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Multiple roles of RBM4 in muscle cell differentiation

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1. ABSTRACT

Muscle cell differentiation involves gene regulation at both transcriptional and post-transcriptional steps. Post-transcriptional control requires various RNA binding proteins. The multi-functional RNA binding motif 4 (RBM4) protein participates in both precursor mRNA splicing regulation and translational control in muscle cells. In myoblasts, RBM4 promotes the expression of many muscle-specific mRNAs from individual genes via its activity in modulating alternative splicing. In addition, RBM4 transiently translocates to the cytoplasm during myogenesis, where it participates in translation control. RBM4 may act in conjunction with a subset of muscle-specific microRNAs to modulate their activity in translation suppression. Overexpression of RBM4 promotes muscle cell differentiation, suggesting that RBM4 acts as a positive myogenic regulator. In this review, we discuss recent data regarding how RBM4 may foster muscle cell differentiation.

2. POST-TRANSCRIPTIONAL REGULATION

Cell type-specific posttranscriptional events in multicellular organisms, including alternative precursor mRNA splicing, mRNA transport/storage/degradation, and translational control, provide an extra degree of complexity to gene expression (1-3). Post-transcriptional gene regulation may impact the expression, abundance or subcellular localization of isoforms encoded by regulated transcripts, and thus determines cell specification and function (4). The above processes are facilitated by RNA binding proteins (RBPs) that exhibit restricted expression or activity in specific cells or stages during development. Moreover, each RBP may play multiple roles in different steps of the post-transcriptional regulation and thereby provide more accurate and efficient control of target gene expression under certain circumstances (5). Therefore, cell type-specific RBPs may increase the genome coding capacity at the post-transcriptional level to achieve a diversity of cell or tissue types and specify their physiological function.

Cis-regulatory elements that are widely distributed over the entire length of many primary transcripts also play important roles in determining the fate of the transcripts in the post-transcriptional process (4). These elements modulate nuclear processing of precursor mRNAs, including splicing and polyadenylation. After splicing, mature mRNAs are exported to the cytoplasm where they undergo a quality control step (6, 7). Subsequently, mature/competent mRNAs are subjected to immediate translation or may be translocated to specific subcellular compartments for regulated translation (6, 8). Moreover, the half-life of some mRNAs may be influenced by cellular signals (9, 10). These cytoplasmic events are largely directed by *cis*-regulatory elements within the untranslated regions of the mRNAs. Conceivably, variation in *cis*-element sequences may have an impact on the fate of the transcripts and consequently alter the protein expression pattern. The interplay between *cis*-regulatory elements and RBPs is a major determining factor in protein expression control. The issue of how this interplay achieves cell type-specific post-transcriptional regulation, particularly in response to the changes in cellular environment, remains to be explored.

In this review, we discuss the multiple roles of the RNA binding protein RBM4, the mammalian homolog of *Drosophila* Lark, in post-transcriptional regulation. Insights from recent studies help to illuminate the physiological function of RBM4 in muscle differentiation.

3. AN OVERVIEW OF MUSCLE DEVELOPMENT

In vertebrates, development of somitic muscles involves highly ordered, temporally distinct events, the control of which is quite complex (11, 12). Initially, mesodermal precursor cells are committed to diverse myogenic lineages followed by cell differentiation and morphogenesis to form different muscle types. External signals specific to myogenic differentiation are transmitted to pluripotent mesodermal cells to induce the expression of the myogenic determination factors MyoD and Myf5 (12-14). Activation of these two master regulators leads to the withdrawal of myoblasts from the cell cycle and triggers a transcriptional cascade involving downstream myogenic regulatory factors such as myogenin and the MEF family members that subsequently activate expression of many structural genes for myoblast differentiation and myotube formation. For skeletal myogenesis, differentiated myocytes then fuse with each other to form multinucleated myotubes and ultimately mature to contractile muscle fibers that express late-stage proteins of mature myocytes, such as myosin heavy chain and muscle creatine kinase and exhibit characteristics muscle tissue (15). Moreover, there exists a distinct population of myoblasts, termed satellite cells, that fails to differentiate into myocytes during myogenesis but associates rather with differentiated myotubes. These myocyte-adhered satellite cells may differentiate into functional myotubes and thus can facilitate regeneration of damaged-musculature caused by trauma (16, 17). To date, two cellular signaling pathways, namely the p38 MAPK and PI3K/AKT pathways, have been implicated in the control of muscle cell differentiation and regeneration (18-20). These two pathways have different targets but may also converge on some common targets involved in myogenesis. Nevertheless, the details of how extracellular cues control myogenesis remain unclear.

