REVIEW ARTICLE

Role of CLIP technologies in understanding RNA–protein interactions in neurological and neuro-oncological disorders: a narrative review

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ABSTRACT

RNA-binding proteins (RBPs) are central regulators of posttranscriptional gene expression, controlling RNA splicing, stability, localization, transport, and translation. Disruption of RBP–RNA regulatory networks is increasingly implicated across neurological and neuro-oncological disorders. Crosslinking and immunoprecipitation (CLIP) technologies enable transcriptome-wide identification of RBP-associated RNAs and, depending on the method, can provide high-resolution information on protein–RNA interaction sites. This narrative review summarizes the fundamental principles, major variants, method-selection considerations, and analytical challenges of CLIP-based approaches, with particular emphasis on HITS-CLIP, PAR-CLIP, iCLIP, eCLIP, irCLIP, and uvCLAP. We critically evaluate disease-specific evidence from amyotrophic lateral sclerosis and frontotemporal dementia, fragile X syndrome, spinal muscular atrophy, myotonic dystrophy, Huntington disease, spinocerebellar ataxias, Alzheimer disease, and neuroblastoma. Particular attention is given to distinguishing direct evidence derived from CLIP experiments from complementary transcriptomic, biochemical, and functional data. We further discuss key methodological and analytical limitations, including crosslinking and RNase biases, antibody specificity, input requirements, reproducibility, normalization, peak calling, and the inability of CLIP alone to establish functional causality. Emerging developments, including low-input and single-cell-compatible strategies, integrative multi-omics, and improved computational approaches, may enhance the resolution and biological interpretability of RBP–RNA interaction maps. Ultimately, CLIP-defined regulatory networks can provide valuable frameworks for identifying disease-relevant RNA targets and prioritizing candidate biomarkers and RNA-targeted therapeutic strategies, but independent functional and clinical validation remains necessary to establish biological and translational relevance.

Keywords: CLIP, RNA-binding proteins, RNA–protein interactions, neurological disorders, neurodegenerative diseases, neuro-oncology, HITS-CLIP, PAR-CLIP, iCLIP, eCLIP, irCLIP, uvCLAP.

Introduction

RNA-binding proteins (RBPs) are central regulators of posttranscriptional gene expression, controlling RNA splicing, stability, localization, transport, and translation. These functions are particularly critical in neurons, where extensive cellular processes, compartmentalized RNA metabolism, and activity-dependent responses require precise spatial and temporal regulation of RNA fate. Consequently, genetic or acquired perturbation of RBP function can disrupt neuronal homeostasis and contribute to a broad spectrum of neurological diseases (1,2).

Although the biological importance of RBP–RNA interactions is well established, their direct

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characterization in cells and tissues remains technically challenging. Conventional RNA immunoprecipitation-based approaches can identify transcripts associated with a given RBP but may not reliably distinguish direct from indirect associations or determine the precise locations of interaction sites. CLIP addresses these limitations by using ultraviolet (UV) irradiation to covalently stabilize protein–RNA contacts before immunoprecipitation, controlled RNA fragmentation, library preparation, sequencing, and computational analysis. Depending on the specific CLIP strategy, these approaches can generate transcriptome-wide maps of RBP-associated RNAs with varying degrees of positional resolution (1,2).

Since the development of high-throughput sequencing-based HITS-CLIP, CLIP methodologies have evolved substantially. Approaches such as PAR-CLIP, iCLIP, eCLIP, irCLIP, and uvCLAP differ in their crosslinking strategies, library-construction schemes, experimental requirements, controls, and analytical characteristics. These methods are therefore not interchangeable, and their relative advantages depend on the biological question, RBP under investigation, experimental material, required positional resolution, available input, and control design. Accordingly, method selection should be guided by experimental objectives and biological context rather than by the assumption that a single CLIP platform is universally superior (1-4).

