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Review

A nucleolar view of neuromuscular disease

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The nucleolus is a master regulator of ribosome biogenesis and cellular homeostasis, as well as an increasingly key determinant of neuromuscular diseases. Across these conditions, diverse genetic and molecular lesions converge on alterations in nucleolar organization and function. These changes impact ribosomal RNA synthesis and reshape translational output, linking nuclear events to cytoplasmic protein homeostasis in disease-relevant contexts. In this review, we propose a comprehensive framework in which the nucleolus integrates RNA dysfunction, genome organization, and translational control across neuromuscular disorders. This perspective provides a conceptual basis for interpreting disease heterogeneity and highlights nucleolar pathways as potential, underexploited targets for therapeutic intervention.

Highlights

The nucleolus emerges as a dynamic regulatory hub, integrating RNA metabolism, genome organization, and translational control across cellular states and stress conditions.

Diverse RNA species, including repeat-derived transcripts, long noncoding RNAs, and small nucleolar RNAs, actively shape nucleolar architecture, condensate properties, and functional output.

Neuromuscular disorders with distinct genetic origins converge on nucleolar dysfunction, revealing a shared layer of RNA-mediated disease mechanisms.

Nucleolar perturbation drives selective translational reprogramming and ribosome specialization, contributing to cell-type-specific vulnerability in muscle and motor neurons.

Targeting RNA-mediated toxicity, ribosome biogenesis, and condensate dynamics represents a promising but still largely unexplored therapeutic strategy.

The nucleolus beyond ribosome production

The nucleolus has traditionally been viewed as the cellular site of ribosome biogenesis. However, growing evidence indicates that this membrane-less nuclear condensate also participates in chromatin organization, RNA surveillance, stress responses, and translational regulation [1–3]. These advances have expanded our understanding of nucleolar function far beyond ribosome production and revealed unexpected links between nucleolar homeostasis and human disease. Notably, studies across multiple neuromuscular and neurodegenerative disorders have identified recurrent alterations in nucleolar structure and function despite highly diverse upstream genetic causes. This convergence suggests that the nucleolus may represent more than a downstream target of disease-associated perturbations and instead function as an active integrator of pathogenic signals arising from defects in RNA metabolism, chromatin regulation, and nuclear organization [4–6] (Figure 1).

This convergence raises a fundamental question: can nucleolar dysfunction represent a common pathogenic node linking distinct molecular defects to shared cellular vulnerabilities?

In this review, we examine recent evidence supporting this concept and discuss how nucleolar integration of RNA dysfunction may influence genome organization, translation, and disease progression. We propose that the nucleolus should be viewed not simply as a ribosome-producing organelle but as an active regulatory hub through which diverse pathogenic mechanisms may converge to shape disease phenotypes. We first summarize emerging evidence linking nucleolar dysfunction to neuromuscular and neurodegenerative disorders, then discuss its impact on genome organization and translation, and finally consider the implications of this framework for disease mechanisms and therapeutic intervention.

A convergent node in neuromuscular disease

Despite arising from distinct genetic and molecular pathogenic mechanisms, several neurodegenerative and neuromuscular disorders are increasingly associated with alterations in nucleolar biology (Table 1). In some cases, disease-associated factors directly localize to nucleolar subcompartments or interfere with rRNA metabolism. In others, nucleolar perturbation emerges

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indirectly through altered RNA processing, chromatin organization, or disruption of nuclear condensates. The major disease-associated mechanisms converging on nucleolar dysfunction are summarized in Figure 2, Key figure and Table 1.

Spinal muscular atrophy (SMA) (see Glossary) provides one of the clearest examples of this link. Although classically defined by reduced levels of the survival motor neuron (SMN) protein and impaired **small nuclear ribonucleoprotein (snRNP)** assembly, SMN function extends beyond spliceosomal pathways. Early studies identified interactions between SMN and nucleolar components such as fibrillarin, as well as the nucleolar localization of SMN in human cell lines [15–17]. More recent work shows that nucleolar reorganization after transcriptional stress, including conditions that impair rRNA synthesis, depends on SMN trafficking between nuclear compartments [18], and that SMN deficiency is associated with increased ribosomal DNA damage in human fibroblasts [11]. Together, these observations suggest that nucleolar alterations may, at least partially, contribute to SMA.

A distinct mechanism leading to nucleolar dysfunction is observed in **amyotrophic lateral sclerosis (ALS)**, particularly in **C9ORF72 repeat expansion**-associated forms. In this case, expanded repeat RNAs and arginine-rich dipeptide repeat proteins perturb the dynamics of membrane-less compartments. These molecules disrupt the assembly and material properties of nuclear condensates, including the nucleolus [7,9,19]. Poly(proline-arginine) [poly(PR)] peptides, in particular, directly interfere with nucleolar function [7]. In ALS, cytoplasmic redistribution and aggregation of **TDP-43** have also been observed and are considered pathological hallmarks of the disease, although whether toxicity primarily derives from toxic gain-of-function effects in the cytoplasm, loss of nuclear function, or a combination of both remains actively debated. Nucleolar stress has been observed prior to the mislocalization of the RNA-binding protein TDP-43 in human motor neurons [8]. Recent studies further place TDP-43 within the broader framework of condensate regulation and nucleolar-associated processes [20–22].

Facioscapulohumeral muscular dystrophy (FSHD) adds a distinct dimension to this convergence. While traditionally interpreted through **DUX4**-driven transcriptional deregulation, recent findings indicate that the **FRG2 long noncoding RNA (lncRNA)** family also contributes directly to nucleolar dysfunction. Abnormally upregulated in FSHD muscle cells, FRG2 transcripts accumulate within nucleolar compartments, where they repress rRNA transcription and impair cytoplasmic translation [10]. This mechanism demonstrates that noncoding transcripts associated with repetitive genomic elements can act as functional regulators of nucleolar output rather than passive byproducts of epigenetic deregulation. Additional evidence, including DUX4-dependent HSATII RNA accumulation and altered proteome dynamics, supports a model in which RNA-driven perturbations extend to nucleolar and translational pathways [23–25].

Myotonic dystrophy type 1 (DM1) represents a prototypical RNA-mediated disorder in which expanded cytosine–uracil–guanine (CUG) repeat transcripts disrupt RNA-binding protein networks and nuclear organization. Although overt nucleolar alterations are less well characterized in DM1, emerging evidence links RNA-mediated pathology to nucleolar-associated processes. **Small nucleolar RNAs (snoRNAs)** can restore muscle differentiation programs in DM1 models [13], and the DM1 locus undergoes dynamic changes in chromatin organization and nuclear positioning during differentiation [26]. These observations indicate that RNA-mediated perturbations may influence nucleolar organization and function even in the absence of overt nucleolar defects.

