteristics. Upper motor neuron lesions like those seen in stroke or cerebral palsy are known to create spastic muscles. This spasticity and altered use often causes muscles to adapt into a pathologic state known as a 'fixed muscle contracture'.⁹⁶ When muscle stiffness, without any active force generation, limits the functional range of motion of a joint, the patient is considered to have a fixed contracture. This pathologic state is not the result of any genetic abnormalities of muscle genes themselves, but represents a maladaptive state.⁹⁷ The mechanism that leads to restricted muscle in contracture is largely unknown. Recent studies on children with cerebral palsy have suggested that many of the functional muscle networks described here are also altered in cerebral palsy.⁹⁸ Many proteins related to ECC are upregulated, such as parvalbumin, a protein involved that assists in relaxing the muscle. The ECM also showed many transcripts that were upregulated including the major component of the basal lamina collagen IV. The muscle from children with cerebral palsy also had conflicting growth signals with both IGF1, a protein that signals hypertrophy, and MSTN, a protein expressed to limit growth, being upregulated.⁹⁸ These results demonstrate how the network approach described here may be used to investigate muscle adaptations and lead to therapeutic interventions to reverse these maladies.^a

^aThis systems approach to understanding complex muscle adaptation in disease was used in the case of children's cerebral palsy. In this approach, the gene expression pattern present in human muscle biopsies was first determined,⁹⁸ and then the biochemical, biomechanical, and structural properties of the tissue itself were also determined.⁹⁹ Using these data, we hope that therapeutic strategies can be developed to combat this devastating muscle adaptation.

FIGURE 1.

Neuromuscular junction (NMJ). Motor neurons release the neurotransmitter acetylcholine, which triggers an action potential. NMJ formation is also induced by motor neuron factors that signal muscle proteins. For all figures, we use the following node conventions: (blue circle) entrez gene symbols, (blue circle within grayed box) complexes, (blue triangle) non-protein molecules, (blue square with rounded edges) modules or functions. There are the following interactions: (plus in white circle) positive, (minus in red circle) negative, (black circle) binding, (double back slash) intermediate, and (question mark with circle) unknown. There are the following lines: (solid thick line) basic, (solid thick line with arrow) A proceeds to B, (horizontal thin line with vertical right end thick line) A does not proceed to B, and (curved arrow) translocation of A. Genes within complexes are listed in Table S1, Supporting Information. Some complexes are interacting proteins; however, others are multiple isoforms of a protein that have the same function.

FIGURE 2.

Excitation–contraction coupling. Action potentials travel into the T-tubule system and induce Ca^{2+} release from the sarcoplasmic reticulum (SR) through the ryanodine receptor. Intracellular Ca^{2+} triggers muscle contraction and is then pumped back into the Sr. Ca^{2+} also plays a role in several critical intracellular signaling pathways that regulate muscle mass and muscle fiber type.

FIGURE 3.

Muscle contraction (MC). Myosin binds to actin and undergoes cross-bridge cycling to produce contractile force. Myosin (thick) and actin (thin) filaments slide past each other during contraction. Sarcomeres are separated by Z-discs. The force generated by the myosin cross-bridge powers active MC.

FIGURE 5.

Extracellular matrix (ECM). Provides the network for intracellular loads to be transmitted extracellularly. The basal lamina is a mesh-like network, while the fibrillar ECM is made up of larger collagen fibrils and associated proteins. Several important growth factors are involved in ECM formation and several enzyme systems regulate its state.

FIGURE 6.

Energy metabolism. Muscles use ATP as their energy source for contraction and much of relaxation. ATP is generated both glycolytically and oxidatively in the muscle from glucose or fatty acids, respectively. Muscle has energy-sensing mechanisms that permit adaptation of metabolic systems to changes in energy demand.

FIGURE 7.

Inflammation. Early macrophages and neutrophils enter damaged muscle to clear debris and produce an inflammatory signal. If present chronically, inflammation can lead to secondary tissue degradation. Secondary macrophages enter to limit inflammatory signals and repair muscle.

FIGURE 8.

Muscle hypertrophy and atrophy. Multiple pathways determine muscle size. IGF1 signals fiber growth and increased protein production. MAPKs can elicit muscle myogenic factors in response to stresses. Autocrine factor MSTN limits muscle growth through the SMAD pathway.

FIGURE 9.

Muscle fiber type (FT). Genes that have similar function but are expressed specifically in either a fast or slow muscle FT are listed here. MYH is the major determinant of FT, but numerous genes exist that are coregulated in the various FTs.