The present review examines how CLIP-based technologies have contributed to the mechanistic understanding of RNA dysregulation across neurological and neuro-oncological disorders. Particular emphasis is placed on evidence derived directly from CLIP experiments and on distinguishing such evidence from complementary transcriptomic, biochemical, and functional findings. The review focuses on disease-specific applications in ALS/FTD, FXS, SMA, myotonic dystrophy, Huntington disease (HD), spinocerebellar ataxias (SCAs), Alzheimer disease (AD), and neuroblastoma. Neuroblastoma is included as a neuro-oncological application because RBP-mediated posttranscriptional regulation has emerged as a biologically relevant component of its molecular pathophysiology (5,6). By integrating methodological considerations with disease-specific evidence, this review aims to highlight both the contributions and limitations of CLIP-based approaches in defining disease-relevant RBP–RNA regulatory networks and to identify emerging opportunities for their application in mechanistic studies and therapeutic target prioritization.

Narrative Review Methodology

This article was prepared as a narrative review. For the revised manuscript, the literature was updated through May 2026 using PubMed/MEDLINE and Google Scholar. The search strategy combined terms related to CLIP and RBP–RNA interactions with methodological and disease-specific terms. Representative search terms included "crosslinking and immunoprecipitation," "CLIP-seq," "HITS-CLIP," "PAR-CLIP," "iCLIP," "eCLIP," "irCLIP," "uvCLAP," "RNA-binding protein," "RNA–protein interaction," "amyotrophic lateral sclerosis," "frontotemporal dementia," "Fragile X syndrome," "spinal muscular atrophy," "myotonic dystrophy," "Huntington disease," "spinocerebellar ataxia," "Alzheimer disease," and "neuroblastoma".

Priority was given to primary studies that directly applied CLIP-based approaches to characterize RBP–RNA interactions in disease-relevant cells, tissues, animal models, or human specimens. Methodological reviews and primary method-development studies were used to describe CLIP principles, technical advances, and analytical considerations. Complementary studies employing RNA sequencing, RIP-seq, functional perturbation, proteomics, or other mechanistic approaches were included when they provided relevant biological context for interpreting CLIP-derived findings; however, these studies were explicitly distinguished from direct CLIP evidence. English-language publications were prioritized. References were selected based on methodological relevance, strength of primary evidence, disease relevance, and their contribution to the central objectives of the review.

As a narrative review, this article did not employ a formal PRISMA-based screening process, meta-analysis, or quantitative risk-of-bias assessment. The literature search was complemented by iterative examination of reference lists from relevant primary studies, methodological reviews, and key method-development papers. Particular attention was given to disease-specific primary CLIP studies and methodological developments relevant to the scope of this review.

Principle of CLIP Technology

CLIP is designed to capture RNA molecules that are physically associated with a selected RBP in cells or tissues. In conventional UV-CLIP, ultraviolet irradiation induces covalent crosslinking between proteins and RNA at or near sites of direct molecular contact. Following cell or tissue lysis, the RBP is immunoprecipitated using a specific antibody or affinity reagent. Controlled RNase digestion then reduces unprotected RNA while retaining short RNA fragments associated with the immunopurified protein (1,2).

The recovered RNA fragments are subsequently ligated to sequencing adapters, converted into sequencing libraries, and analyzed by high-throughput sequencing. Bioinformatic processing typically includes quality filtering, removal of technical artifacts, alignment to a reference genome or transcriptome, identification of enriched RNA regions or crosslink-associated signatures, and annotation of binding sites relative to transcript features. The resulting interaction map represents an experimental profile of RBP-associated RNA and should not be interpreted as a complete catalog of all functional RBP–RNA interactions (1,2).

Interpretation of CLIP data depends strongly on the specific assay and experimental design. Crosslinking chemistry can introduce sequence- and structure-dependent biases, whereas RNase digestion can influence the observable binding landscape by affecting which RNA fragments are retained for sequencing. Antibody specificity and immunoprecipitation efficiency can further affect enrichment, while sequencing depth

and computational peak-calling strategies influence the number and distribution of reported binding sites. Therefore, CLIP-derived signals should be interpreted using appropriate experimental controls and, where possible, supported by independent biochemical or functional validation (1,2).