Duchenne muscular dystrophy (DMD) provides a less straightforward but intriguing example. While primarily attributed to the loss of dystrophin and consequent membrane instability [27],

Glossary

Amyotrophic lateral sclerosis (ALS): a progressive neurodegenerative disease affecting motor neurons, associated with RNA-binding protein dysfunction and altered proteostasis.
C9ORF72 repeat expansion: GGGGCC hexanucleotide repeat expansion produces toxic RNAs and dipeptide repeat proteins that disrupt nucleolar function and translation.
CX-5461: a small-molecule inhibitor of RNA polymerase I that suppresses rRNA transcription and nucleolar activity.
Duchenne muscular dystrophy (DMD): X-linked disorder caused by dystrophin mutations, leading to progressive muscle degeneration.
DUX4: a transcription factor aberrantly expressed in FSHD, driving transcriptional deregulation and RNA-mediated pathogenic pathways.
Facioscapulohumeral muscular dystrophy (FSHD): a genetic muscle disorder linked to D4Z4 derepression and the aberrant expression of toxic transcripts.
FRG2: noncoding RNA with nucleolar localization that modulates rRNA transcription and translational output.
FUS: RNA-binding protein that regulates RNA metabolism and phase separation; mutations alter condensate properties.
Integrated stress response: a cellular pathway that reduces global protein synthesis in response to stress through the phosphorylation of eIF2α.
Intergenic spacer: noncoding region located between adjacent rRNA transcription units within ribosomal DNA arrays.
Intergenic spacer transcripts: noncoding RNAs produced from the intergenic spacer regions of rDNA arrays that participate in nucleolar organization, stress responses, and the sequestration of regulatory proteins.
Lamina-associated domains (LADs): genomic regions that interact with the nuclear lamina and are typically transcriptionally repressed.
Long noncoding RNA (lncRNA): ncRNAs longer than 200 nucleotides that can modulate transcription, chromatin architecture, and nuclear organization.
mTOR pathway: a central regulator of cell growth and protein synthesis, integrating nutrient and energy signals.
Myotonic dystrophy type 1 (DM1): a disorder caused by CTG repeat

recent findings point to altered RNA processing, including nuclear retention of dystrophin transcripts with enrichment near nucleolar regions [14]. Although still preliminary, these observations raise the possibility that defects in RNA trafficking and nuclear organization may thereby impact nucleolar function.

Collectively, these disorders show how distinct pathogenic mechanisms converge on nucleolar dysfunction. In some cases, pathogenic factors directly perturb nucleolar components or ribosomal DNA (rDNA)-related processes, whereas in others, broader alterations in RNA homeostasis progressively destabilize nucleolar integrity. This convergence identifies the nucleolus as a critical hub linking RNA dysregulation to translational dysfunction and cellular vulnerability, particularly in muscle cells and motor neurons, which are highly dependent on sustained protein synthesis and RNA homeostasis.

RNA-mediated perturbations of nucleolar function

If nucleolar dysfunction is a shared feature of neuromuscular disease, the next question is, what molecular class most directly drives this dysfunction? Emerging evidence increasingly implicates RNA. Rather than acting solely as a downstream readout of disease, RNA can directly shape nucleolar architecture, influence ribosomal RNA metabolism, and modulate translational output.

This idea is consistent with the physical architecture of the nucleolus itself. As a multiphase condensate [28,29], the nucleolus depends on extensive RNA–protein and RNA–RNA interactions for its assembly, internal organization, and material properties [2,3,30]. Changes in RNA abundance, processing, or localization can therefore directly alter nucleolar behavior. Experimental approaches used to investigate RNA-dependent nucleolar organization and translational control are summarized in Box 1.

Several classes of RNA are particularly relevant in this context. Repeat-derived RNAs can disrupt condensate dynamics and nucleolar functions both directly and through their unconventional translation products [7–9,19,23,50]. LncRNAs provide a more spatially restricted mechanism, as a growing number of lncRNAs localize to nucleolar compartments and modulate ribosomal gene transcription or nucleolar organization [30,51]. In this framework, FRG2 lncRNAs represent a disease-relevant example of nucleolar-targeted noncoding transcripts that directly repress rRNA transcription and impair translation [10]. More broadly, repeat-containing lncRNAs, including those enriched in Alu elements, display intrinsic architectural properties that promote multivalent interactions and nuclear compartmentalization, supporting a general role for repeat-derived RNA sequences as structural scaffolds of nuclear condensates [2,52].

Importantly, nucleolar-associated RNAs are not limited to canonical rRNA species. RNA polymerase II-derived noncoding transcripts, including **promoter-associated RNAs (pRNAs)** and stress-induced antisense transcripts such as **promoter and pre-rRNA antisense (PAPAS)**, contribute directly to nucleolar organization and ribosomal gene regulation [53–55]. In parallel, rDNA-derived **intergenic spacer transcripts** modulate the transcriptional and epigenetic state, as well as the structural dynamics, of ribosomal DNA arrays, particularly in response to cellular stress [56,57]. Together, these findings support a structural and regulatory role for RNA in nucleolar organization that extends beyond classical gene regulation.

SnoRNAs add a further layer of complexity. Beyond their established role in rRNA modification, specific snoRNAs can influence ribosome biogenesis, senescence, and differentiation programs [13,58], indicating that endogenous nucleolar RNA populations actively participate in cell-state transitions and stress adaptation.

expansion, leading to toxic RNA accumulation and splicing defects.
Nucleolus-associated domains (NADs): regions of the genome that physically associate with the nucleolus and are typically transcriptionally repressed.
Phase separation: a biophysical process by which biomolecules self-organize into membraneless compartments (condensates).
Promoter and pre-rRNA antisense (PAPAS): a stress-responsive long noncoding RNA transcribed antisense to rDNA loci that regulates rRNA transcription through chromatin remodeling and nucleolar repression.
Promoter-associated RNAs (pRNAs): small noncoding RNAs transcribed from rDNA promoter regions that contribute to rDNA silencing, chromatin organization, and the regulation of nucleolar activity.
RAN translation: noncanonical translation mechanism producing peptides from repeat expansion RNAs without AUG initiation.
Rapamycin: an mTOR inhibitor that modulates protein synthesis, autophagy, and cellular metabolism, with context-dependent effects on muscle homeostasis.
Ribosome-associated quality control (RQC): a surveillance pathway that detects stalled ribosomes and promotes the degradation of aberrant proteins.
Small nuclear ribonucleoproteins (snRNPs): RNA–protein complexes essential for pre-mRNA splicing, and their assembly depends on SMN function.
Small nucleolar RNA (snoRNA): a class of ncRNAs that guide chemical modifications of rRNA and contribute to ribosome biogenesis.
Spinal muscular atrophy (SMA): a neuromuscular disease caused by SMN deficiency, affecting RNA metabolism and motor neuron survival.
TDP-43: an RNA-binding protein involved in RNA processing and condensate dynamics; its mislocalization is a hallmark of ALS.