4. POST-TRANSCRIPTIONAL REGULATION OF MYOGENESIS

Beyond transcriptional control, post-transcriptional gene regulation also plays an important role in myogenesis; the most important steps include alternative mRNA splicing, mRNA turnover and translational control.

4.1. Alternative mRNA splicing

Alternative splicing provides a powerful mechanism to generate functionally distinct proteins from one gene as well as regulate mRNA expression level at a post-transcriptional step. Alternative splicing events prevail in brain, testis, spleen, skeletal muscle and heart (21). In muscles, numerous genes that are essential for muscle development produce multiple isoforms via alternative splicing (22, 23). For example, each of the troponin T genes can generate several distinct mRNA isoforms by alternative use of exons in different muscle types or at different stages of embryonic development (24, 25). The resulting troponin isoforms confer differential sensitivity of myofilaments to calcium or have altered ability to inhibit the actomyosin ATPase activity. Therefore, alternative splicing of troponin T mRNAs may directly affect the contractile properties of the myofibrils and thus myocardial contraction (25).

Alternative splicing is determined by the interplay between *trans*-acting splicing factors and *cis*-elements of the regulated transcripts (26, 27). Despite numerous studies, there is still limited information about the underlying mechanisms of alternative splicing regulation. Recent genome-wide discovery of *cis*-regulatory motifs by deep mRNA sequencing and exon profiling has begun to offer a more global view of tissue-specific regulatory programs (28-30). However, such approaches have mainly focused on a few splicing factors such as polypyrimidine tract-binding protein (PTB), Nova and Foxl/2. Therefore, the details of how a large repertoire of splicing factors is orchestrated to regulate alternative splicing warrants further investigation.

4.2. mRNA stability

mRNA stability control modulates the expression of several critical factors that regulate muscle development and function. As myogenesis is initiated, the RNA-recognition motif-containing RNA-binding protein HuR accumulates in the cytoplasm and stabilizes MyoD and myogenin mRNAs via the AU-rich element in each of their 3' untranslated regions (3' UTRs) (31, 32). These mRNAs are also stabilized by the double-stranded RNA binding protein NF90 (33). Cell cycle withdrawal accompanies myocyte differentiation. Coincidently, the stability of the cell cycle inhibitor p21 mRNA is also regulated by HuR and NF90 (31, 32). Muscle cell differentiation also involves two competitive RNA degradation pathways, namely Staufen1-mediated decay

(SMD) and nonsense-mediated mRNA (NMD) (34). SMD regulates the stability of Pax3 mRNA (34). Pax3 is a critical transcriptional factor in undifferentiated muscle progenitor cells and functions to promote myoblast proliferation. Down-regulation of Pax3 level allows differentiation to proceed. It is noteworthy that myogenin mRNA is a target of NMD, and SMD outcompetes NMD for the Upf1, which is the central effector of NMD and also participates in SMD. Therefore, the increase of Staufen1 protein as well as SMD activity during myogenic differentiation may possibly down-regulate Pax3 expression but stabilize myogenin mRNA, although contradictory arguments remain (34-36).

4.3. Translation control

Various signaling pathways, such as those involving the p38 kinase or mammalian target of rapamycin (mTOR), regulate translation activity of proliferating and differentiating muscle cells via modulating translation factors, but details of the underlying mechanisms have not been very clear. Besides translation factors, RNA binding proteins also participate in translational control. CUGBP1 enhances the translation of the mRNA encoding the cell cycle regulator p21 via binding to the GC-rich sequences in its 5' UTR (Iakova et al., 2004). Thus, a higher level of CUGBP1 in myotonic dystrophy type 1 patients may account for the delay of skeletal muscle differentiation (37). Recent studies have implicated miRNA-mediated translational control in myogenic differentiation (14, 38, 39). Expression of a set of miRNAs, such as miR-1 and related miR-206, is particularly induced by the myogenic regulatory factors during myogenesis. In addition, some miRNAs, although not muscle specific, may also participate in regulation of muscle cell differentiation or function (40-42). It is conceivable that miRNAs may fine-tune the expression of their target mRNAs, which encode transcription factors that function in different stages of muscle cell differentiation, and hence affect the differentiation process. Indeed, ablation of the miRNA processing factor Dicer in the myogenic compartment reduces skeletal muscle mass and induces abnormal muscle morphogenesis during embryogenesis, indicating the critical role of miRNAs in skeletal muscle development (43)