Major CLIP Variants and Method Selection

Conventional UV-CLIP provides the methodological foundation for several subsequent CLIP variants. It typically uses 254-nm ultraviolet irradiation to induce covalent crosslinking between an RBP and directly associated RNA, followed by immunoprecipitation, controlled RNA digestion, library preparation, and sequencing. Although conventional UV-CLIP can provide transcriptome-wide information on RBP-associated RNAs, subsequent CLIP variants were developed to improve positional resolution, experimental efficiency, background assessment, scalability, or workflow flexibility (1-4).

HITS-CLIP couples UV CLIP with high-throughput sequencing to generate transcriptome-wide maps of RBP-associated RNAs. It remains useful when the primary objective is to define a broad RBP target landscape, although its positional resolution is generally lower than that of approaches specifically designed to exploit reverse-transcription termination or crosslink-induced sequence signatures (1,2).

PAR-CLIP incorporates photoreactive ribonucleoside analogs, such as 4-thiouridine (4SU), into newly synthesized RNA before ultraviolet irradiation. Crosslinking can generate characteristic nucleotide substitutions during reverse transcription, providing molecular signatures that facilitate the identification of protein–RNA interaction sites. However, the requirement for metabolic labeling can limit its application in primary tissues and certain in vivo settings (1,2,7).

iCLIP exploits reverse-transcription termination at or near protein–RNA crosslink sites and captures truncated cDNA products, enabling high-resolution mapping of interaction positions. However, nucleotide-level resolution should be regarded as a methodological capability rather than an unconditional outcome, as mapping precision can be influenced by crosslinking chemistry, reverse-transcription behavior, library complexity, sequencing depth, and computational processing (1,2).

eCLIP improves scalability and background assessment through standardized library construction and the use of size-matched input controls. It is particularly suited to comparative profiling of multiple RBPs and large-scale studies of RBP–RNA interactions. Nevertheless, its effective positional resolution is not intrinsically equivalent to that of iCLIP and depends on crosslinking efficiency, library preparation, sequencing depth, and peak-calling strategies (1,2,8).

irCLIP uses infrared dye-conjugated adapters to enable non-radioactive visualization of RNA–protein complexes and facilitate efficient detection during library preparation. Subsequent developments, including irCLIP-RNP and Re-CLIP, have extended CLIP-based approaches toward the characterization of RNA-associated RBP assemblies and shared RNA targets. uvCLAP combines ultraviolet crosslinking with affinity purification and provides a non-radioactive approach for identifying RBP-associated RNAs that can support multiplexed experimental designs. Although these newer platforms offer increased experimental flexibility and workflow efficiency, they remain subject to biological and analytical biases associated with crosslinking chemistry, affinity purification, RNA digestion, sequencing depth, and computational analysis. The major characteristics, advantages, limitations, and applications of the principal CLIP variants are summarized in Table 1 (2,4).

Choosing the appropriate CLIP method

Method selection should begin with the biological question rather than with the assumption that a single platform is universally optimal. HITS-CLIP is appropriate for broad transcriptome-wide target discovery when precise positional mapping is not essential. PAR-CLIP is advantageous when metabolic labeling is feasible and crosslink-associated nucleotide substitutions can assist in site assignment. iCLIP is particularly useful when high-resolution localization relative to splice sites or other regulatory elements is central to the biological question. eCLIP is well suited to standardized comparative profiling and large-scale studies involving multiple RBPs. irCLIP can be advantageous when non-radioactive visualization, efficient workflow monitoring, or limited input material is important, whereas uvCLAP is useful when affinity purification strategies and multiplexed experimental designs are practical.

The choice of method should also consider the biological material, sample availability, antibody or affinity-reagent performance, required positional resolution, sequencing depth, availability of appropriate controls, and computational infrastructure. These considerations are particularly important in disease-relevant systems, where primary tissue, patient-derived cells, or other biologically informative samples may impose substantial limitations on experimental design and input requirements (1-4,7).