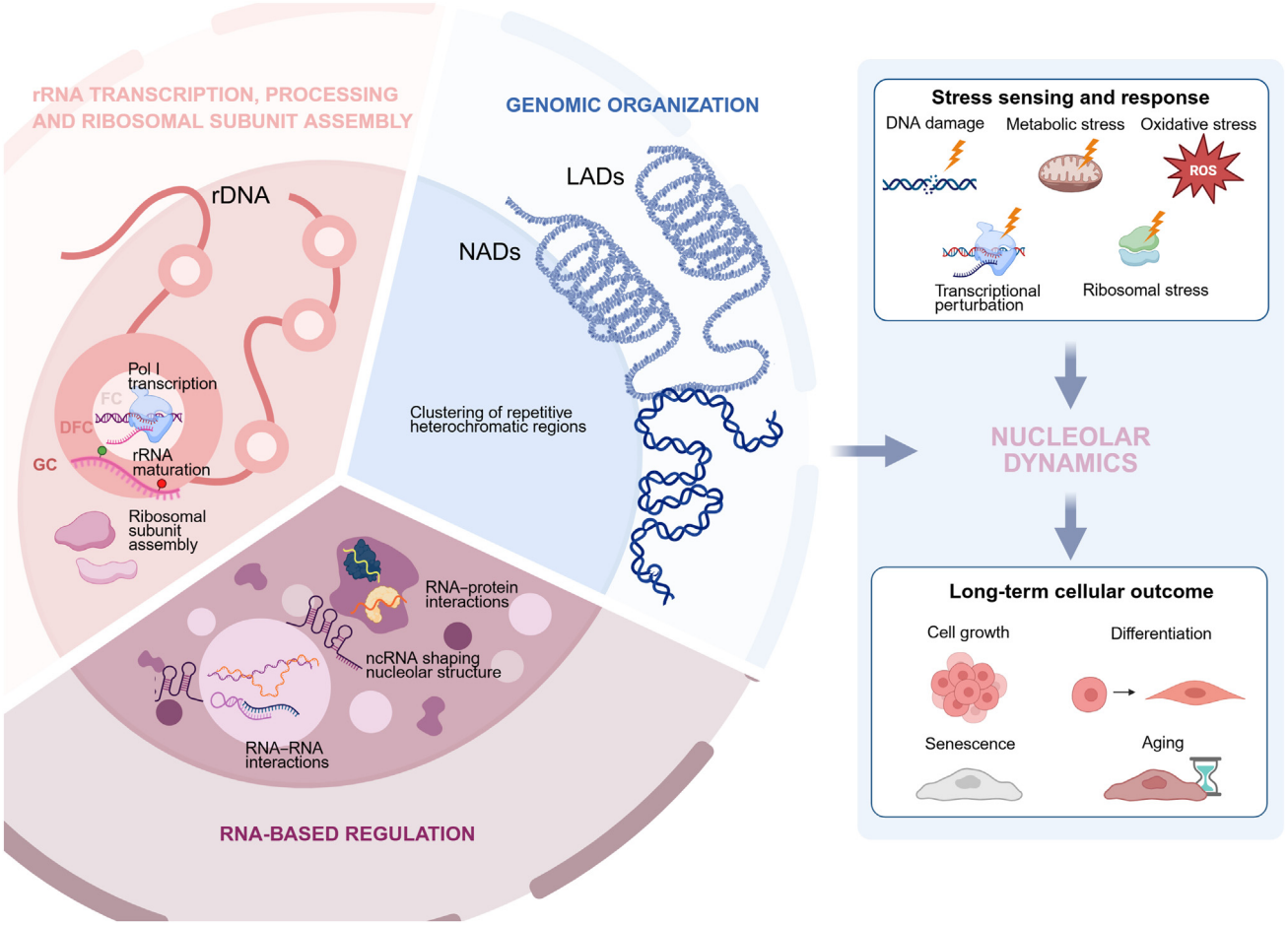

Figure 1. The nucleolus as a multifunctional hub linking ribosome biogenesis, genome organization, RNA-based 60 regulation, and stress responses. The nucleolus is a dynamic nuclear compartment that coordinates rRNA transcription, processing, and ribosomal subunit assembly while also contributing to higher-order genome organization through interactions with nucleolus-associated domains, lamina-associated domains, and repetitive heterochromatic regions. RNA-mediated mechanisms, including RNA–RNA interactions, RNA–protein assemblies, and noncoding RNA-dependent scaffolding, further shape nucleolar architecture and function. In response to cellular stresses such as DNA damage, metabolic and oxidative stress, transcriptional perturbation, and ribosomal stress, nucleolar dynamics are remodeled, influencing long-term cellular outcomes including growth, differentiation, senescence, and aging. ROS, reactive oxygen species.

Moreover, RNA-binding proteins connect RNA dysregulation and nucleolar dysfunction. Proteins such as TDP-43 and **FUS** do not merely bind RNA targets; they also regulate condensate dynamics and the spatial organization of nuclear RNA networks [20–22,50]. When mutated or mislocalized, their effects propagate beyond individual transcripts to alter the physical state of nuclear compartments, including the nucleolus [7,19,50].

A key concept integrating these observations is **phase separation**. Since nucleolar organization depends on a finely balanced network of RNA-dependent interactions [2,3,50,59], disease-associated RNAs and altered RNA-binding proteins can shift the nucleolus toward less dynamic and dysfunctional states. The result is not only structural disorganization but also an alteration in rRNA metabolism, defective ribosome biogenesis, and impaired translation regulation [28,29,60,61].

Table 1. Nucleolar dysfunction across disease models: a literature-based overview

