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RNA-binding proteins and gene regulation in myogenesis

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Abstract

Skeletal muscle development, repair and function are dependent on highly coordinated expression of many genes. RNA-binding proteins are critical determinants of gene expression in the health and disease of various tissues, including skeletal muscle. A variety of RNA-binding proteins are associated with a transcript during its life cycle and define the lifetime, cellular localization, processing and rate at which that transcript is translated and ultimately degraded. The focus of this review is to highlight the roles of the best-characterized RNA-binding proteins in muscle including HuR, KSRP, CUGBP1, PABPN1, Lin-28 and TTP. Recent studies indicate key functions for these RNA-binding proteins in different aspects of muscle physiology. Understanding the role of specific RNA-binding proteins in skeletal muscle will provide insights not only into basic mechanisms regulating gene expression in muscle, but also into the etiology and pathology of muscle disease.

Post-transcriptional control of myogenesis

Skeletal muscle is crucial for movement, breathing and metabolism, and therefore changes in muscle function due to disease, injury or aging lead to alterations in mobility and lifespan. Proper muscle function is dependent on myofibers, which are multinucleated cells formed during muscle development, growth or regeneration in a process called myogenesis (Figure 1). In the developing mammalian embryo, precursor cells in the somites become determined for the myogenic lineage and give rise to proliferating myoblasts which terminally differentiate, withdraw from the cell cycle, and fuse to one other to form multinucleated myofibers [1]. During post-natal muscle regeneration and growth, myoblasts are derived from resident muscle precursor cells, called satellite cells. Satellite cells are mitotically quiescent and reside in a niche between the basal lamina and the sarcolemma of their associated myofibers. Upon a growth stimulus or injury, satellite cells activate, proliferate and give rise to myoblasts, which differentiate and withdraw from the cell cycle, subsequently fusing either to existing damaged myofibers for repair or to one another for new myofiber formation [2]. A fraction of satellite cells self-renew and return to quiescence underneath the basal lamina for future rounds of muscle repair [2].

The progression of myogenesis from mononucleated precursor cell to a functional myofiber depends on highly regulated changes in gene expression. The switching on and off of

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distinct sets of genes during myogenesis is orchestrated in part by four myogenic regulatory factors (MRFs), which are muscle-specific transcription factors [1]. MRFs regulate the transcription of promyogenic genes required to establish muscle cell identity and control terminal differentiation [1]. As illustrated in Figure 1, the MRFs myoD and myf5, act early in myogenesis to determine myogenic fate and regulate proliferation, whereas myogenin and MRF4 act at later stages of myogenesis such as differentiation, fusion and growth. In addition, other non-muscle specific transcription factors, such as p21 and MEF2, are also important at specific stages of myogenesis (Figure 1). Although regulatory events in myogenesis are well described at the transcriptional level, post-transcriptional events are also important for muscle development and maintenance [3, 4].

In the life cycle of an mRNA transcript, every facet is subject to elaborate control (Figure 2). Many of the processing events such as addition of the 5'cap and splicing out of introns occur co-transcriptionally [5, 6]. During RNA synthesis, various RNA-binding proteins are specifically deposited on the nascent transcript, generating mRNA-bound ribonucleoprotein complexes (mRNPs). Following the transcription of the 3'UTR of a transcript, the pre-mRNA is cleaved at the polyadenylation signal by a cleavage and polyadenylation complex and a poly(A) tail is added to the 3' end of the transcript [7, 8]. The mRNP complex, in which the mRNA is assembled, is very dynamic and undergoes extensive remodeling. Numerous proteins in this complex have the capacity to influence downstream events such as mRNA export to the cytoplasm, localization, translation and degradation [9, 10]. In eukaryotes, mRNA can be degraded by multiple pathways, but the bulk of mRNA is degraded by a pathway initiated by poly(A) tail shortening, with the deadenylation rate being the main factor determining mRNA half-life [11]. The interplay between RNA-binding proteins and *cis*-elements within an mRNA ultimately determines the rate at which the mRNA will be degraded [11]. The regulation of mRNA stability is important in the control of overall gene expression, offering a rapid and additional modulation of mRNA levels in response to a variety of stimuli and, consequently, the levels of protein in the cell.