Applications in Neurological and Neuro-Oncological Disorders

The disease-specific literature demonstrates that the greatest value of CLIP lies in integrating RBP–RNA binding maps with complementary transcriptomic, biochemical, and functional evidence. Accordingly, the following sections focus on the biological question addressed by each study, the experimental model in which RBP–RNA interactions were measured, and the extent to which the resulting evidence supports a mechanistic interpretation. CLIP-derived binding maps are therefore interpreted as evidence of RBP occupancy, whereas functional consequences and disease causality require independent experimental validation, as summarized in Table 2 (1,2).

Amyotrophic lateral sclerosis and frontotemporal dementia

TDP-43 and FUS are key RBPs implicated in amyotrophic lateral sclerosis (ALS) and frontotemporal

Table 1. *Comparison of major CLIP-based technologies, including their core principles, effective resolution, key advantages, limitations, and best-fit applications.*

Method	Core principle	Effective resolution	Key advantages	Key limitations	Best-fit use
HITS-CLIP	254-nm UV crosslinking + IP + sequencing	Regional to moderate	Foundational, direct in vivo mapping	Low crosslink efficiency; positional ambiguity	Global target discovery in cells/tissues (1,2)
PAR-CLIP	Photoreactive nucleoside labeling + 365-nm UV	High when mutation signatures are informative	Strong crosslinking; diagnostic RT signatures	Metabolic labeling; nucleotide bias	Cultured cells and controlled labeling experiments (1,2,7)
iCLIP	RT truncation captured at crosslink sites	High / near-nucleotide	Precise site mapping; strong for splicing	Technically demanding; computational complexity	Splicing and fine-scale RBP occupancy (1,2)
eCLIP	CLIP + size-matched input controls	High but context-dependent	Scalable; improved background control	Requires substantial sequencing and bioinformatics	Comparative RBP profiling (1,2,8)
irCLIP	Infrared adapter-based CLIP	Comparable to underlying CLIP design	Non-radioactive; rapid QC; quantitative visualization	Still dependent on antibody and crosslink performance	Low-input/QC-sensitive workflows and extensions (2,3)
uvCLAP	UV crosslinking + affinity purification	Method-dependent	Robust, non-radioactive, multiplexable	Requires suitable affinity strategy	Tagged RBPs and multiplexed target discovery (2,4)

Table 2. *Disease-specific applications of CLIP-based technologies in neurological and neuro-oncological disorders.*

Disorder	RBP	CLIP platform	Model / material	Principal CLIP evidence	Interpretive level
ALS/FTD	TDP-43	UV-CLIP; iCLIP	Human neuronal cells / human brain	UG-rich and intronic binding; altered occupancy in FTD brain	Direct binding + functional interpretation (9-11)
Fragile X syndrome	FMRP	HITS-CLIP	Multiple brain regions	Broad neuronal target repertoire	Direct binding; functional relevance requires validation (12,13)
SMA	SMN	HITS-CLIP	SH-SY5Y neuronal cells	SMN-associated RNAs including *ANXA2*	Direct binding; disease relevance requires validation (14,15)
Myotonic dystrophy	MBNL2	HITS-CLIP	Human DM1/DM2 brain	Binding to expanded repeat RNA and loss from normal targets	Direct binding evidence integrated with complementary functional data (16,17)
Huntington's disease	RBM5	HITS-CLIP	WT and R6/2 mouse brain	Genotype-associated changes in RBM5 target landscape	Direct model-based evidence (18)
SCA8	CUGBP1	CLIP	Mouse hindbrain	Candidate targets including *Gabt4*	Direct binding + functional follow-up (19)
Alzheimer's disease	nELAVL; G3BP1/2	HITS-CLIP; eCLIP	Human brain; SH-SY5Y cells	Altered nELAVL binding in AD brain; broad G3BP target maps	Direct binding; translational relevance remains prospective (20,21)
Neuroblastoma	nELAVL; NONO	HITS-CLIP for nELAVL; complementary NONO studies	Neuroblastoma cell models	nELAVL-associated RNA regulation; complementary evidence for NONO-dependent RNA regulation	Neuro-oncological application; direct CLIP and complementary evidence should be distinguished (5,6,20)