Disease/context	Key study	Model(s) used	Nucleolar alteration/ associated process	Main supporting experiments	Strength of evidence
C9ORF72-ALS/FTD	White *et al.*, 2019 [7]	*In vitro* condensates; HeLa and U2OS cells expressing poly(PR)	Disruption of NPM1-dependent nucleolar phase separation	NPM1 phase-separation assays; FRAP; nucleolar localization; rRNA/nucleolar component redistribution	Very strong/direct
C9ORF72/sporadic ALS	Aladesuyi Arogundade *et al.*, 2021 [8]	Human iPSC-derived spinal motor neurons; ALS patient material	Nucleolar stress preceding TDP-43 mislocalization	Nucleolar stress markers; TDP-43 localization; temporal analysis	Strong/direct
C9ORF72 poly(PR) toxicity	Cicardi *et al.*, 2023 [9]	Primary neurons and transfected mammalian cell lines	Nucleolar accumulation of poly(PR) associated with nucleolar stress	Nucleolar localization; stress-response assays; rescue by reducing nuclear PR	Strong/supportive
FSHD	Salsi *et al.*, 2025 [10]	Primary FSHD myoblasts and differentiated myotubes	FRG2A accumulation at the nucleolar periphery associated with NAD remodeling and repression of rRNA transcription	RNA-FISH; ChIRP-seq/interactome; NAD-associated chromatin mapping; EU-based rRNA transcription assays; translation assays	Very strong/direct
SMA	Karyka *et al.*, 2022 [11]	SMN-deficient human fibroblasts and cellular SMA models	Increased rDNA damage associated with impaired rRNA synthesis and translation	γH2AX/rDNA damage assays; rRNA synthesis assays; puromycin-based translation assays	Moderate-to-strong/direct
SMA/stress response	Musawi *et al.*, 2023 [18]	HeLa cells under genotoxic/transcriptional stress	Defective nucleolar recovery following stress	SMN trafficking; RNAP1/FBL relocalization; nucleolar reassembly assays	Moderate; stress-context dependent
SMA	Lauria *et al.*, 2020 [12]	SMA mouse tissues	Altered selective translation linked to specialized ribosome populations	RiboLace; ribosome profiling; biochemical ribosome isolation; translational analyses	Strong translational evidence; indirect nucleolar link
DM1	Bogard *et al.*, 2025 [13]	DM1 myogenic cellular models	Dysregulation of snoRNA-associated pathways with potential nucleolar relevance	snoRNA manipulation; differentiation assays; transcriptomic analysis	Preliminary/indirect
DMD	Falzarano *et al.*, 2023 [14]	Primary DMD myogenic cells and muscle biopsies	Partial nucleolar localization and nuclear retention of dystrophin transcripts	RNA *in situ* hybridization; subcellular localization analysis	Preliminary/indirect

C9ORF72: chromosome 9 open reading frame 72; ChIRP: Chromatin Isolation by RNA Purification; EU: 5-ethynyl uridine; FBL: fibrillarin; FRAP: fluorescence recovery after photobleaching; FTD: frontotemporal dementia; iPSC: induced pluripotent stem cell; poly(PR): poly(proline-arginine) dipeptide repeat protein; RNAP1: RNA polymerase I; TDP-43: TAR DNA-binding protein 43.

In this model, the nucleolus acts as an integrative hub that converts RNA imbalance into defects in ribosome production and translational output.

Genome organization and nucleolar interactions

RNA-mediated changes in nucleolar state are unlikely to remain confined to ribosome biogenesis alone. The nucleolus is also a major organizer of higher-order chromatin architecture, and its organization is tightly linked to RNA-driven interactions and broader 3D genome topology [3,31]. Perturbations in nucleolar structure or composition can therefore reshape genome–nucleolus interactions, heterochromatin organization, and nuclear compartmentalization, with direct consequences for gene expression and regulatory element accessibility [4–6,62].

Key figure

Nucleolar dysfunction as a convergent pathogenic hub in neuromuscular and neurodegenerative disease

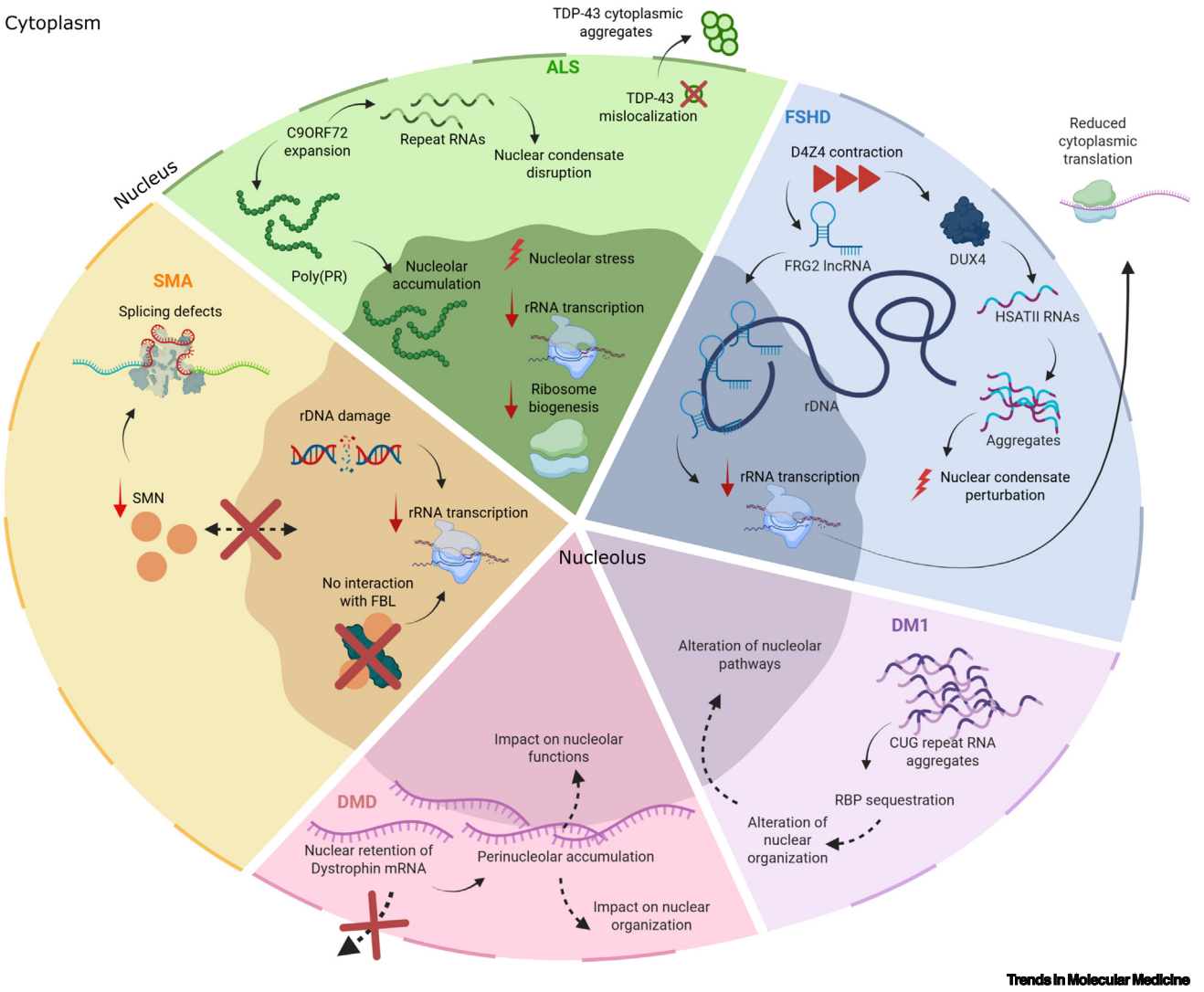

Figure 2. Distinct disease-associated genetic and RNA-mediated alterations converge on the nucleolus, leading to defects in nuclear organization, rRNA transcription, ribosome biogenesis, and translational control. In ALS, repeat-derived RNAs and dipeptide repeat proteins associated with C9ORF72 expansions disrupt nuclear condensates and induce nucleolar stress. In FSHD, D4Z4 contraction promotes expression of FRG2 lncRNAs, DUX4, and HSATII RNAs, perturbing nucleolar organization and translation. In DM1, toxic CUG-repeat RNA aggregates sequester RNA-binding proteins and alter nuclear architecture. In DMD, nuclear retention and perinucleolar accumulation of dystrophin mRNA affect nucleolar function, whereas in SMA, SMN deficiency disrupts nucleolar integrity and ribosome biogenesis. Together, these findings support a model in which nucleolar dysfunction represents a central and potentially unifying mechanism underlying selective cellular vulnerability across neuromuscular disorders.

