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Review

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Alternative splicing orchestration in skeletal muscle: from developmental programming to therapeutic targets in muscle disorders

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Abstract: Alternative RNA splicing represents a fundamental post-transcriptional regulatory mechanism that generates diverse mRNA transcripts from single genes, thereby expanding protein diversity beyond genomic constraints. In mammalian systems, over 95% of multi-exon genes undergo alternative splicing, with skeletal muscle exhibiting one of the highest frequencies of tissue-specific splicing events. This sophisticated regulatory process governs critical aspects of skeletal muscle development, regeneration, and functional maintenance. Dysregulation of alternative splicing pathways is intimately linked to devastating muscle disorders, including Duchenne muscular dystrophy (DMD), myotonic dystrophy (DM), and sarcopenia. This comprehensive review synthesizes current understanding of alternative splicing mechanisms, examines their pivotal roles in muscle growth and regeneration, and explores emerging therapeutic strategies that target splicing abnormalities in muscle disease.

Key words: Alternative RNA splicing; skeletal muscle; myogenesis; muscle regeneration; muscle diseases.

1 Introduction

Alternative splicing is a fundamental post-transcriptional regulatory mechanism that massively expands transcriptome and proteome diversity, and plays an indispensable role in skeletal muscle development, homeostasis, and disease(Howard et al., 2013; Reddy et al., 2013). The human genome contains approximately 19,000 protein-coding genes, yet alternative splicing generates an estimated 100,000-200,000 distinct protein isoforms. This mechanism enables a single gene to encode multiple protein isoforms, significantly increasing the diversity and functional complexity of the proteome. Abnormal alternative splicing can modify or eliminate protein function, leading to various human diseases such as cancer(Bradley and Anczuków, 2023), cardiovascular disease(Cao et al., 2024), diabetes(Zhang et al., 2024), myasthenia gravis(Suzuki et al., 2008), neurological disorders, and immune system diseases(Jin et al., 2018). The remarkable expansion of coding potential is especially pronounced in skeletal muscle, where tissue-specific splicing events contribute to the specialized functions of different muscle-fiber types and the dynamic processes of muscle development and regeneration(Merkin et al., 2012; Tapial et al., 2017). Recent studies have shown that transcriptional regulation and post-transcriptional RNA alternative splicing regulation are equally important for skeletal muscle growth and development, and that abnormal RNA alternative splicing can lead to muscle-development

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disorders(Grifone et al., 2021; Hu et al., 2023; Falcucci et al., 2025; Xu et al., 2025). Understanding these mechanisms not only illuminates fundamental principles of muscle biology but also provides crucial insights into therapeutic strategies for muscle disorders.

2 Molecular Mechanisms of Alternative Splicing

The main types of RNA alternative splicing include (1) retained intron (RI), which refers to the retention of intron regions in mature mRNA; (2) exon skipping (ES), which refers to the excision of an exon from the initial transcript; (3) mutually exclusive exons (MXE), where only a single exon is retained in the mature mRNA among multiple adjacent exons; (4) alternative 5' splice site (A5SS), which involves selective usage of 5' splice sites; and (5) alternative 3' splice site (A3SS), which features selective usage of 3' splice sites (Fig. 1a). These transcript variants of the same gene have distinct functional roles and may even exert opposing biological functions (Abaji et al., 2022; Chang et al., 2024). Thus, alternative splicing is a key mechanism driving the functional and regulatory complexity of cellular processes.

Alternative splicing is precisely controlled by a highly coordinated regulatory system consisting of cis-acting elements and trans-acting splicing factors, which jointly determine splice-site selection and splicing outcome(Nikonova et al., 2019). Cis-acting elements are regulatory sequences located within pre-mRNAs that modulate the recognition of splice sites by trans-acting splicing factors, and their regulatory function is restricted to the same pre-mRNA molecule. Three typical types of cis-acting elements include promoters, enhancers, and silencers. According to their locations and functional features, splicing-related cis-regulatory elements are further classified into four categories: exon-splicing enhancers (ESEs), intron-splicing enhancers (ISEs), exon-splicing silencers (ESSs), and intron-splicing silencers (ISSs) (Fig. 1b). Distributed in exons and introns of pre-mRNAs, these sequences regulate splice-site selection by recruiting specific trans-acting splicing factors. Trans-acting factors are proteins or RNA factors that can bind to cis-acting elements and regulate them, and that promote or inhibit specific splicing events by binding to cis-regulatory elements. In contrast to cis-acting elements, trans-acting factors are not restricted to specific RNA regions and can directly or indirectly modulate alternative splicing events of multiple genes through complex regulatory networks. Trans-acting factors primarily include various splicing factors and RNA-binding proteins (RBPs) that regulate alternative splicing. Key RBPs involved in skeletal muscle alternative splicing are summarized in Table 1, and are primarily serine and arginine-rich (SR) proteins, the heterogeneous nuclear ribonucleoprotein (hnRNP) family, the CUGBP Elav-like family (CELF) family, RNA binding Fox-1 homolog 1/2 (RBFox1/2), and the muscleblind-like (MBNL) family, which cooperatively control splice-site selection and splicing efficiency in skeletal muscle.