At the core of mRNPs are the RNA-binding proteins that directly associate with elements in the mRNA sequence through one or more RNA-binding domains (RBDs). More than 1100 annotated genes in humans are involved in RNA metabolism [12], with 40 different types of domains implicated in RNA recognition. Most RNA-binding proteins recognize particular structures or sequences present in specific target mRNAs. Studies of mRNP assembly from various species support the view that mRNAs that encode proteins with similar functions may be recognized by the same RNA-binding protein, allowing a specific RNA-binding protein to target an entire mRNA network and control the stability and translation of mRNAs encoding proteins involved in common functions [13, 14].

Although RNA-binding proteins play key roles in RNA metabolism and a large number of these proteins have been identified, very few RNA-binding proteins have been studied specifically in skeletal muscle. Several recent findings demonstrate that RNA-binding proteins play key roles in regulating gene expression during myogenesis both *in vitro* and *in vivo* (Figure 3). In addition, some RNA-binding proteins are either mutated or their levels altered in patients with specific forms of muscular dystrophy. Thus, an overview of RNA-binding proteins and their control of myogenesis in health and disease is timely and will stimulate further research into the varied roles of RNA-binding proteins in controlling muscle cell physiology, as well as possibly provide new therapeutic modalities for muscle disease. In this review, we discuss the roles of the best-characterized RNA-binding proteins HuR, KSRP, CUGBP1, PABPN1, Lin-28 and TTP in skeletal muscle and examine how they crucially impact different stages of myogenesis.

HuR stabilizes critical myogenic transcripts required for differentiation

The human antigen R (HuR) is a RNA-binding protein from the ELAV-1 family (embryonic lethal abnormal vision in *Drosophila*) that has multiple roles at different stages of mRNA metabolism including splicing, polyadenylation, nucleo-cytoplasmic transport, translation and degradation of mRNA [15]. The HuR protein contains three highly conserved RNA recognition motifs (RRMs) with a variable basic hinge region located between RRM2 and RRM3 (Table 1). The HuR RRM3 mediates binding to a heterogeneous group of *cis*-elements, including U- and AU-rich elements (AREs) of specific target mRNAs. AREs are the most common *cis*-element and are responsible for rapid mRNA decay in mammalian cells and for maintaining a precise level of short-lived transcripts encoding cytokines, chemokines, transcription factors, proto-oncogenes, and cell-cycle regulators [16, 17]. As many as 10% of 3'UTRs in human mRNAs contain AREs [18].

HuR post-transcriptionally regulates the expression of genes involved in multiple cellular processes, including proliferation, survival, inflammation and stress responses in many cells [15]. HuR also plays a crucial role in myogenesis (Figure 3) [3, 19]. HuR function in muscle cells is primarily regulated by dynamic compartmentalization mediated by the nuclear import receptor transportin 2 (TRN2). HuR is almost exclusively nuclear in proliferating myoblasts, but upon differentiation, it accumulates in the cytoplasm [3]. During differentiation, caspase-mediated cleavage of HuR generates a 24 kDa inhibitory fragment, which associates with TRN2 and consequently blocks import of intact HuR to the nucleus, leading to the cytoplasmic accumulation of full-length HuR [19]. Cytoplasmic accumulation of HuR results in subsequent stabilization and increased levels of MyoD and myogenin mRNAs, leading to increased levels of the respective proteins thus promoting myogenesis [19]. siRNA-mediated depletion of HuR from myoblasts leads to a significant reduction in the expression of MyoD and myogenin and complete inhibition of differentiation, demonstrating the crucial role of HuR in myogenesis [20].