Because the nucleolus is itself an RNA–protein condensate, its ability to organize chromatin most likely depends on scaffold-like interactions mediated by nucleolar RNAs and proteins of the granular compartment [63]. A recent study has shown that nucleophosmin 1 (NPM1), a major

Box 1. Tools to dissect RNA-nucleolus-translation interplay

- Mapping genome-nucleolus architecture

Chromosome conformation approaches, such as Hi-C and nucleolus-centered Hi-C, together with TSA-seq and DamID-based mapping, enable the identification of NADs and their relationship with LADs, revealing how genome architecture couples with nucleolar function [4–6,31].

- Profiling RNA localization and molecular neighborhoods

RNA-FISH and RNA-centric capture approaches, including ChIRP, RAP, and CHART, which differ in probe design, target accessibility, and hybridization stringency, identify nucleolar-enriched transcripts and their chromatin and protein interactions [32–34]. By contrast, O-MAP, conceptually related to APEX-based proximity labeling, resolves the local protein, RNA, and genomic environment of target RNAs *in situ* and is applicable to primary and low-input systems [35].

- Interrogating nucleolar RNA architecture and dynamics

Recent approaches enable direct mapping of RNA-dependent nucleolar organization and rRNA-processing landscapes. These methods identify RNAs associated with specific nucleolar compartments, characterize their local molecular environment, and determine how RNA perturbations reshape nucleolar architecture and function [3,35,36].

- Measuring ribosome biogenesis and ribosomal efficiency

rRNA transcription assays, nascent RNA labeling, and ribosome profiling (Ribo-seq) quantify ribosome production and translational output [37,38]. Complementary approaches, such as polysome profiling, RiboLace [39,40], and RiboMeth-seq [41,42], provide additional resolution on ribosome engagement, active translation, and rRNA modification status. Recent methods, such as ribosome-associated protein identification by affinity to sulfhydryl-charged resin (RAPIDASH) [43] and spatially resolved mapping of translational machinery, enable the characterization of ribosome composition, associated factors, and subcellular organization, revealing dynamic and context-dependent remodeling of translational machinery [44,45].

- Resolving translational control across layers

Integrated analysis of transcriptomics, translatomics (e.g., translation efficiency and ribosome occupancy from Ribo-seq), and quantitative proteomics enables the identification of discrepancies between RNA abundance and protein output [38,41]. This multilayered approach reveals gene-specific translational regulation and disease-associated reprogramming of protein synthesis and supports emerging frameworks that redefine translated regions beyond canonical annotations [46].

- Resolving repeat-rich and RNA-driven mechanisms

Long-read sequencing, particularly Oxford Nanopore Technologies [47], combined with repeat-aware genomic frameworks such as T2T assemblies [48] and pangenome references [49], is essential for accurately capturing repeat-derived transcripts and structurally complex loci, enabling the study of RNA-driven nucleolar and translational dysfunction across multiple regulatory layers.

APEX: engineered ascorbate peroxidase proximity labeling; CHART: capture hybridization analysis of RNA targets; ChIRP: Chromatin Isolation by RNA Purification; DamID: DNA adenine methyltransferase identification; Hi-C: chromosome conformation capture coupled to high-throughput sequencing; O-MAP: oligonucleotide-mediated proximity-interactome mapping; RAP: RNA antisense purification; RNA-FISH: RNA fluorescence *in situ* hybridization; TSA-seq: tyramide signal amplification sequencing.

component of the nucleolar granular compartment, mediates the association of **nucleolus-associated domains (NADs)** with the nucleolus and contributes to the establishment of repressive chromatin states [36]. Thus, nucleolar condensates may provide a dynamic anchoring surface for heterochromatic and repeat-rich domains, whose positioning can be redistributed between nucleolar- and lamina-associated compartments [**lamina-associated domains (LADs)**] according to cellular state [5,6,64]. Through this NAD–LAD interplay, nucleolar dynamics may influence chromatin accessibility, affect long-range chromatin interactions, and modulate the activity of transcription-associated regulatory hubs, thereby contributing to gene regulation across multiple scales of 3D genome organization.

Neuromuscular disorders are beginning to reveal this connection. In FSHD, the 4q35 region displays altered long-range chromatin interactions, supporting the view that repeat-linked disease mechanisms can reshape higher-order genome organization [65,66]. More recent observations further suggest that D4Z4-like regions and ribosomal DNA clusters can engage in dynamic interchromosomal contacts involving nucleolar regions, reinforcing the possibility that the nucleolus participates in organizing repeat-rich pathogenic landscapes [67].

Such reorganization may influence the transcriptional activity of repeat elements and nearby genes, promote aberrant RNA production, and contribute to the establishment of disease-specific chromatin states [52,68]. In parallel, the repeat-rich and epigenetically labile architecture of the FSHD locus itself supports the idea that disease-associated RNAs and chromatin states may be integrated within nucleolar regulatory environments [10,69].

In DM1, RNA toxicity appears to be linked to a broader reorganization of chromatin and nuclear architecture occurring during differentiation [26]. In ALS, repeat expansion disorders such as C9ORF72 are increasingly linked to epigenetic and 3D genome alterations, raising the possibility that nucleolar dysfunction and chromatin rewiring may be mechanistically connected rather than parallel consequences of the disease [70].

Although these links remain less well resolved than canonical studies of ribosome biogenesis, they point toward an emerging view in which the nucleolus participates in structuring disease-relevant chromatin environments.

This architectural dimension is likely to be especially important in repeat-associated and RNA-mediated disorders, in which local chromatin perturbations, long-range interactions, and condensate behavior are intertwined. Understanding how neuromuscular disease mechanisms reshape genome–nucleolus interactions may therefore be essential for explaining how nuclear organization contributes to selective vulnerability and downstream translational defects.

From nucleolar dysfunction to translation defects

The key pathogenic question is not simply whether the nucleolus is altered structurally and functionally, but how that disruption is converted into cellular dysfunction. The most immediate answer lies in ribosome biogenesis.