Recent studies have revealed that RNA Pol II directly binds to U2AF1 via its RPB9 subunit, recognizing the 3' splice site (AG dinucleotide) in nascent RNA; simultaneously, its subunits RPB2 and RPB12 bind to U1 snRNP, recognizing 5' splice sites (GU dinucleotides). This enables Pol II to dynamically regulate the recruitment and release of splicing factors during transcription, and perform real-time linear scanning of both 5' and 3' splice sites on newly transcribed pre-mRNA to ensure splicing accuracy and precise regulation of gene expression (Shao et al., 2025). Epigenetic modifications, transcription elongation rate, and RNA secondary structure also participate in regulating RNA alternative splicing (Prochazka et al., 2014).

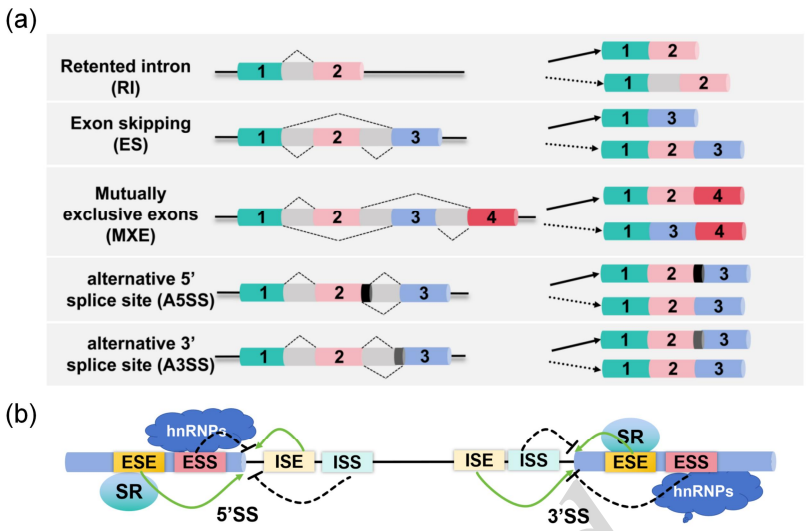

Fig. 1 Major types of RNA alternative splicing and their regulatory mechanisms. (a) The five main types of alternative splicing. (b) Cis-acting RNA elements that positively or negatively influence splice-site selection. Depending on their location and functions, they are referred to as exonic splicing enhancers (ESEs), exonic splicing silencers (ESSs), intronic splicing enhancers (ISEs), or intronic splicing silencers (ISSs). (C) Exon-bound SR proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) have opposite effects on recognition of adjacent splice sites by the core splicing machinery.

Table 1 Key RNA-binding proteins (RBPs) which regulate alternative splicing in skeletal muscle

RBP Family	Specific Members	Core Functions in Skeletal Muscle Development and Disease	Representative References
SR proteins	SRSF1, SRSF3, SRSF9	Facilitate splice-site recognition and exon inclusion; govern myoblast differentiation; abnormal expression linked to muscular dystrophy	(Jeong, 2017)
hnRNP family	hnRNPA1, hnRNPC, hnRNPI	Dual regulatory effect on exon splicing; modulate myofiber typing; dysregulation induces muscle atrophy	(Geuens et al., 2016)
CELF family	CELF1, CELF2, CELF6	Drive tissue-specific splicing pattern during muscle maturation; aberrant activity contributes to myopathy	(Nikonova et al., 2024)
RBFox family	RBFox1, RBFox2	Dominantly regulate contractile gene splicing; essential for normal muscle growth; mutation causes muscle dysfunction	(Conboy, 2017)
MBNL family	MBNL1, MBNL2	Coordinate splicing switch in muscle development; functional loss correlates with myotonic dystrophy	(Weskamp et al., 2021)

3 Alternative splicing in myogenesis

Myogenesis proceeds as a sequential, multi-stage process characterized by cell cycle exit and the expression of critical myogenic molecules, consisting of two distinct groups: myogenic regulatory factors (MRFs), and muscle-specific structural genes exemplified by myosin, actin, and troponin. Collectively, these factors and genes trigger myoblast differentiation and subsequent fusion into multinucleated myotubes(Buckingham et al., 2003; Bentzinger et al., 2012; Sun et al., 2012; Chal and Pourquié, 2017). Alternative splicing plays an important role in skeletal muscle development and tissue specificity and occurs throughout different stages of muscle development(Kim et al., 2018; Chen et al., 2019). Many splicing regulators are dynamically expressed during muscle differentiation, including polypyrimidine tract-binding protein 1 (PTBP1)(Hu et al., 2018), RBFox1(Sebastian et al., 2013) and MBNL(Vogan et al., 1996). These can all trigger exon inclusion or exclusion depending on the position of their homologous cis-elements relative to the exon(Fig. 2b). Both RBFox(Runfola et al., 2015) and MBNL(Kalsotra et al., 2008) are upregulated during myogenesis, resulting in increased retention of alternative exons in transcripts encoding Myocyte enhancer factor 2D (MEF2D). PTBP1 suppresses muscle differentiation by modulating splicing events in myoblasts. miR-133 negatively regulates PTBP1 expression, and the reduced level of PTBP1 further facilitates the progression of myogenic differentiation(Boutz et al., 2007). Splicing and transcription regulatory factors serine and arginine-rich splicing factor 2 (SRSF2) also participate in regulating the alternative splicing of genes associated with human skeletal muscle disease, such as bridging Integrator 1 (BIN1), myotonic dystrophy 1 protein kinase (DMPK), four and a half LIM domains 1(FHL1), and LIM domain-binding 3 gene (LDB3)(Sha et al., 2024).