Cell cycle withdrawal by proliferating myoblasts is a key facet of muscle differentiation and HuR may also play an important role in regulating cell cycle withdrawal [21]. HuR stabilizes the ARE-containing mRNA encoding the cyclin-dependent kinase inhibitor p21. p21 transcription is induced by MyoD and p21 protein plays an important role in coupling irreversible withdrawal from the cell cycle with differentiation during myogenesis [21]. HuR associates with p21 mRNA during muscle differentiation [4] and overexpression of HuR in differentiating muscle cells increases the steady-state levels of p21, accelerating muscle differentiation [3]. Therefore, HuR likely has an important role in the cell cycle arrest of myoblasts during myogenesis through regulating p21 expression. HuR also binds to and stabilizes Cyclin D1 mRNA, which encodes a key cell cycle control factor for myoblast proliferation [22]. The interaction between HuR and Cyclin D1 mRNA is stabilized by another factor, Pitx2. However, during differentiation, Pitx2 is phosphorylated and the complex Pitx2/HuR/Cyclin D1 mRNA dissociates leading to destabilization of Cyclin D1 mRNA and subsequent cell cycle arrest [23]. Together, these studies establish HuR as a major modulator of the expression of myogenic regulatory factors and other key regulatory proteins important for different stages of myogenesis. Future studies on HuR function in myogenesis are likely to reveal other ARE-containing mRNAs that are modulated by HuR binding and contribute to myogenesis.

KSRP counteracts HuR effects by destabilizing myogenic mRNAs

The KH-type splicing regulatory protein (KSRP) is a protein of the far upstream element (FUSE)-binding protein (FBP) family that is involved in several steps of mRNA metabolism [24]. KSRP was initially described as an alternative splicing regulator for *c-src* [24].

However, later studies demonstrated that KSRP also binds to ARE-containing mRNAs and recruits the exosome, a multi-subunit protein complex with 3'-5' exonucleolytic activity, thereby promoting rapid decay of such transcripts [24]. KSRP recognition of AREs in the 3'UTR of specific transcripts is mediated by four contiguous K homology (KH) domains (Table 1). KSRP also binds to select miRNA precursors and modulates their processing [24]. These multiple functions of KSRP are important in the regulation of different steps of mRNA metabolism and consequently, post-transcriptional regulation of gene expression. KSRP competes with HuR for binding to ARE-containing mRNAs [25]. Because these factors have opposing roles in regulating ARE-containing transcripts and their binding to ARE sequences is mutually exclusive, the steady state levels of transcripts are determined by the ratio of HuR and KSRP-bound to the mRNA pool at any given time [25].

Of crucial importance to myogenesis, KSRP, like HuR, regulates turnover of MyoD, myogenin and p21 mRNAs (Figure 3) [26]. In proliferating myoblasts, KSRP binds to the ARE sequences of these myogenic transcripts and recruits the decay machinery, leading to rapid mRNA decay. Upon myoblast differentiation, phosphorylation of KSRP by the Ser/Thr protein kinase p38 leads to a marked reduction in KSRP binding to MyoD, myogenin and p21 mRNAs and attenuation of its decay-promoting function [26]. Coordinately, the increase in the cytoplasmic levels of HuR, which binds ARE sequences in MyoD, myogenin and p21 mRNAs, leads to stabilization of the transcripts. The interplay of HuR and KSRP in the modulation of MyoD, myogenin and p21 mRNA decay regulates the transition of myoblasts from proliferation to differentiation, promoting the myogenic differentiation program. Further studies are needed to determine whether HuR and KSRP act in concert to modulate transcripts required for other aspects of skeletal muscle physiology besides proliferation and differentiation. Given the expanding knowledge of the importance of miRNAs in skeletal muscle development, growth and regeneration, together with the involvement of KSRP in the processing of miRNAs, further studies on the role of KSRP function in regulating miRNA during myogenesis is a major goal for the future.

Changes in the levels of CUGBP1 contribute to myotonic dystrophy type 1

CUG-binding protein 1 (CUGBP1) is a member of the CELF (CUG-BP- and ETR-3-like factors) family of RNA-binding proteins. CUGBP1 binds, via three RRM domains, to a variety of mRNA cis-elements including UG-rich elements, GC-rich elements, GU-rich elements (GREs) and AREs, and has been implicated in the regulation of mRNA alternative splicing as well as the control of mRNA translation and stability [27, 28].