Ribosome biogenesis integrates signals from growth, stress, and metabolic pathways, and its disruption underlies a group of disorders known as ribosomopathies [71,72]. These conditions highlight a key paradox: defects in a ubiquitous process can produce highly tissue-specific phenotypes. This paradox has helped motivate the concept of ribosome heterogeneity and specialization, in which variation in ribosome composition or availability selectively affects the translation of particular mRNA subsets [73–75]. Recent work has further expanded this framework by showing that, in addition to ribosome number [76], ribosome diversity can also arise from variation at the rRNA level and that the translational machinery displays dynamic subcellular organization at single-molecule resolution, reinforcing the view that translational control is both compositional and spatially regulated [44,45]. Within this model, nucleolar dysfunction is likely to be better understood as a qualitative shift in translational control rather than a purely quantitative defect. Indeed, alterations in rRNA transcription, processing, and modification may influence not only the number of ribosomes produced but also their composition and functional properties. As a result, specific subsets of mRNAs may be translated with different efficiencies, leading to selective changes in protein synthesis rather than a uniform reduction in translational output.

In neuromuscular disorders, multiple lines of evidence support this view. In FSHD, FRG2 lncRNAs repress rRNA transcription and reduce ribosome output, leading to impaired cytoplasmic translation [10]. These effects are not uniform but appear to selectively impact muscle-relevant gene expression programs. Similarly, DUX4 expression is associated with global suppression of translation alongside selective induction of stress-responsive transcripts, suggesting a rewired translational landscape [25]. Proteomic analyses further reveal discrepancies between transcriptional and protein-level changes, supporting the idea that post-transcriptional and translational controls are key regulatory layers in disease, in addition to post-translational ones [24].

A comparable principle applies to ALS, where distinct upstream mechanisms converge on translational dysfunction [77]. In C9ORF72-associated disease, arginine-rich dipeptide repeat proteins bind ribosomal components, interfere with ribosome function, and perturb nucleolar organization [78,79]. These effects are compounded by defects in **ribosome-associated quality control**

Box 2. Routes to nucleolar stress: causes and consequences

Nucleolar stress arises from diverse perturbations that affect ribosome biogenesis at different levels. Despite their distinct origins, these stresses converge on common outputs, including nucleolar reorganization, altered rRNA metabolism, and changes in translational control.

- Transcriptional stress

Cause: Inhibition or dysregulation of RNA polymerase I and rRNA synthesis (e.g., actinomycin D; signaling pathways affecting rDNA transcription).

Output: Rapid nucleolar segregation, reduced rRNA production, and decreased ribosome biogenesis [60,84].

- Genotoxic stress

Cause: DNA damage at rDNA loci, including double-strand breaks and replication stress.

Output: Nucleolar reorganization, formation of nucleolar caps, activation of DNA damage responses, and impaired rRNA transcription [61].

- Proteotoxic and condensate stress

Cause: Accumulation of misfolded proteins or aberrant species that alter phase-separated compartments (e.g., arginine-rich dipeptide repeat proteins in ALS).

Output: Disruption of nucleolar material properties, altered condensate dynamics, and impaired ribosome function [7,19].

- Metabolic and oxidative stress

Cause: Changes in energy availability, nutrient sensing, and redox balance (e.g., mTOR/AMPK signaling).

Output: Downregulation of ribosome biogenesis, adaptive reduction of translational capacity, and nucleolar remodeling [85].

- RNA-mediated stress

Cause: Accumulation or mislocalization of noncoding RNAs, including repeat-derived transcripts and nucleolar-enriched lncRNAs.

Output: Perturbation of RNA–protein interactions, altered nucleolar architecture, and dysregulation of rRNA transcription and translation [51,52].

These stress types are not mutually exclusive and often coexist, generating composite nucleolar states. Their convergence highlights the nucleolus as a central hub that translates diverse upstream perturbations into shared defects in ribosome biogenesis and translational output.