During myogenesis, different splicing isoforms generated by alternative splicing of a single gene exhibit complex regulatory mechanisms in promoting or inhibiting myogenic differentiation. MEF2D is expressed in two subtypes in muscle tissue by MXE splicing, MEF2Dα1 and α2. The inclusion of exon α1 (MEF2Dα1) results in a functional transcriptional repressor, while the alternative inclusion of the mutually exclusive exon α2 (MEF2Dα2) produces a transcriptional activator(Sebastian, et al., 2013). Splicing timing analysis indicates that production of the transcriptionally active MEF2Dα2 subtype occurs during the late stages of muscle-terminal differentiation, activating the late muscle gene expression program(Sebastian, et al., 2013) (Fig. 2a). Genome-wide alternative splicing profiles have been systematically investigated in skeletal muscle across multiple species. In pigs, Wang et al. constructed a comprehensive splicing atlas using transcriptomes from 27 developmental stages and 10 tissues, and identified over 3000 dynamic alternative splicing events, 280 skeletal muscle-specific events, and 49 specific development-related events(Wang et al., 2024). In chickens, Mortazavi et al. compared fast- and slow-growing strains at embryonic days 14 and 20, which revealed 230 and 373 differential alternative splicing events, respectively(Mortazavi et al., 2008). Cross-species studies have further highlighted the conservation and tissue specificity of alternative splicing in muscle: approximately 80% of sarcomeric proteins undergo differential expression or splicing in Drosophila muscle, and distinct dominant splicing types vary across mammals, with intron retention predominant in cattle and alternative 3' splicing common in pigs and chickens. These studies provide a valuable global view of alternative splicing regulation in vertebrate skeletal muscle development(Nikonova et al., 2020). During myogenic differentiation, the 15th exon of the FMR1 autosomal homolog 1 (Fxr1) gene pre-mRNA undergoes alternative splicing, resulting in two Fxr1 subtypes: Fxr1E15− and Fxr1E15+. RBM24 has been demonstrated to critically regulate the alternative splicing of Fxr1 exon 15 during this process (Fig. 2b). Functional studies show that the Fxr1E15- subtype enhances myoblast proliferation, while the Fxr1E15+ subtype promotes myoblast differentiation and fusion, ultimately influencing myotube formation(Wang, et al., 2024)(Fig. 2b). In vivo studies in the African clawed toad have indicated that mis-splicing of exon 15 of Fxr1 leads to segment-formation defects, while in vitro inhibition of exon 15 retention does not affect myotube formation (Smith et al., 2020). Chen Genghua et al. (Chen et al., 2024) identified two alternative splicing transcripts of the RNA-binding protein human transformer 2 beta homolog (transformer 2 Beta, TRA2β), TRA2β-L and TRA2β-S, by mapping the alternative-splicing regulatory landscape of muscle-fiber formation in broiler chickens. Further studies revealed that the TRA2B-S subtype

inhibited the TGF-β pathway by promoting skipping of the 4th and 5th exons of transforming-growth-factor beta receptor II (TGFβR2), thereby inhibiting the myogenic differentiation program (Fig. 2c). LIM domain-binding 3 (LDB3) is a striated muscle-specific Z-band alternative splicing protein that plays an important role in mammalian skeletal muscle development[55, 56]. By reanalyzing RNA sequencing data from chicken skeletal muscle tissue, Wei Chengjie et al. [57] identified three variants (LDB3-X, LDB3-XN1, and LDB3-XN2) resulting from LDB3 gene alternative splicing, with LDB3-XN1 and LDB3-XN2 being new variants. They also found that LDB3-X inhibits myotube formation, whereas LDB3-XN1 and LDB3-XN2 promote myotube formation (Fig. 2d). LIM and calponin homology domains 1 (LIMCH1) is a stress-fiber-associated protein that can be alternatively spliced to produce uLIMCH1 (a widely expressed subtype) and mLIMCH1 (a skeletal-muscle-specific subtype that includes six additional exons in mice after birth). Myofibers that are deficient in mLIMCH1 exhibit disruption of calcium handling, a mechanism that leads to muscle weakness(Penna et al., 2023).