CUGBP1 regulates mRNA stability and translation of several factors that play key roles in myogenesis (Figure 3). For example, CUGBP1 upregulates translation of the transcript encoding the transcription factor myocyte enhancer factor 2A (MEF2A) via direct interaction with a GC-rich element located within MEF2 mRNA, delaying myogenesis [29]. Similarly, CUGBP1 controls cell cycle progression during myogenesis by regulating the translation of p21 and Cyclin D1 mRNAs. During myoblast proliferation, CUGBP1 binds to and upregulates the translation of Cyclin D1 mRNA, promoting cell proliferation [30]. Conversely, during differentiation, CUGBP1 binds to and upregulates the translation of p21 mRNA, leading to cell cycle withdrawal [30]. Moreover, CUGBP1 binds to AREs in the tumor necrosis factor α (TNF α) mRNA, resulting in transcript destabilization [31]. The role of TNF α in myogenesis is multi-faceted, depending on the duration of expression and the stage of the regenerative process. Prolonged exposure to TNF α , such as during inflammation or aging, is an inducer of muscle wasting [32], and may have inhibitory effects on the transition between early differentiation and terminal differentiation of myoblasts [33]. By contrast, studies in TNF- α null mutant mice and TNF- α receptor mutant mice suggest that TNF- α positively influences both the proliferative and early differentiation stages of muscle

regeneration [33]. With regard to other targets of CUGBP1, high-throughput analysis of mRNA half-life in myoblasts indicated that many labile mRNAs contain GREs. These GRE-containing mRNAs such as Smad7, MyoD and Rnd3 mRNAs, which are all linked with myogenesis [34, 35], are bound and destabilized by CUGBP1 [36].

The function of CUGBP1 is linked to myotonic dystrophy type 1 (DM1), a disease affecting multiple organ systems; major symptoms include skeletal muscle wasting and impaired relaxation (myotonia), cardiac arrhythmia, early-onset cataracts, insulin resistance, hyperinsulinemia and altered central nervous system function [37]. DM1 is caused by the expansion of CTG repeats in the 3' UTR of the dystrophin myotonia-protein kinase (DMPK) gene but the phenotype does not result from altered DMPK function [37]. The phenotype observed in this disease is partially due to sequestration of the splicing factor, MBNL1 (Muscle-blind), via binding to the CUG expansion within the DMPK mRNA; however, the mechanism by which sequestration of MBNL1 leads to DM1 is unknown [37]. The mutant DMPK transcript containing the CUG expansion can also form stable complexes with CUGBP1, which does not occur with wild-type DMPK mRNA. This interaction leads to the stabilization of CUGBP1, possibly explaining the increased levels of this protein observed in DM1 patients [37]. Increased levels of CUGBP1, as well as decreased levels of MBNL1, lead to aberrantly spliced mRNAs encoding muscle-specific chloride channel 1, insulin receptor, and cardiac troponin T, which could underlie the disease symptoms such as myotonia and insulin resistance [37]. In addition, overexpression of CUGBP1 upregulates the translation of several mRNAs important for muscle differentiation, such as p21 and MEF2A, similar to what is observed in DM1 patients, probably leading to dysregulation of normal muscle development [29, 37]. Given the function of CUGBP1 in regulating mRNA stability, increased levels of this protein in DM1 patients could also have deleterious effects through altering the stability of additional mRNA targets. CUGBP1 function may also be altered in other neuromuscular diseases, because it is sequestered in nuclear inclusions in Oculopharyngeal Muscular Dystrophy [38] and Fragile-X-Associated Tremor/Ataxia Syndrome [39], or over-expressed in Spinal Bulbar Muscular Atrophy [40], suggesting a key role for this protein in muscle physiology.