It is clear that the balance between proliferation and differentiation of muscle progenitors and myogenic differentiation and maturation involve multi-layer control of gene expression. Moreover, different control mechanisms likely act in concert to reach the precise level and timing of expression of a particular gene. Although much has been learned, the mechanisms underlying post-transcriptional regulation of myogenesis must be further clarified.

5. THE ROLES OF RBM4 IN MUSCLE CELL DIFFERENTIATION

5.1. Basic characteristics of RBM4

The RNA binding motif 4 (RBM4) proteins are homologs of the *Drosophila Lark* gene product. *Drosophila Lark* is essential for development and has been implicated in regulation of the circadian rhythm (44). In the human genome, two copies of closely related RBM4 genes, designated *RBM4a* and *RBM4b*, are located closely on chromosome 11q13.2. Our previous study showed that expression of both RBM4a and RBM4b mRNAs was ubiquitous in human tissues but was relatively higher in brain, heart, skeletal muscle and testis (45). By using antibodies specific for RBM4a and RBM4b, RBM4a was shown to be highly expressed in liver as well as in several other tissues including skeletal muscles and heart; by contrast, RBM4b was detected only in liver (46). Nevertheless, whether RBM4 proteins play any specific role in these tissues remains unclear and must be addressed by studies with RBM4 knockout mice.

RBM4 proteins of all species contain two RNA recognition motifs at the N-terminus, followed by a CCHC-type zinc knuckle. The C-terminal region of RBM4s is less conserved across species, however. Mammalian RBM4 proteins contain several alanine-rich stretches whereas *Drosophila* Lark contains proline-rich sequences as well as many arginine/serine dipeptides. We have previously demonstrated that the C-terminal domain of human RBM4 is crucial for its nuclear entry and localization in splicing factor-enriched nuclear speckles and also contributes to RBM4's activity in alternative splicing control (47). Moreover, the RBM4 protein continuously shuttles between the nucleus and cytoplasm, and its nucleocytoplasmic transport and subcellular localization are probably controlled by cellular signaling cascades (48). RBM4 is phosphorylated under cell stress conditions and at the onset of muscle cell differentiation (48). Phosphorylated RBM4 accumulates in the cytoplasm and moreover localizes to cytoplasmic stress granules and microRNP (miRNP)-containing granules (48). The MKK/p38 kinase pathway is responsible for stress-induced phosphorylation and cytoplasmic accumulation of RBM4 (48). Indeed, localization of RBM4 to such granules agrees well with the activity of RBM4 in translational control (see below for detail).

5.2. Role of RBM4 in muscle cell-specific splicing

Using alternative splicing reporters, we initially found that RBM4 may not be essential for splicing but rather can modulate alternative splice site selection (47). In this regard, RBM4 and the serine/arginine-rich protein ASF/SF2 (SRSF1) act oppositely. For example, RBM4 overexpression favors distal 5' splice site utilization of the adenovirus E1a transcript, whereas ASF/SF2 activates the proximal 5' splice site. Immunoprecipitation of RBM4 followed by differential display analysis revealed that the α-tropomyosin transcript is a potential target of RBM4 (45). Indeed, RBM4 overexpression enhances utilization of skeletal muscle-specific exon 2b and terminal exons 9a/9b, whereas knockdown of RBM4 induces exon skipping. A minigene reporter analysis revealed that RBM4 activates exon 9a inclusion via binding to multiple intronic CU-rich sequences immediately downstream of exon 9a. Moreover, by binding to these *cis*-elements, RBM4 could antagonize the suppressive effect of PTB in exon 9a/b inclusion (45) (Figure 1). Besides, RBM4 overexpression could induce exon inclusion in the transcripts of *MAPT/Tau* and *SMN2*, both of which are implicated in hereditary