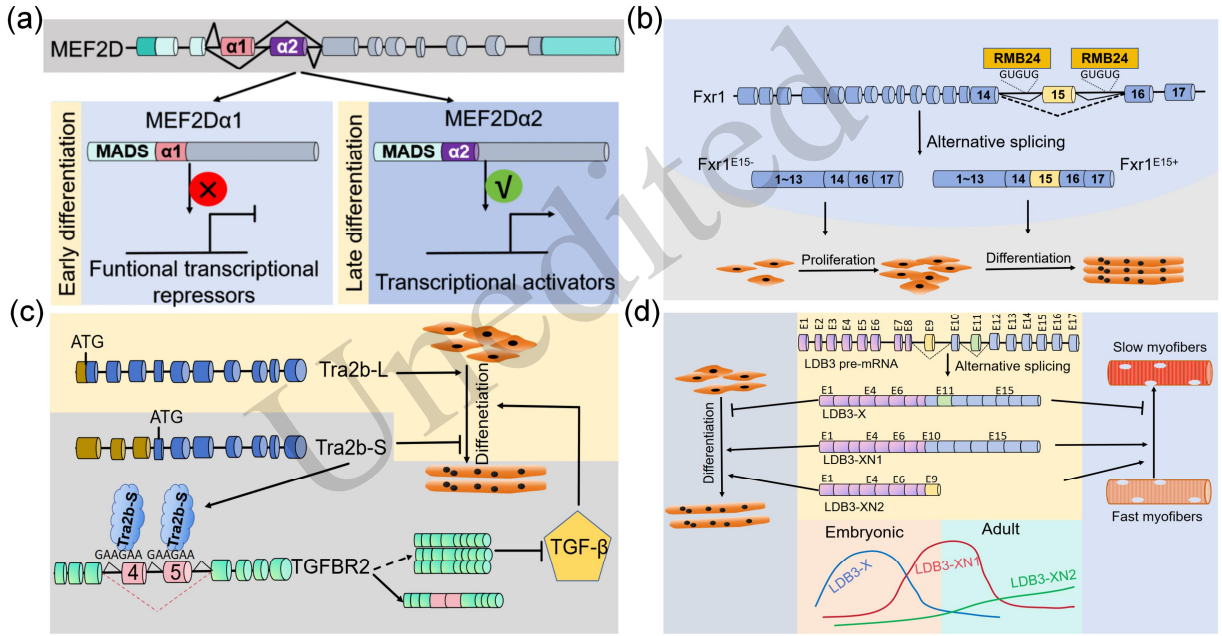

Fig. 2 Roles of alternative splicing in myoblast proliferation and differentiation. (a) MEF2D is expressed in muscle as two subtypes: MEF2Dα1 and α2. The inclusion of exon α1 results in a functional transcriptional repressor, while inclusion of the mutually exclusive exon α2 produces a transcriptional activator. Generation of the transcriptionally active MEF2Dα2 subtype occurs during the late stages of muscle-terminal differentiation, activating the late muscle gene-expression program. (b) The 15th exon of Fxr1 pre-mRNA undergoes alternative splicing, producing two subtypes: Fxr1E15− and Fxr1E15+. The FXR1E15- subtype enhances myoblast proliferation, whereas the FXR1E15+ isoform promotes myoblast differentiation and fusion, ultimately affecting myotube formation.(c) Two differentially spliced transcripts of TRA2β exist, TRA2β-L and TRA2β-S; the TRA2β-S subtype can inhibit the TGF-β pathway by promoting skipping of the TGFβR2 exon, ultimately inhibiting the myogenic differentiation program.(d) Three variants of LDB3-LDB3-X, LDB3-XN1, and LDB3-XN2 are produced by alternative splicing of the LDB3 gene. Studies have shown that overexpression of LDB3-X and knockout of LDB3-XN1 and LDB3-XN2 inhibit myotube formation, while the opposite promotes it.

Skeletal muscle consists of myofibers and exhibits high plasticity. The proportion of different myofiber types significantly influences an animal's locomotor ability, muscle size, and overall glycolipid metabolism(Mok et al., 2024). Muscle fiber-type conversion is subject to complex regulation at both the transcriptional and post-transcriptional levels (Majesky, 2007; Guo et al., 2010; Kalsotra and Cooper, 2011; Nayak and Amrute-Nayak, 2020; Nikonova, et al., 2020). The characteristic developmental transitions of myosin, troponin, and myosin-binding protein-C, as well as muscle-type-specific splicing patterns in vertebrate

skeletal and cardiac muscle, demonstrate the widespread role of alternative splicing in regulating muscle structure (Nakka et al., 2018; Nikonova, et al., 2024)

RBFox1 can influence transcript levels and splicing, and exhibits differential expression between oxidative and glycolytic myofiber. It is required for the development of adult Drosophila fibers and tubular muscle fibers, and interacts with the CELF 1/2 family homologs Bruno1 (Bru1, Arrest) to define fiber-type-specific patterns of alternative splicing(Nikonova et al., 2022). Alternative splicing of LDB3 generates three transcripts, among which LDB3-X inhibits conversion of fast glycolytic myofibers to oxidative myofibers, while LDB3-XN1 and LDB3-XN2 promote conversion of glycolytic myofibers to oxidative myofibers(Wei et al., 2024). Mutations that disrupt the nonsense-mediated mRNA decay (NMD) pathway impair its physiological function and induce muscle defects, and such genetic abnormalities are closely associated with various neuromuscular diseases (Jagannathan et al., 2019; Xu et al., 2019; Gheller et al., 2020) As an essential RNA surveillance mechanism, NMD eliminates abnormal transcripts bearing premature stop codons produced by alternative splicing, gene insertions, deletions, and nonsense mutations, effectively restricting the accumulation of toxic truncated proteins in skeletal muscle (Weskamp, et al., 2021). Alternative splicing can further modulate mRNA abundance by mediating the NMD process. Relevant research shows that functional NMD prevents harmful truncated protein buildup in patients carrying uniparental truncation variants (Gohlke et al., 2024). Further exploration of NMD-regulated alternatively spliced mRNA variants is therefore essential for understanding muscle pathogenesis. For instance, the TTN splice variant 117 identified in this study involves completely skipping exon 117; such exon-skipping events can introduce premature termination codons and trigger NMD, thereby lowering levels of dysfunctional transcripts. Similarly, three additional patients with intron 242 splice-acceptor variants showed low-level skipping of exon 243 without increased intron retention. Skipping of these exons disrupts the structural and regulatory functions of titin in organizing thick filament length, which may elicit NMD-mediated degradation of abnormal transcripts and ultimately contribute to myopathies(Tonino et al., 2017; Bennett et al., 2020).