Poly(A)-binding protein PABPN1 is mutated in oculopharyngeal muscular dystrophy

The nuclear poly (A)-binding protein 1 (PABPN1) is a nuclear protein that binds to mRNA and plays an important role in mRNA polyadenylation. PABPN1 binds directly to the mRNA polyadenosine (polyA) tail and this interaction is mediated by an RRM in the central region of the protein (Table 1) [41]. PABPN1 is part of the polyadenylation complex that directly interacts with cleavage and polyadenylation specificity factor (CPSF) and poly(A) polymerase (PAP) [42]. PAP on its own has low affinity for its RNA substrate and acts distributively, dissociating from the RNA after each nucleotide addition [43]. However, upon direct binding, PABPN1 recruits PAP to the mRNA, converting PAP from a distributive to a rapid, processive enzyme thereby enhancing the activity of the polymerase [44]. In addition, PABPN1 controls poly(A) tail length by decreasing PAP elongation activity beyond 250 adenine residues [42]. PABPN1 is predominantly nuclear at steady state, however it shuttles between the nucleus and cytoplasm in an RNA-independent manner [45]. siRNA-mediated depletion of PABPN1 in myoblasts leads to nuclear accumulation of poly(A) mRNA, suggesting an important role for this protein in nuclear export of mature mRNAs [46].

Although PABPN1 is ubiquitously expressed and has basic functions in mRNA metabolism, mutation of the N-terminus that results in expansion of a 10 alanine stretch to 12–17 alanines leads to Oculopharyngeal Muscular Dystrophy (OPMD). This muscular dystrophy

is a late onset, autosomal dominant disease characterized primarily by progressive eyelid drooping (ptosis) and difficulties in swallowing. Additional weakness is noted in other facial muscles and proximal limb [47]. Complications include choking, regurgitation, aspiration and pneumonia. The pathological hallmark of the disease is the presence of nuclear aggregates of PABPN1 in muscle [47].

Why the expansion of the N-terminal polyalanine tract in the ubiquitous PABPN1 protein leads to a muscle-specific disease is currently unknown and, as with other polyalanine diseases, controversy exists over the effects of nuclear aggregates and the mechanism underlying pathology [47]. In autosomal dominant OPMD, one mutant allele of PABPN1 replaces one normal allele of PABPN1. Thus, disease pathology could be due to the loss of one normal allele, the gain of function of the mutant allele or the combination of both events. Furthermore, due to the fact that PABPN1 appears to play an essential role in RNA metabolism, any impairment of its function should, in theory, affect numerous cell and tissue types, but some intrinsic characteristics of skeletal muscle may make this tissue more vulnerable to the effects of alanine-expanded PABPN1. Depletion of PABPN1 in myoblasts causes defects in cell proliferation and differentiation (Figure 3) [46]. These defects are likely due to the observed shortening of poly(A) tails and impairment of mRNA export, which can drastically impact mRNA stability and translation and, consequently, gene expression, altering important cellular events during myogenesis. Further investigation is needed to determine muscle-specific roles of PABPN1 as well as why muscle is particularly susceptible to alanine expansion in PABPN1 in order to provide critical understanding of the pathogenesis of OPMD.

Lin-28 regulates translation of transcripts essential for differentiation

Lin-28 is a small RNA-binding protein with a unique combination of RNA-binding motifs, a cold-shock domain (CSD) and two CCHC zinc finger motifs (Table 1). Lin-28, which is predominantly cytoplasmic and found associated with mRNPs, polysomes, P-bodies and stress granules [48, 49], is widely expressed in embryonic tissues and stem cells. Lin-28 plays a central role in the generation and/or maintenance of stem cells, making this factor a marker of “stemness” [50–52]. Lin-28 inhibits the processing of let-7 microRNAs, which have been implicated in regulation of cell growth and differentiation in embryonic cells [53]. In contrast, Lin-28 is down-regulated during cell differentiation in most mammalian models, but its expression is maintained in adult cardiac and skeletal muscles [54], suggesting a specific function of Lin-28 in these tissues. The expression of Lin-28 is up-regulated during *in vitro* myoblast differentiation and *in vivo* skeletal muscle regeneration [55]. In addition, myoblast differentiation is increased in cells overexpressing Lin-28 [55]. During myoblast differentiation *in vitro*, Lin-28 binds several mRNAs involved in myogenesis, including MyoD and insulin-like growth factor 2 (IGF-2), a factor indispensable for efficient muscle differentiation both *in vitro* and *in vivo* (Figure 3) [56, 57]. Lin-28 associates with polysomes and upon binding to IGF-2 mRNA enhances the translation of this transcript in cultured myoblasts [55]. Although the mechanism of mRNA recognition by Lin28 is currently unknown, Lin-28 appears to regulate gene expression either directly, by binding to target mRNAs to modulate translation, or indirectly by regulating miRNA processing. Further studies are needed to shed light on how Lin-28 binds to transcripts and functions in regulating gene expression. Given the role of Lin-28 in other stem cell populations, the identification of Lin-28 targets may point to other roles for this protein in satellite cell quiescence, proliferation or self-renewal during myogenesis which could be exploited therapeutically for modulating muscle repair in disease or aging.