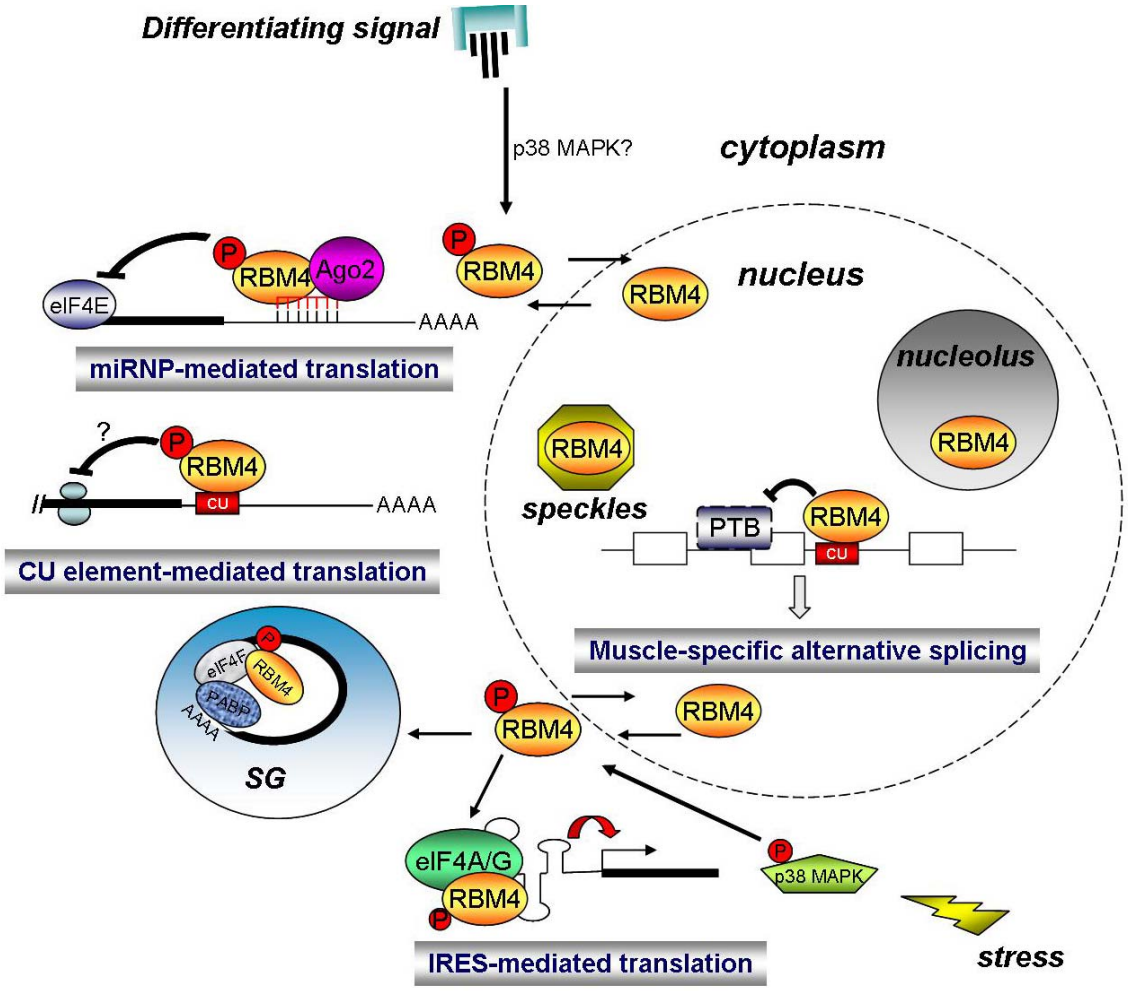

Figure 1. Model for RBM4 function in alternative splicing and translational control during muscle cell differentiation. In the nucleus, RBM4 has a fundamental activity in regulating alternative splicing. Via binding to exonic or intronic CU-rich elements, RBM4 modulates alternative splice site selection. Current data suggest that RBM4 regulates exon selection of muscle cell transcripts. In general, RBM4 antagonizes the activity of PTB and thereby promotes expression of myocyte-specific isoforms. Further, RBM4 transiently localizes to nucleoli during myogenesis, but the functional significance is unclear. Meanwhile, RBM4 is phosphorylated and translocates to the cytoplasm perhaps upon activation of the p38 MAPK kinase pathway in response to differentiation signals. In muscle cells, RBM4 can potentiate the activity of certain muscle-specific miRNAs in translation suppression via its interaction with Ago2 (miRNP-mediated translation). For example, RBM4 down-regulates cyclin D1 mRNA translation via collaboration with miRNA-1 in C2C12 myoblasts. Moreover, RBM4 can inhibit translation of CU-rich element-containing mRNAs (bulk translation) and activates translation of certain IRES-containing mRNAs (IRES-mediated translation) by recruiting Ago2 and eIF4A/eIF4G, respectively. Note that RBM4-induced IRES translation has been observed in stressed cells but has not yet been confirmed in muscle cells. In regards to translation control, the mRNA targets of RBM4 need to be identified by future experiments.

neurodegenerative disorders (49, 50). The activity of RBM4 in promoting SMN2 exon 7 inclusion could be abrogated by an isoform of the Wilms' tumor suppressor WT1, which functions as a splicing factor, but its underlying mechanism is unclear (50). These findings have provided hints for the role of RBM4 in modulating alternative splicing in muscles and neurons.

Our recent report provides a more comprehensive picture of the interplay between RBM4 and PTB in programming skeletal muscle-specific alternative splicing (51). Using a computational search for potential substrates of RBM4 in muscle cells, we identified a set of transcripts that undergo muscle cell-specific alternative splicing and also contain CU-rich elements around the regulated exons. The majority of these transcripts are indeed targets of PTB probably because PTB, like RBM4, preferentially binds to CU-rich sequences (52). It has been shown that PTB can negatively autoregulate its own expression via alternative splicing-coupled NMD regulation. PTB overexpression promotes exon 11 skipping in its own transcripts, leading to NMD of the resulting