Previous studies have identified numerous exons that undergo specific alternative splicing in muscle tissue, including both cardiac and skeletal muscle, and these exons fill critical functional roles in muscle development and physiology. Cross-species analyses that include humans, mice, pigs, chickens, and other animals have revealed both evolutionary conservation and obvious tissue- and cell-type specificity of these splicing events in skeletal muscle. Such conserved and specific splicing patterns not only reflect fundamental regulatory mechanisms shared across species but also contribute to the unique physiological characteristics of skeletal muscle in different animals, highlighting the importance of alternative splicing in fine-tuning muscle function and development.

4 Alternative splicing in skeletal muscle regeneration

Upon muscle injury, alternative splicing is rapidly reprogrammed to support satellite-cell activation, myogenic differentiation, and functional regeneration, thus representing a key regulatory layer in tissue repair. Studies have shown that RNA alternative splicing plays an important regulatory role in the regeneration process following skeletal muscle injury by affecting myocyte enhancer factor 2C (MEF2C)(Baruffaldi et al., 2017), MEF2D(Sebastian, et al., 2013), quaking(QKI)(Montañés-Agudo et al., 2023), RBFox2(Singh et al., 2014; Cao et al., 2021), and nascent peptide-associated complex subunit alpha (Naca)(Park et al., 2010), as well as expression of other regulatory factors, thereby participating in the regulation of skeletal muscle regeneration.

RNA-binding motif protein 24 (RBM24) is a muscle-specific alternative splicing regulator(Yang et al., 2014). The absence of RBM24 leads to defects in myogenic fusion and differentiation, delaying muscle regeneration(Zhang et al., 2020). Specific knockout of the RBM24 gene in muscle satellite cells results in mice exhibiting regeneration defects(Zhang, et al., 2020). Additionally, RBM24 participates in regulating the alternative splicing of MEF2D, Naca, Rho-associated protein kinase 2 (Rock2), and LRRFIP1- LRR-binding

FLII-interacting protein 1 (Lrrfip1)(Zhang, et al., 2020); alternative splicing of these genes is essential for muscle differentiation and muscle regeneration (Fig. 3). Furthermore, RBM24 can regulate alternative splicing of Fxr1, producing two transcripts encoding different proteins, with the Fxr1E15+ subtype promoting muscle regeneration in vivo (Wang, et al., 2024). Insulin-like growth factor I (IGF-1) promotes skeletal muscle regeneration by promoting myofiber formation and inhibiting inflammatory response (Musarò, 2005; Vassilakos and Barton, 2018; Kok and Barton, 2021). While the most well-characterized form of IGF-I is the mature protein, alternative splicing and post-translational modification complexity lead to several additional forms of IGF-I. Yangyi E Luo et al(Luo et al., 2024) utilized CRISPR-Cas9 gene editing to mutate the IGF-I glycosylation site (2ND) or its cleavage site (3RA) in mice. Their results showed that 3RA mice displayed impaired muscle regeneration, whereas 2ND mice possessed superior regenerative capacity relative to 3RA mice.. YTH structural domain family protein YTH N6-Methyladenosine RNA binding protein C1 (YTHDC1) is a unique m6A reader that has post-transcriptional regulatory functions such as pre-mRNA splicing(Xiao et al., 2016; Kasowitz et al., 2018), mRNA export(Roundtree et al., 2017; Lesbirel et al., 2018), and mRNA stabilization due to its preferential localization in the nucleus. Studies have shown that YTHDC1 deficiency in satellite cells significantly alters target mRNA splicing (such as Ythdf2, Ythdf3, and Pi4kb), inhibits satellite cell activation and proliferation, and delays acute-injury-induced muscle regeneration (Qiao et al., 2023).