dementia (FTD), in which nuclear depletion, cytoplasmic mislocalization, and aggregation are associated with widespread abnormalities in RNA metabolism and processing. Early UV-CLIP and subsequent iCLIP studies

established transcriptome-wide binding landscapes for TDP-43 and demonstrated preferential association with UG-rich RNA regions, including extended intronic regions and disease-relevant transcripts. In human FTD brain, iCLIP further revealed altered TDP-43 binding to non-coding RNAs and deep intronic regions, providing direct evidence of disease-associated changes in TDP-43 RNA occupancy (9-11).

More recent studies have extended this mechanistic framework by linking nuclear TDP-43 loss in ALS/FTD to widespread changes in alternative polyadenylation. These findings provide complementary evidence for downstream consequences of TDP-43 dysfunction but do not, in isolation, identify the direct TDP-43 binding sites responsible for individual changes. This distinction is critical: CLIP establishes RBP occupancy, whereas transcriptomic and functional experiments are required to determine whether altered occupancy contributes to a specific molecular or disease phenotype (11).

Fragile X syndrome

Fragile X syndrome (FXS) results from the loss or functional deficiency of FMRP, an RBP with important roles in neuronal RNA transport, localization, and translation. HITS-CLIP studies performed across multiple brain regions identified a broad repertoire of FMRP-associated transcripts and revealed regional differences in FMRP RNA binding. These findings expanded the known FMRP target landscape beyond a limited set of synaptic mRNAs and identified transcripts involved in neuronal development and synaptic function (12).

The major contribution of CLIP to understanding FXS is therefore not simply the identification of additional FMRP targets, but the demonstration that FMRP associates with a distributed network of neuronal transcripts. However, CLIP-derived target enrichment can be influenced by transcript abundance, transcript length, and tissue-specific expression. Consequently, functional studies remain necessary to distinguish disease-relevant regulatory targets from associations that primarily reflect underlying neuronal expression patterns (12,13).

Spinal muscular atrophy

Spinal muscular atrophy (SMA) is primarily caused by insufficient levels of survival motor neuron (SMN) protein. Because SMN participates in multiple aspects of RNA metabolism, characterization of SMN-associated RNAs provides a mechanistic perspective beyond its canonical role in small nuclear ribonucleoprotein (snRNP) assembly. Ottesen et al. used HITS-CLIP in neuronal SH-SY5Y cells to identify cellular RNAs associated with SMN, including transcripts involved in ribosome biogenesis and cytoskeletal regulation, and independently validated a direct interaction with *ANXA2* mRNA (14).

These findings provide evidence for direct SMN–RNA interactions but should not be interpreted as demonstrating that all identified targets are pathogenic drivers of SMA. Importantly, the CLIP experiments were performed in a neuronal cell line rather than in primary motor neurons from patients with SMA. More recent studies have linked altered presynaptic Munc13-1 mRNA localization and local translation to synaptic abnormalities in mouse and patient-derived motor neurons, providing complementary functional evidence that impaired RNA localization and local translation may contribute to SMA pathogenesis (15).

Myotonic dystrophy

Myotonic dystrophy provides an important example of an RNA-mediated disease mechanism in which CLIP can directly inform the molecular model. In myotonic dystrophy type 1 (DM1) and type 2 (DM2), expanded repeat-containing RNAs sequester MBNL proteins and disrupt normal RNA processing. MBNL2 HITS-CLIP in human hippocampus and frontal cortex demonstrated enrichment of MBNL2 binding at the expanded *DMPK* CUG repeat in DM1 and the *CNBP* CCUG repeat in DM2, together with depletion of MBNL2 from its normal RNA targets (16).