Satellite cells in regenerating muscle upregulate the expression of TTP

Tristetraprolin (TTP) is a zinc-finger protein member of the TIS11 (TPA-inducible sequence 11) family of RNA-binding proteins (Table 1). TTP binds directly to ARE sequences via two CCCH zinc-finger domains and triggers rapid mRNA decay by recruiting enzymes involved in mRNA turnover [58–60]. TTP was originally believed to bind only to a subset of AREs, mostly present in transcripts encoding immune and inflammatory mediators such as TNF α , granulocyte/macrophage colony-stimulating factor, interleukin 2 and immediate early response 3 [61–63]; however, genome-wide analysis has identified 250 putative target mRNAs that are stabilized in TTP null mouse embryonic fibroblasts [63]. Furthermore, RNA-immunoprecipitation revealed that TTP associates with more than 100 mRNAs [64], suggesting that TTP may control a broader set of transcripts than originally thought. Like KSRP, TTP activity is also modulated by phosphorylation; however, upon phosphorylation, TTP remains bound to AREs although it loses its ability to trigger deadenylation of tethered mRNA by preventing the recruitment of cytoplasmic deadenylases and leading to mRNA stabilization [65–67]. The fact that TTP remains associated with its target mRNA upon phosphorylation indicates that TTP may be poised to rapidly reactivate mRNA decay when dephosphorylated, or TTP may act by protecting the mRNA from decay triggered by preventing other RNA-binding protein from binding to the ARE [67].

TTP is acutely induced within 30 minutes following skeletal muscle injury and is expressed in a spatial pattern consistent with activated satellite cells [68], suggesting this protein may function in muscle repair (Figure 3). Early induction of TTP after muscle injury likely regulates TNF α expression, but TTP may also regulate the stability of other as yet unidentified ARE-containing transcripts important for satellite cell function at early stages of muscle repair. Identification of such new TTP-regulated transcripts could provide greater insight into regulatory mechanisms controlling satellite cell function.

Concluding remarks

As this review has highlighted, post-transcriptional regulation of gene expression by RNA-binding proteins is an integral part of myogenesis. However, compared to other fields such as inflammation and cancer, research on RNA-binding proteins in skeletal muscle biology is still in its infancy. The most studied role for RNA-binding proteins in skeletal muscle is in regulating the expression of key myogenic regulators such as the transcription factors myoD and myogenin, and the cell cycle regulator p21, which are essential for the differentiation process. Defining other mRNA targets for RNA-binding proteins will be critical for expanding our knowledge of regulatory mechanisms in myogenesis. To date, functions for RNA-binding proteins in myogenesis have been predominantly identified in myoblast proliferation and differentiation. However, this bias likely reflects the limitations inherent to cell culture assays in recapitulating *in vivo* aspects of skeletal muscle biology. Emphasis should be directed towards identifying functions for the RNA-binding proteins discussed in this review during myogenesis *in vivo*. Furthermore, given the large number of RNA-binding proteins that are encoded in the genome, other RNA-binding proteins in addition to the ones discussed in this review are likely to have key roles in multiple stages of myogenesis. Determining how multiple RNA-binding proteins modulate myogenic transcripts will provide an integrative understanding of the regulatory mechanisms controlling gene expression in myogenesis. Furthermore, little work has been done on the role of RNA-binding proteins in muscle physiology *in vivo* during development, regeneration, aging or disease. Similarly, little work has been performed on the role of these proteins in satellite cells or other stem cell populations in skeletal muscle. Given the importance of these tissue-specific stem cells for muscle regeneration and growth in the adult, further research on RNA-binding proteins in satellite cells will delineate critical