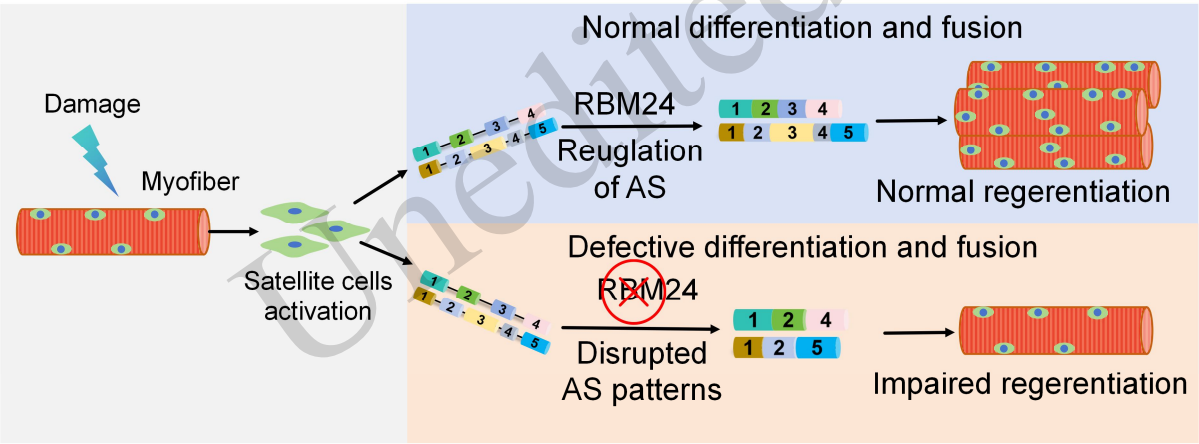

Fig. 3 Aworking model of RBM24 regulating muscle regeneration by modulating alternative splicing. Following skeletal muscle injury, satellite cells are activated and differentiate into myoblasts. Under physiological conditions, RBM24 modulates the alternative splicing pattern of precursor mRNAs to promote myoblast fusion and the formation of nascent myofibers with centralized nuclei, thereby ensuring normal skeletal muscle regeneration. In contrast, loss of RBM24 function causes dysregulation of alternative splicing, which impairs myoblast fusion and nascent myofiber formation, ultimately leading to defective skeletal muscle regeneration.

5 Alternative splicing in skeletal muscle disease and therapeutic approaches

Dysregulated alternative splicing can lead to abnormal expression levels of multiple transcript isoforms derived from the same gene, or may even generate novel pathogenic isoforms. These aberrant alterations contribute to a progressive decline in muscle function and play a pivotal role in the pathogenesis of numerous devastating muscle disorders, such as spinal muscular atrophy (SMA), myotonic dystrophy (DM), and Duchenne muscular dystrophy (DMD) (Fig. 4) (Narasimhan et al., 2018; Donega et al., 2025).

Spinal muscular atrophy (SMA) is considered a mild splicing-related disorder characterized by severe degeneration of motor neurons in the spinal cord. It leads to progressive muscle weakness, paralysis, and death(Boulisfane et al., 2011; Lotti et al., 2012). The most common form of SMA is caused by survival mutations from the survival of the motor neuron 1 (SMN1) gene located on chromosome 5q(Schmutz et al.,

2004). Liang Li et al. (Li et al., 2020) used genome-editing technology to directly demonstrate that defects in minor splicing (U12-type) can lead to the SMA phenotype. They identified a large number of splicing events sensitive to minor spliceosomes, proving that three sensitive retained intron genes—phosphate cytidylyltransferase 2-ethanolamine (Pcyt2), zinc finger MYND-type containing 10 (Zmynd10), and fatty acid synthase 3 (Fas3), directly promote SMA development. Additionally, they discovered that many splicing sites within the sensitive introns can be recognized by both the small and the large spliceosome, suggesting a new mechanism for regulating splicing through competition between the small and large spliceosomes(Li, et al., 2020). Although humans have a second nearly identical paralog on chromosome 5 in the centromeric region, namely survival of motor neuron 2 (SMN2), it cannot compensate for the loss of SMN1 because the 7th exon of most SMN2 transcripts is abnormally skipped. The 68-kDa SRC-associated mitotic RNA-binding protein Sam68—the prototype member of the steroid hormone synthesis acute regulatory protein family—and hnRNP A1 jointly bind the proximal region of SMN2 exon 7's central domain. Nadal et al. (Nadal et al., 2023) demonstrated this dual binding interaction and indicated that inhibition of Sam68 homodimerization could act as a drug target for SMA and additional splicing disorders Since 2016, splice-conversion therapies have been used in clinical practice for SMA and DMD(Takeshima et al., 1995; Matsuo, 2021; Nishio et al., 2023; Roberts et al., 2023). The antisense oligonucleotide drug nociceptin sodium (Spinraza), the first global treatment for SMA, enhances expression of full-length SMN protein by acting on the splicing recognition site near exon 7 of the SMN2 gene, thereby improving clinical symptoms(Neil and Bisaccia, 2019; De Waele and Servais, 2024).

Myotonic dystrophy (DM) is a common group of autosomal dominantly inherited myopathies with a prevalence of approximately 12/100,000. It is most often characterized by progressive muscle weakness, muscle ankylosis, and multiorgan damage outside of the skeletal muscular system(Udd and Krahe, 2012). There are two types of DM: type 1 and type 2. DM1 is caused by an amplification of CTG sequences in the DMPK gene, while DM2 is triggered by an amplification of CCTG repeats in the gene for the cellular nucleic-acid-binding protein (CNBP) (Liquori et al., 2001). DM1 is a splicing disorder characterized by accelerated muscle aging and sarcopenia(Malatesta et al., 2009; Vihola et al., 2010; Malatesta, 2012; Mateos-Aierdi et al., 2015; Mukund and Subramaniam, 2020). In DM1, MBNLs are isolated on toxic RNAs containing amplified CUG repeats, which leads to disruption of MBNL-regulated processes and the disease characteristics of DM1. Penna et al. (Penna, et al., 2023) showed that mLIMCH1 requires MBNL activity and that LIMCH1 splicing is disrupted in patient-derived DM1 skeletal muscle tissue, which means that deletion of mLIMCH1 expression is a novel splicing dysregulation event in DM1 muscle. Nitschke and Cooper (Nitschke and Cooper, 2024) systematically examined the combined effects of DM1-associated mis-splicing patterns in vivo, identifying the synergistic interaction of mis-spliced calcium- and chloride-channel proteins as a major contributor to DM1 skeletal muscle damage. This demonstrated the therapeutic potential of blocking this synergistic interaction via calcium-channel modulation for treat muscular disorders(Nitschke and Cooper, 2024).