mRNA products by NMD (53, 54). RBM4 overexpression also has the same effect as PTB for alternative splicing and expression level regulation of PTB via binding to CU-rich elements within and adjacent to exon 11 of PTB (51). Note that RBM4 is expressed in both undifferentiated and differentiated mouse C2C12 muscle cells, whereas PTB expression is gradually suppressed as differentiation proceeds. Our data indicate that RBM4 contributes at least in part to the reduction of PTB expression levels during C2C12 cell differentiation. In addition to PTB transcripts, RBM4 modulates alternative exon selection in the transcripts encoding critical myogenic factors such as MEF2c and myocargin, cytoskeletal proteins such as α-topomyosin, α-actinin, troponin T and vinculin, and also regulators of muscle cell function such as insulin receptor and ryanodine receptor. Although RBM4 can act as either an activator or repressor in alternative exon selection, it constantly induces the expression of skeletal muscle-specific isoforms, which is consistent with its potential role in promoting muscle cell differentiation (51). However, it appears that PTB acts oppositely to RBM4 in modulating alternative splicing of the transcripts that are expressed in fully differentiated myocytes (Lin and Tarn, 2010). Nevertheless, RBM4 down-regulates PTB expression and antagonizes PTB activity in alternative splicing regulation, suggesting a hierarchical role for RBM4 in promoting muscle cell differentiation via a splicing cascade.

Numerous studies have unveiled the molecular mechanisms involved in the regulation of splicing, but our knowledge in this regard is still rudimentary. Nevertheless, recent systems analysis of alternative splicing has provided a more complete description of how alternative exon selection is dictated by locations of *cis*-elements and modulated by different combinations of RNA binding proteins (27-30). For example, the most extensively characterized PTB binding motifs often are located upstream of repressed exons but downstream of activated exons (55, 56). From a limited list of known RBM4 targets, we have deduced a model of RBM4-medated splicing regulation, which is similar to that of PTB. While binding immediately downstream of regulated exons, RBM4 may promote exon inclusion. Perhaps RBM4 enhances the efficiency of 5' splice site utilization via its association with the U1 snRNP protein U1C (57). However, when RBM4 binding sites appear in regulated exons as well as upstream or downstream introns, RBM4 may induce exon skipping. Because RBM4 can self-interact (47), it may behave similarly to PTB in either looping out the suppressed exon or forming a repressive zone across such an exon upon dimerization or even multimerization. Moreover, the activity of splicing regulatory factors can be modified under different cellular conditions. For example, PTB, while forming a complex with KSRP and hnRNP H and F proteins, can repress c-src N1 exon inclusion in neurons (58). It is also plausible that the activity of RBM4 in splicing regulation is affected by its associated factors. Thus, more detailed information of RBM4-mediated splicing regulation mechanisms will be gleaned from future identification of RBM4 targets and biochemical investigation.