regulatory pathways necessary for stem cell function. Finally, understanding the role of specific RNA-binding proteins in skeletal muscle will provide insights into the etiology and pathology of muscle diseases such as OPMD and DM1. Thus, modulation of post-transcriptional activity through RNA-binding proteins will open new avenues for therapeutic approaches to the treatment of muscle disease and aging.

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Glossary

MRFs (Myogenic Regulatory Factors)	a family of transcription factors consisting of Myf-5, MyoD, myogenin, and MRF4 that plays key regulatory roles in the development of skeletal muscle during myogenesis
Myoblast	a proliferating progenitor cell in muscle tissue that differentiates and fuse to form multinucleated, post-mitotic myofibers
mRNPs (mRNA-bound ribonucleoprotein complexes)	complex composed of fully processed mRNA and an array of RNA-binding proteins that mediate the post-transcriptional events of gene expression
AREs (U and AU-rich elements)	mRNA elements containing repeats of AUUUA or UUAUUUAUU which range in length from 50 to 150 nucleotides. AREs are functionally involved in mediating mRNA decay
GREs (GU-rich elements)	mRNA elements determined by the conserved 11-mer sequence UGUUUGUUUGU, which are functionally involved in the mediating mRNA decay

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Figure 1. Main regulatory steps during myogenesis in skeletal muscle

Myogenesis occurs during embryonic development and adult muscle regeneration. Schematic shows the main cellular transitions that occur during myogenesis and the key regulatory proteins (right) that control these transitions. In the embryo, precursor cells in the somites become determined for the myogenic lineage and proliferate to give rise to myoblasts. The transcription factors MyoD and Myf5 are critical for determination. In the adult, precursor cells called satellite cells begin to proliferate in response to injury and give rise to myoblasts. MyoD and Myf5 also regulate the proliferation of myoblasts in both embryos and adults. During later stages of myogenesis, myoblasts differentiate, withdraw from the cell cycle and fuse to form myofibers which grow in size. Transcription factors such as myogenin, MEF2 and MRF4 and the cell cycle inhibitor p21 play key roles during these later stages of myogenesis.

Figure 2. Post-transcriptional control of RNA metabolism occurs at multiple steps

Post-transcriptional regulation can occur throughout the RNA life cycle, from processing and nuclear export to translation and ultimately degradation. These processes are regulated by the dynamic association of RNA-binding proteins (RBPs, showed in colored shapes) with mRNA, which define a transcript's lifetime, cellular localization, editing, polyadenylation and rates of translation and decay, ultimately determining the levels of protein produced.

Figure 3. Roles of selected RNA-binding proteins in myogenesis

Shown schematically are the RNA-binding proteins discussed in this review together with their respective mRNA targets in skeletal muscle. For each RNA-binding protein, the cis-element within the mRNA to which it binds is shown. These RNA-binding proteins mainly regulate proliferation or differentiation of myoblasts. See text for details.

Table 1

The best characterized RNA-binding proteins involved in myogenesis.

RPB	RNA-binding Domain	Cis- Elements	Effect on Transcripts
CUGBP1		UG-rich ARE, GRE GC-rich	alternative splicing destabilization translation enhancing
HuR		ARE	stabilization
KSRP		ARE	destabilization
Lin-28		n/d	translation enhancing
PABPN1		poly(A)	poly(A) length control nuclear export
TTP		ARE	destabilization

Abbreviations: RRM - RNA recognition motif; KH - K homology domain; CSD - cold-shock domain; ZF – zinc finger domain, ARE – AU-rich element; GRE - GU-rich element; poly(A) – polyadenosine; n/d – not determined.