Duchenne muscular dystrophy (DMD) is a severe disease caused by mutations in the antimyotrophic protein gene, which results in loss of functional antimyotrophic protein in muscle. Skeletal muscle fibrosis is one of the most prominent pathological features of DMD. Trundle et al.(Trundle et al., 2024) found that the carboxyl-terminal region of Periostin (Postn) contains an alternative splicing domain, and the sequence encoded by Exon 17 localizes to this carboxyl-terminal segment. This region harbors an RGD motif that mediates binding to integrins αvβ3/αvβ5, as well as potential TGF-β1 activation sites. In skeletal muscle cells from patients with DMD, TGF-β1-induced Smad3 phosphorylation directly promotes the binding of Smad3 to the mPostn gene promoter, thereby upregulating expression of the e17+ Periostin isoform (Postne17+). Then, Postne17+ interacts with latent TGF-β binding protein 1 (LTBP1), which facilitates extracellular release and activation of TGF-β1. This forms a positive feedback loop that further exacerbates skeletal muscle fibrosis while underscoring the potential of exon-skipping targeting strategies as a promising therapeutic approach for mitigating fibrosis-related complications. Currently, targeted therapeutic drugs such as phosphorodiamidate morpholino oligomers (PMOs) are used to treat DMD patients with exon skipping occurring at exons 45, 51,

and 53 of the dystrophin (DMD) gene(Syed, 2016; Heo, 2020; Shirley, 2021), but their efficacy is often limited by poor muscle delivery. Usue Etxaniz et al.(Etxaniz et al., 2025) developed an antibody-oligonucleotide coupling, AOC 1044, which induced exon 44 skipping and restored myostatin protein in a DMD clinical model. These studies indicate that abnormal alternative splicing of RNA has an important impact on muscle growth and development, as well as the occurrence of muscle disease, and that targeting abnormal alternative splicing events and their associated regulatory proteins offers great research potential for the treatment of muscle disease.

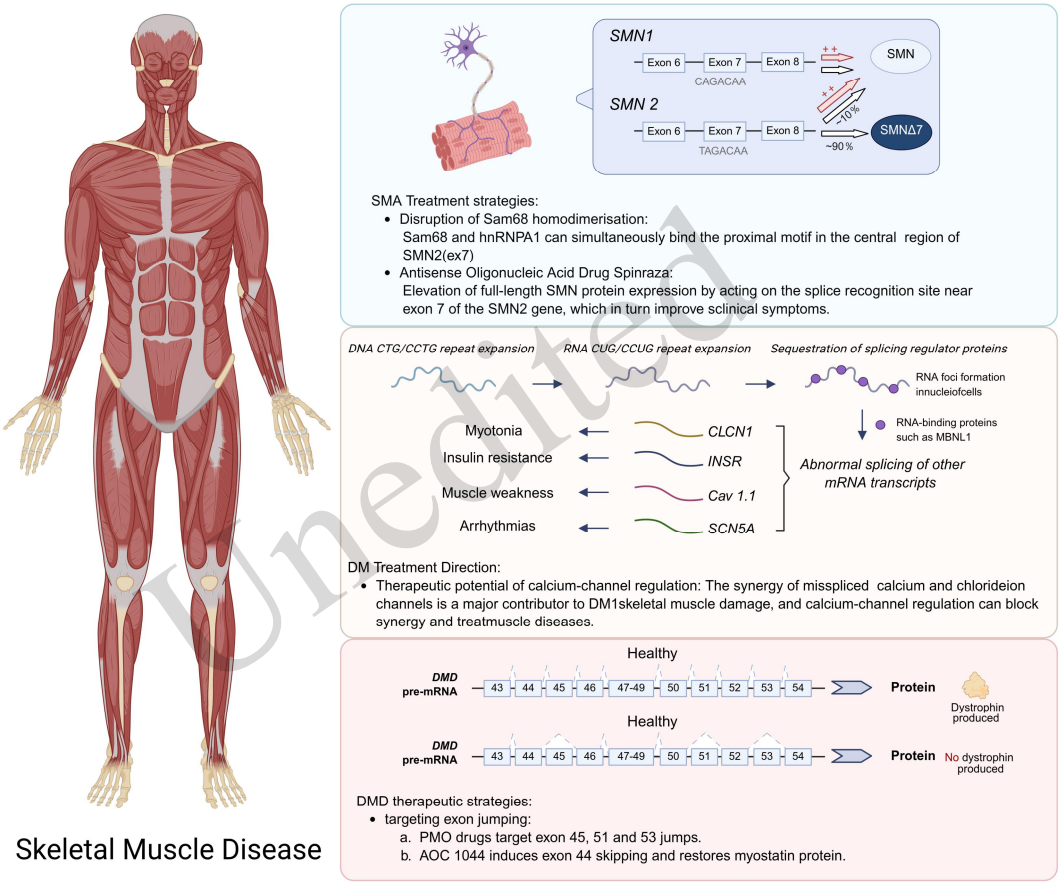

Fig. 4 Schematic representation of aberrant regulation of alternative splicing in SMA, DM, and DMD diseases and therapeutic strategies. For SMA, strategies include disrupting Sam68/hnRNP A1 binding to SMN2 exon 7 or using the antisense oligonucleotide Spinraza to promote full-length SMN protein expression. DM arises from expanded repeat sequences forming RNA foci and aberrant gene splicing; therapies target calcium-channel regulation to block muscle damage. For DMD, exon skipping via PMO drugs (targeting exons 45, 51, 53) or AOC 1044 (targeting exon 44) restores functional dystrophin expression.(Created with BioRender.com)