Results to date suggest an important role for RBM4 in modulating alternative splicing of muscle cell-specific or neuron-specific transcripts. In particular, RBM4 modulates the expression of two critical splicing factors, PTB and its neuronal analog nPTB (51), suggesting its pivotal role in alternative splicing programming during differentiation or modulating cellular functions.

5.3. Role of RBM4 in translational control

Using reporter assays, we found that RBM4 can suppress translation of mRNAs containing CU-rich elements in the 3' UTR (). Recombinant RBM4 could also reduce the translation of CU-rich element-containing mRNAs *in vitro* (59). We postulate that when RBM4 is exported to the cytoplasm, it functions to suppress the translation of mRNAs containing its responsive elements. Recently, two independent studies revealed that RBM4 is an interacting partner of Argonaute2 (Ago2), the functional effector of miRNPs. By identifying the components of Ago2-containing complexes, Hock *et al.* found that RBM4, together with several other RNA binding proteins, is associated with active Ago2/mRNP complexes (60). While studying RBM4 in mouse myoblasts, we found that RBM4 translocates to the cytoplasm and colocalizes with Ago2 in cytoplasmic granules (51). We demonstrated that RBM4 indeed directly interacts with Ago2, and that Ago2 knockdown compromises the ability of RBM4 to suppress translation (59). Therefore, RBM4 may recruit Ago2 for translation suppression. However, the details of how Ago2 participates in RBM4-mediated translation inhibition require further study.

The interaction between RBM4 and Ago2 also suggests the possible involvement of RBM4 in miRNA-guided gene silencing. The first evidence came from the observation that RBM4 knockdown increased luciferase activity of an miRNA-responsive reporter vector, as compared to the control, (60). Our group found that RBM4 selectively bound to muscle-specific miR-1 and miR-206 but not miR133 during myoblast differentiation (59). However, the possibility still remains that the association of RBM4 with miRNAs, whose target sites are relatively CU-rich (e.g. miR-1/206) is in part mediated by the target mRNAs. Nevertheless, using artificial miR-1 response elements or an miR-1 site-containing cyclin D1 3' UTR as a reporter, we observed that RBM4 overexpression potentiated the suppressive effect of miR-1 in translation by promoting Ago2 association with target mRNAs (59). Accordingly, knockdown of RBM4 compromised the effect of miR-1, which further supports the role of RBM4 in potentiating the activity of miRNAs. It is now widely accepted that the activity of miRNPs can be modulated by various associated RNA binding proteins (61, 62). Therefore, the underlying mechanism and biological significance of RBM4/miRNA-mediated translation regulation, particularly during myogenesis, remain to be demonstrated.

Environmental stress activates the p38 MAP kinase pathway, which leads to phosphorylation of RBM4 and triggers cytoplasmic accumulation of RBM4 (48). We previously reported that, during cell stress, RBM4 could associate with the encephalomyocarditis virus internal ribosomal entry site (IRES) and several cellular IRES elements such as those in the Bcl2 and c-myc 5' UTRs. RBM4 could robustly activate encephalomyocarditis virus IRES-mediated translation by recruiting the translation initiation factors eIF4A/eIF4G to IRESs. The p38 kinase cascade, which also plays an important role in myogenesis, can induce phosphorylation of RBM4 during muscle cell differentiation (59). Notably, expression of several IRES-containing mRNAs, for example fibroblast growth factor 1, insulin-like growth factor II and utrophin A, is important for proper myoblast differentiation and myotube formation and physiology. Therefore, the possibility that RBM4-mediated IRES translation takes place during myogenesis remains interesting and testable.

RBM4 represents a multi-faceted regulator of translational control. It can activate or suppress translation via interaction with different translation factors or regulatory machineries. However, the identities of the target mRNAs of RBM4-mediated translation control are yet unclear. Moreover, many other important questions remain to be answered, such as how cellular signaling pathways dictate RBM4 function in translation control, and how RBM4 can exhibit opposite regulatory activities in translation.