Integration of HITS-CLIP with RNA-processing analyses linked toxic repeat RNA, RBP sequestration, and downstream abnormalities in alternative splicing and polyadenylation. This provides a strong example of how CLIP-derived occupancy data can contribute to a mechanistic disease model. Nevertheless, causal interpretation depends on complementary functional experiments and disease models rather than on CLIP alone (16,17).

Huntington disease

HD is caused by an expanded CAG repeat in the *HTT* gene. Although transcriptional and RNA-processing abnormalities have been extensively reported in HD, evidence describing disease-associated alterations in RBP regulation remains comparatively limited. Mullari et al. characterized the RNA-binding protein landscape of the mammalian brain and identified dysregulation of RBM5 in mouse models of HD, supporting a potential role for altered RNA-binding protein regulation in HD molecular pathology (18).

These findings should be interpreted cautiously because the available evidence was derived from mouse disease models rather than human HD brain. RBM5 dysregulation therefore represents a candidate molecular alteration requiring further investigation in human tissue and through functional perturbation studies. More broadly, the study illustrates how systematic characterization of RNA-binding proteins can identify candidate alterations in RNA regulation that may contribute to HD pathology, although additional experimental evidence is required to establish direct mechanistic and disease-specific consequences (18).

Spinocerebellar ataxias

SCAs comprise a heterogeneous group of inherited neurodegenerative disorders, several of which involve repeat expansions and RNA-mediated pathogenic mechanisms. SCA8 provides an example in which CLIP was used to identify candidate RBP-associated RNAs relevant to disease. Daughters et al. used CLIP in mouse hindbrain to map CUGBP1-associated

RNAs and identified transcripts, including *Gabt4*, that were subsequently investigated in relation to altered GABAergic signaling and RNA processing in SCA8 models (20).

The SCA8 study illustrates the appropriate interpretation of CLIP in repeat-expansion disorders: CLIP can identify candidate RBP-associated transcripts, whereas expression, splicing, biochemical, and functional studies are required to determine whether these targets contribute directly to disease phenotypes. More broadly, an integrated CLIP-based strategy may help distinguish disease-relevant RBP–repeat RNA interactions from secondary alterations in neuronal RNA metabolism (20).

Alzheimer disease

The Alzheimer disease (AD) literature provides disease-specific evidence that CLIP can detect changes in RBP occupancy in human brain. Scheckel et al. performed nELAVL HITS-CLIP together with RNA sequencing in human brain samples from controls and individuals with advanced AD. The study identified thousands of nELAVL binding sites and demonstrated disease-associated changes in nELAVL occupancy accompanied by alterations in RNA splicing, including changes involving disease-relevant transcripts. Remodeling of nELAVL association with noncoding Y RNAs was also observed, raising the possibility that altered RBP–RNA interactions contribute to changes in RNA homeostasis in AD (21).

More recent eCLIP experiments in neuronal SH-SY5Y cells demonstrated that the stress-granule-associated RBPs G3BP1 and G3BP2 bind numerous mRNAs and long non-coding RNAs, with several AD-associated transcripts represented among stress-granule-associated RNA populations. These findings provide complementary evidence linking RBP–RNA interactions with stress-granule biology and RNA homeostasis relevant to AD. However, because these experiments were conducted in neuronal cell models rather than AD patient brain, their direct relevance to human disease requires further validation (22).

Accordingly, the available evidence should be interpreted cautiously. CLIP can establish RBP–RNA occupancy, but it cannot independently establish that a particular interaction initiates AD pathology, represents a clinically validated biomarker, or constitutes a therapeutic target. Establishing disease causality requires integration with human genetic and longitudinal evidence, experimental perturbation, and functional rescue studies (1,21,22).

Neuroblastoma: a neuro-oncological application

Neuroblastoma is a pediatric malignancy derived from the neural crest and sympathetic nervous system lineage and is therefore considered a neuro-oncological rather than a classical neurogenetic disorder. It is included in this review because posttranscriptional RNA regulation and RBP-mediated mechanisms have emerged as relevant components of neuroblastoma biology. The available evidence should nevertheless be distinguished according to whether findings were obtained directly using CLIP or through complementary functional and molecular approaches (5,6).