Concluding remarks

RNA alternative splicing modulates skeletal muscle growth, regeneration and disease through diverse molecular mechanisms—including remodeling of protein structure and function, shifts in gene expression, alterations to cellular signaling, cytoskeletal organization, and metabolic homeostasis—all of which are central to the progression of multiple skeletal muscle disorders. In transcriptomics studies, next-generation sequencing (RNA-seq) is commonly used to identify alternative splicing, but it has read-length limitations and is prone to

error. In recent years, third-generation long-read-sequencing technologies, such as Oxford Nanopore Technologies (ONT) and PacBio SMRT sequencing, have provided more advanced tools for accurate identification of alternatively spliced transcripts (Logsdon et al., 2020; Oehler et al., 2023). Long-read sequencing does not require transcript fragmentation and can directly obtain full-length transcriptome information from the 5' to 3' ends of mRNA, enabling accurate identification of alternative splicing, gene fusion, alternative polyadenylation (APA), and allele-specific expression (Nurk et al., 2022). Combined analysis of long-read and second-generation sequencing shows clear advantages in identifying alternatively spliced transcripts and their expression levels. This lays a foundation for the diagnosis and treatment of diseases caused by abnormal alternative splicing (Garalde et al., 2018; Rhie et al., 2021; Lebrigand et al., 2023; Xiang et al., 2024)

Alternative splicing exhibits high spatio-temporal specificity. Single-cell long-read sequencing technologies such as SMOOTH-seq and ScISOr-Seq enable identification of cell-type-specific RNA splicing patterns (Logsdon, et al., 2020; Oehler, et al., 2023; Huang et al., 2024). Spatial transcriptomics based on long-read sequencing will provide novel tools for precise characterization of disease-associated alternative splicing (Foord et al., 2025). These technical advances allow more precise dissection of the relationship between alternative splicing abnormalities in myogenic cells and muscular disorders.

In addition, multiple bioinformatics tools support accurate identification of RNA isoforms and splicing events, including variant-analysis tools such as FLAIR and NanoCount (Depledge et al., 2019; Tang et al., 2020), quality-control tools such as SQANTI3 (Tardaguila et al., 2018), and quantitative tools such as Salmon (Patro et al., 2017) and IGV software (Robinson et al., 2011). Recently, Gao et al. developed a novel programmable trans-splicing riboregulator (SENTRs), which achieves programmable RNA trans-splicing through engineered external guidance sequences (EGS) and thus provides a new tool for synthetic biology and potential RNA-targeted therapeutics (Gao et al., 2025).

Despite these advances, key scientific questions remain to be addressed. Existing studies of alternative splicing have mainly focused on protein-coding genes, whereas research on non-coding RNA splicing is still in its infancy. Alternatively spliced non-coding RNAs, including long non-coding RNAs and circular RNAs, are important in disease development (Khan et al., 2021; Liu et al., 2023). For example, in hepatocellular carcinoma, DDX17 induces intron retention of the lncRNA PXN-AS1, generating a transcript that promotes MYC activation and cancer metastasis (Zhou et al., 2022).

Many alternatively spliced transcripts are pathogenic, and targeting abnormal splicing represents a promising therapeutic strategy (Lv et al., 2025). For instance, the small-molecule drug Risdiplam promotes exon -7 inclusion in SMN2 pre-mRNA and increases functional SMN protein, thus treating spinal muscular atrophy (Ratni et al., 2018).CRISPR-based RNA editing technologies also provide powerful tools for correcting aberrant splicing (Lenharo, 2024). CRISPR-Cas13-mediated cleavage, base editing, and RNA-level editing systems such as SCISSOR allow targeted modification of mis-spliced transcripts (Sun et al., 2025). Skeletal muscle metabolic disorders and atrophy are accompanied by abnormal alternative splicing; thus, targeting pathogenic splicing events represents a key future direction for treating muscular disease.

Looking ahead in anticipation of continuous improvement in sequencing technologies, bioinformatic pipelines, and gene-therapy platforms, our assessment is that precise identification of pathogenic alternative splicing events and development of targeted interventions will open new avenues for clinical therapy of RNA-related diseases.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Wenhui CHENG and Hao CHEN were involved in writing – original draft and editing. Xin'e SHI and Jianjun JIN were involved in writing – review and editing. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Wenhui CHENG, Hao CHEN, Xin'e SHI and Jianjun JIN declare that they have no conflicts of interest. This review does not include any research with human or animal subjects performed by any of the authors.

Declaration on the use of generative AI tools

No generative AI tools were used in the preparation of this manuscript.

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