5.4. Potential function of RBM4 in muscle differentiation

In light of higher expression levels of RBM4 in muscle and heart, we hypothesized that RBM4 confers specific biological functions in these tissues. Past studies have clearly indicated that RBM4 participates in alternative splicing regulation and translational control, but its biological function remains largely uncharacterized. Our recent studies have shown that the cellular level of RBM4 is essentially constant throughout myoblast differentiation, and that it downregulates PTB and counteracts the effect of PTB on a set of muscle cell-expressed transcripts, particularly those encode late myocyte proteins (51). Therefore, an increase in the molar ratio of RBM4/PTB may multiply the effect of RBM4 in promoting the expression of skeletal muscle-specific isoforms. Accordingly, we observed that RBM4 overexpression induces morphological changes in C2C12 myoblasts towards a myocyte phenotype, supporting the role of RBM4 as an activator of muscle differentiation.

Most splicing factors are encoded by single-copy genes; as such, complete knockout of a specific splicing factor gene often results in embryonic lethality (63). Unlike those splicing factors, RBM4 has two copies in vertebrate genomes. To reveal the physiological role of RBM4, we recently generated homozygous mutant mice, of which either *RBM4a* or *RBM4b* was deleted. Although the resulting knockout mice survived without apparent developmental defects, the splicing patterns of many potential RBM4 targets were profoundly altered in embryonic fibroblasts or adult muscles (Lin and Tarn, unpublished data). For example, RBM4 knockout induced exon 11 skipping of insulin receptor transcripts and therefore promoted the expression of alternatively spliced mRNAs encoding insulin resistant insulin receptor (unpublished data). This finding coincided with a previous result that RBM4 overexpression enhanced the inclusion of insulin receptor exon 11 in cultured mouse myoblasts (51). Interestingly, the shift in the insulin receptor isoform expression in RBM4 knockout mice is similar to that observed in models of myotonic dystrophy type 1 (Lin and Tarn, unpublished data). Therefore, we hypothesize that RBM4 may modulate muscle activity or play a role in muscle regeneration in adult skeletal muscle.

6. CONCLUSION AND PERSPECTIVES

Muscle development requires the balance between self-renewal and differentiation of myogenic progenitors. It is clear that the control and fine-tuning of the differentiation process involve not only transcriptional regulation but also various post-transcriptional regulation events. RBM4 modulates the expression level of PTB/nPTB in part via alternative splicing-coupled NMD and promotes expression of certain muscle cell-specific alternatively spliced isoforms, such as insulin receptor and α-tropomyosin (51). Therefore, RBM4 likely plays a hierarchical role in muscle cell differentiation via alternative splicing control. A similar but not identical scenario for splicing cascade control has been observed with differential PTB/nPTB expression in different cell types. However, evidence so far reveals that these two splicing regulator analogs exhibit largely overlapping activities as repressors of alternatively expressed exons (54, 64). Therefore, substitution of a factor such as PTB with its functional analogs at specific times or in specific tissues may not substantially impact the expression of alternatively spliced mRNAs, although it is possible that other steps of mRNA metabolism may be affected. Nevertheless, our data that RBM4 can amplify its effect on expression of alternatively spliced muscle cell mRNAs by downregulating its antagonistic factors provides a novel example of hierarchical splicing control (51). Moreover, RBM4 plays a multifaceted role in translation control. The association of RBM4 with certain muscle-specific miRNAs suggests that RBM4 can modulate translation during muscle cell differentiation (59). Perhaps RBM4 can differentially control the translation of distinct categories of mRNAs in muscle cells, such as IRES-containing mRNAs or mRNAs harboring CU-rich elements or miRNA-targeting sites in their 3' UTR. In this regard, systematic identification of RBM4 target mRNAs or analysis of the proteomic profile of RBM4-knockdown cells is required for furthering understanding of the role of RBM4 in translation control.

Finally, investigation through transgenic or knockout mouse models would benefit our understanding of the biological function of RBM4. Our preliminary data that single-copy RBM4 knockdown has no apparent effect on survival but affects alternative splicing of some muscle cell transcripts (unpublished data) raises a number of

questions such as whether RBM4 is dispensable for development and whether RBM4 indeed exerts any biological function in muscle. Therefore, conditional knockout of *RBM4a* and/or *RBM4b* would be necessary to distinguish the role(s) of the individual RBM4 genes in animals.

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