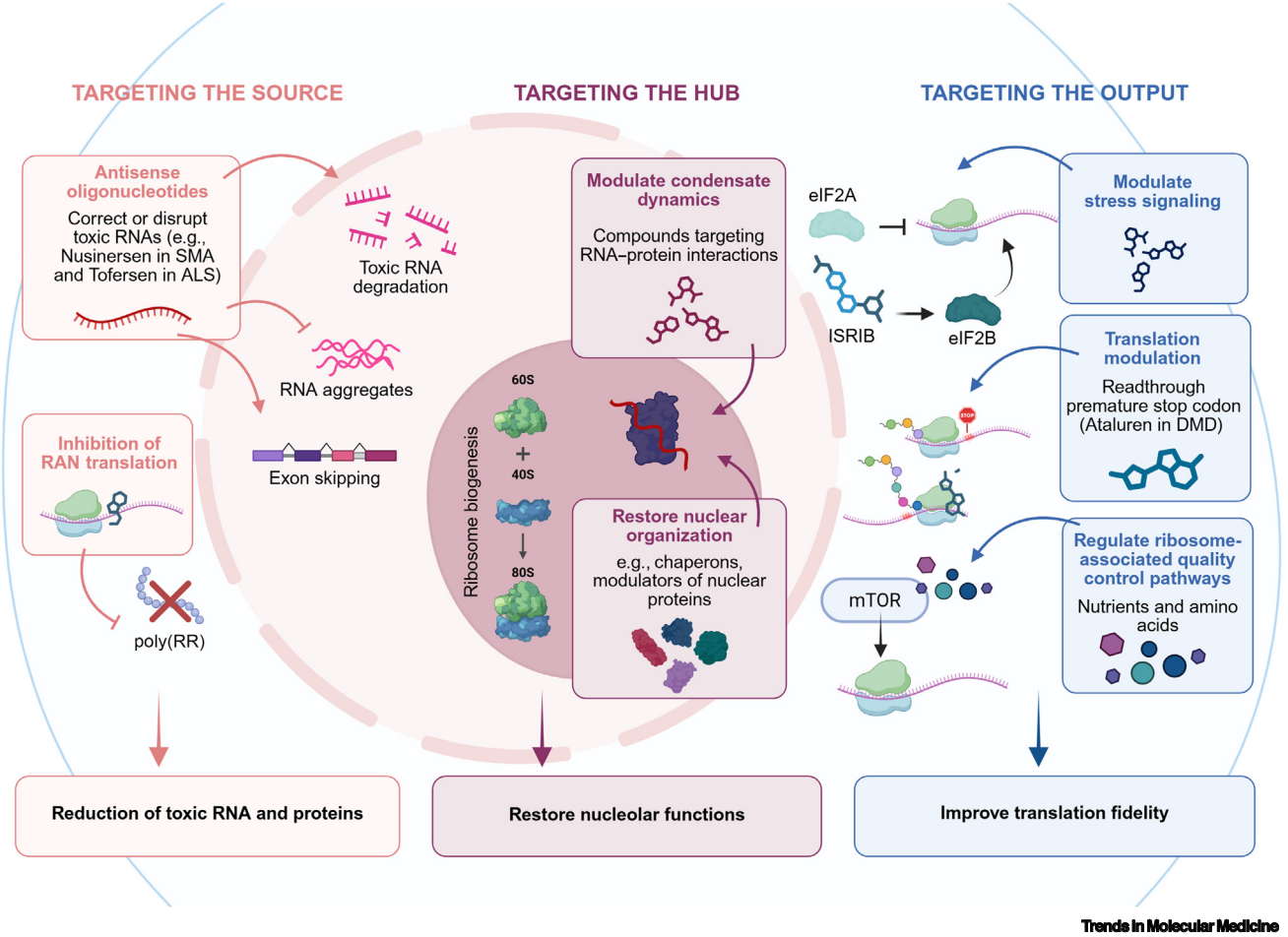

Figure 3. Therapeutic strategies targeting RNA toxicity, nucleolar dysfunction, and translational impairment in neuromuscular disease. Schematic overview of emerging therapeutic approaches acting at distinct levels of the pathogenic cascade. Strategies targeting the source include antisense oligonucleotides, exon skipping, toxic RNA degradation, and inhibition of repeat-associated non-AUG (RAN) translation to reduce pathogenic RNAs and proteins. Approaches targeting the nucleolar hub aim to restore nucleolar organization and condensate dynamics through modulation of RNA–protein interactions, molecular chaperones, or factors involved in ribosome biogenesis. Downstream interventions targeting the translational output include modulation of stress-response signaling, translation fidelity, ribosome-associated quality control pathways, and mTOR-dependent translational regulation. These interventions illustrate how RNA metabolism, nucleolar homeostasis, and translational control represent interconnected and potentially druggable pathways in neuromuscular disorders.

(RQC) pathways, leading to both impaired protein synthesis and increased proteotoxic stress. As a result, neurons experience a dual burden of reduced translational capacity and accumulation of aberrant translation products [80,81].

In SMA, SMN deficiency primarily alters RNA processing and ribonucleoprotein (snRNP) assembly [12], with additional reported effects on ribosome biogenesis and ribosomal DNA stability [11]. Recent studies further suggest a functional interplay between SMN pathways and ribosome function [12,82]. However, whether nucleolar or translational alterations represent central pathogenic drivers in SMA remains unclear. It is plausible that, given the high demand for protein synthesis in motor neurons, even relatively modest perturbations in translational homeostasis could contribute to neuronal vulnerability. This aligns with broader evidence that nucleolar stress can influence pathways controlling apoptosis and cellular resilience.

Across these conditions, a consistent theme emerges: translational output is not a passive reflection of mRNA abundance [83], but rather a regulated and dynamic layer of gene expression that is highly sensitive to the nucleolar state. Nucleolar dysfunction can therefore decouple transcription and translation, generating mismatches between RNA and protein levels. Such discrepancies are particularly detrimental to cells with specialized proteomes, including muscle fibers and motor neurons.

An additional layer of complexity arises from the integration of nucleolar function with cellular stress responses. The nucleolus acts as a sensor of diverse stress signals, including DNA damage, oxidative stress, and metabolic imbalance [61,84]. The major routes leading to nucleolar stress and their downstream consequences are summarized in Box 2.

Perturbations in nucleolar integrity can activate stress pathways that resemble ribosomopathies and directly impact translational capacity, linking alterations in nucleolar dynamics to broader functional consequences [85]. Chronic perturbation of nucleolar activity can therefore reinforce maladaptive translational programs, amplifying disease-associated phenotypes over time.

The clinical relevance of this framework, including its implications for patient stratification, functional biomarkers, and treatment monitoring, is discussed in the Clinician's corner.

From disease mechanisms to therapeutic opportunities

This framework suggests three complementary levels of therapeutic intervention: targeting the upstream RNA lesion, modulating the nucleolar hub, or reshaping the downstream translational response. These therapeutic intervention points are illustrated in Figure 3.

Targeting the source: RNA-mediated pathogenic mechanisms

Growing efforts aim to neutralize toxic RNA species more directly. In repeat-expansion disorders, for example, antisense oligonucleotides and small molecules can reduce toxic RNA accumulation, disrupt pathogenic RNA–protein interactions, or inhibit repeat-associated non-AUG **RAN translation**. More broadly, the reported success of RNA-directed therapies, including nusinersen in SMA, exon-skipping approaches in DMD, and tofersen for SOD1-associated ALS [86], demonstrates that disease-associated RNA pathways can be effectively targeted *in vivo* [87,88], although they have not been specifically developed to target nucleolar dysfunction.

In disorders such as DM1, oligonucleotide- and small molecule-based strategies targeting expanded CUG repeat RNAs are actively being explored [13]. In C9ORF72-associated disease, inhibition of RAN translation using antisense oligonucleotides or small molecules can reduce toxic dipeptide repeat production and attenuate downstream cellular stress [89].

Within the framework proposed here, these approaches are conceptually relevant not because they directly target the nucleolus but because they act upstream of RNA-mediated processes that may converge on nucleolar dysfunction, altered ribosome biogenesis, and translational imbalance.

Targeting the hub: nucleolar activity and condensate behavior

A second level of intervention involves the nucleolus itself. Ribosome biogenesis is, in principle, pharmacologically tractable, as shown by RNA polymerase I inhibitors such as **CX-**

Clinician's corner

Neuromuscular disorders are traditionally interpreted within a gene-centric framework, in which a primary mutation is expected to determine disease onset and progression. In clinical practice, however, variability often exceeds what can be explained by the causal genetic lesion alone. Patients carrying the same genetic defect may display markedly different phenotypes, rates of progression, and responses to therapy, indicating that additional layers of regulation shape disease expression. These issues challenge the one gene–one disease paradigm and necessitate a paradigm shift. Indeed, increasing evidence supports a view in which disease phenotypes emerge from the interplay between genome regulation, RNA-dependent processes, and protein synthesis. Rather than acting as independent modules, these processes form an integrated system that defines cell state. Within this framework, the nucleolus occupies a central position, linking RNA imbalance to alterations in ribosome production and translational output. Changes in nucleolar function, therefore, provide a plausible mechanism through which upstream variation is converted into downstream cellular dysfunction.

This perspective has direct implications for patient stratification. Individuals with comparable genetic lesions may differ in how these regulatory layers are engaged, resulting in distinct molecular states that influence disease severity and progression. Such differences are not readily captured by DNA-based diagnostics alone but may become accessible through integrated analyses that reflect functional cellular output.

From a clinical standpoint, this challenges the sufficiency of candidate gene-based approaches and suggests the need for models that account for system-level variability. It also opens the possibility of identifying biomarkers that reflect the functional state of the cell rather than the presence of a mutation alone, with potential applications in prognosis and treatment monitoring.

Finally, this framework expands therapeutic opportunities. In addition to mutation-specific interventions,

5461 and BMH-21, which have been extensively explored in oncology [90,91]. In the case of neuromuscular disease, the goal should not be to inhibit rRNA synthesis but rather to selectively rebalance nucleolar production in conditions where ribosome production and nucleolar dynamics are pathologically altered.

An emerging parallel strategy concerns condensate behavior. Because nucleolar organization depends on phase separation, compounds that modulate protein–RNA interactions or restore condensate material properties may help recover functional compartmentalization. Experimental work suggests that disease-associated factors, such as TDP-43 and arginine-rich dipeptide repeats, can be modulated through chaperone pathways or compounds affecting condensate dynamics [21,92]. Although still in their early stages, these approaches target the physical state of pathogenic condensates rather than single downstream effectors.

targeting downstream regulatory states may provide alternative or complementary strategies, particularly for patients who do not respond to current treatments. The central challenge will be to define how these regulatory states can be modulated in a controlled and disease-relevant manner.

Targeting the output: translation, stress signaling, and proteostasis

A third area of focus concerns the downstream translational response to nucleolar dysfunction. The **integrated stress response** is especially relevant here because it links cellular stress to global translational repression. Integrated stress response inhibitor (ISRIB), which restores translation downstream of eIF2α phosphorylation by stabilizing eIF2B, indicates that stress-linked translational defects can be pharmacologically bypassed [93,94]. In neurodegenerative settings, ISRIB improves proteostasis and cellular function, suggesting that similar strategies may help counter the consequences of nucleolar stress.

Translation can also be modulated more directly. Ataluren, which promotes readthrough of premature stop codons, illustrates the broader principle that defects in translational decoding can be targeted for therapeutic purposes, although its clinical benefit remains debated [95,96]. In parallel, RQC pathways represent a particularly relevant target in disorders such as ALS, where impaired translation and proteotoxic stress coexist [80,97,98]. Enhancing RQC or broader proteostasis mechanisms may reduce the burden of aberrant translation products while improving translational fidelity.

Finally, translational control is tightly integrated with metabolism. Pathways such as **mTOR** couple nutrient availability to ribosome biogenesis and protein synthesis, making them natural candidates for therapeutic modulation. Amino acid sensing, mTOR signaling, and **rapamycin**-sensitive pathways influence muscle homeostasis and neuromuscular junction stability [99–101], illustrating how metabolic regulation intersects with nucleolar and translational dysfunction.

These therapeutic axes are unlikely to be mutually exclusive. Since nucleolar dysfunction integrates multiple upstream insults, the most effective strategies may lie in combinatorial approaches combining RNA-directed interventions with those targeting stress signaling, condensate dynamics, or translational control. At the same time, the essential nature of the nucleolus imposes a major constraint: treatment will need to restore balance rather than suppress core biosynthetic functions globally.

A nucleolar perspective, therefore, does more than nominate a single target. It reframes neuromuscular disease as a network problem involving RNA toxicity, ribosome biogenesis, nuclear organization, stress adaptation, and translational control, and it identifies multiple points at which that network may be therapeutically rewired.

Concluding remarks

A comprehensive message emerges from the disorders discussed in this review: the nucleolus is not merely a passive victim of cellular stress but an active regulatory hub through which diverse pathogenic mechanisms converge to impair ribosome biogenesis, translation, and cellular homeostasis. Across neuromuscular diseases, abnormalities in repeat-derived RNAs, RNA-binding proteins, chromatin organization, and condensate behavior repeatedly intersect at the level of nucleolar structure and activity.

This perspective places RNA at the center of nucleolar regulation and identifies translational dysfunction as a direct consequence of an altered nucleolar state. Importantly, this view extends beyond global defects in protein synthesis. Alterations in rRNA transcription, processing, and modification may generate ribosomes with distinct compositions and functional properties, contributing to the emergence of specialized ribosome populations with selective mRNA preferences.

In this framework, nucleolar dysfunction is not only a quantitative constraint on ribosome production but may also reshape ribosome heterogeneity. Disease-associated perturbations of the ribosome pool may selectively impact subsets of transcripts that are critical for muscle and motor neuron function, providing a mechanistic basis for cell-type-specific vulnerability. This vulnerability may be particularly pronounced in skeletal muscle fibers and motor neurons, which experience limited cellular turnover and are, therefore, especially sensitive to cumulative defects in nucleolar homeostasis, proteostasis, and translational control during aging. In these cells, chronic perturbation of nucleolar activity may progressively amplify defects in RNA metabolism and stress adaptation, ultimately contributing to the late onset and progressive nature of many neuromuscular disorders.

A nucleolar framework may, therefore, help explain a key feature of neuromuscular disease: why diverse upstream lesions can produce overlapping downstream phenotypes despite distinct genetic origins.

This view also opens new therapeutic spaces. Approaches that modulate pathogenic RNAs, nucleolar activity, condensate behavior, or translational control may complement mutation-specific strategies and offer broader opportunities for intervention. The central challenge now is to define when nucleolar dysfunction is causal, how it becomes cell-type specific, and whether disease-associated ribosome states can be selectively modulated without compromising essential cellular functions (see Outstanding questions). Addressing these questions will be essential if the nucleolus is to move from an emerging concept in disease biology to a clinically actionable framework.

Outstanding questions

How do distinct classes of RNA, including repeat-derived transcripts, cooperate or compete to shape nucleolar architecture and function?

To what extent do RNA-mediated changes in nucleolar organization directly drive disease phenotypes rather than reflect secondary stress responses?

How do alterations in rRNA transcription, processing, and modification influence ribosome composition and contribute to selective mRNA translation in neuromuscular cells?

Can nucleolar dysfunction explain the disconnect between the transcriptome and proteome observed across neuromuscular disorders?

Does nucleolar dysfunction drive the emergence of specialized ribosomes that selectively regulate mRNA translation in disease?

What determines cell-type-specific vulnerability to nucleolar perturbations, particularly in muscle fibers and motor neurons?

How do disease-associated RNAs and RNA-binding proteins alter the material properties of nucleolar condensates, and can these changes be reversed?

Are nucleolus-associated chromatin domains dynamically reshaped in disease, and how does this impact genome organization and transcriptional regulation?

Can targeting nucleolar pathways, such as ribosome biogenesis, RNA processing, or condensate dynamics, restore translational homeostasis without compromising essential cellular functions?

How can emerging technologies, including long-read sequencing and spatial transcriptomics, be leveraged to resolve nucleolar RNA dynamics in disease-relevant contexts?

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Declaration of interests

All authors have no conflicts of interest associated with this study.

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