The nELAVL HITS-CLIP study by Scheckel et al. included IMR-32 neuroblastoma cells as a complementary experimental model and demonstrated that neuronal ELAV-like proteins participate in the regulation of RNA abundance and splicing (21). In addition, studies of NONO in neuroblastoma have provided functional evidence that its RNA-binding activity contributes to the maintenance of SREBP expression and cholesterol biosynthesis, while other work has implicated NONO in the regulation of super-enhancer-associated transcripts, including *GATA2* and *HAND2*. These findings support a role for RBP-dependent RNA regulation in neuroblastoma but should not be classified as CLIP-derived evidence unless the corresponding experiments directly employed a CLIP-based method (5,6).

Taken together, neuroblastoma is best viewed as a neuro-oncological extension of CLIP-based RNA biology rather than as a classical neurogenetic disorder. This distinction allows direct CLIP-derived evidence to be evaluated within the broader context of RBP-mediated posttranscriptional regulation while maintaining a clear separation between direct RNA-binding evidence and complementary functional observations.

Limitations and Analytical Challenges

CLIP is a powerful technology for mapping RBP–RNA occupancy, but the observed signal is shaped by both biological properties and experimental design. UV crosslinking is inefficient and chemically selective, meaning that RNA sequence composition and structural context can influence the probability of crosslink formation. PAR-CLIP introduces additional sources of bias through metabolic labeling and crosslink-associated nucleotide substitutions. RNase digestion can further influence the observed binding landscape because RNA accessibility affects susceptibility to cleavage and the size of RNA fragments recovered for sequencing (1,2,4,7).

Antibody specificity is another major determinant of CLIP data quality. Off-target immunoprecipitation can produce nonspecific enrichment and false-positive peaks, whereas insufficient affinity or poor immunoprecipitation efficiency can reduce sensitivity. Where feasible, experimental designs should incorporate appropriate controls, including independent antibodies, tagged endogenous proteins, no-UV controls, IgG controls, and size-matched input controls, depending on the CLIP platform. Biological replicates are also essential because technical reproducibility cannot substitute for biological replication (1,2).

Library complexity and PCR amplification can distort apparent binding strength and contribute to the overrepresentation of individual molecules. Unique molecular identifiers (UMIs) can assist in identifying amplification-derived duplicates in workflows that incorporate them. Sequencing depth, read-mapping strategy, transcript annotation, and peak-calling algorithms can also influence the number and genomic or transcriptomic locations of reported binding sites.

Differences in normalization procedures and peak definitions may further complicate comparisons between datasets generated using different CLIP variants. Standardized analytical pipelines and public deposition of raw and processed sequencing data can improve reproducibility and facilitate independent reanalysis (1,2,22,23).

Finally, CLIP measures RBP occupancy rather than biological function. A reproducible binding event may represent a functional regulatory interaction, a context-dependent association, or incidental occupancy without a measurable downstream effect. Functional interpretation therefore requires integration with complementary approaches, including perturbation experiments, RNA sequencing, splicing or polyadenylation analyses, proteomics, reporter assays, and, where feasible, rescue experiments. In heterogeneous brain tissue, single-cell or spatially resolved approaches may additionally be required to distinguish cell-type-specific and region-specific RBP–RNA interactions that can be obscured in bulk tissue measurements (1,2).

Future Perspectives

The next stage of CLIP development is likely to focus increasingly on resolving biological context rather than simply increasing the number of detectable RBP-binding sites. Low-input and streamlined workflows may facilitate the application of CLIP to patient-derived neurons and other clinically relevant specimens in which biological material is limited. Single-cell and spatially resolved approaches could potentially connect RBP occupancy with neuronal subtype, anatomical location, and disease stage. However, these applications should currently be regarded as emerging methodological directions rather than established clinical technologies (1,3).

Computational analysis is also becoming increasingly important for CLIP interpretation. Probabilistic and machine-learning approaches can integrate biological replicates, input controls, sequence features, transcript abundance, and other experimental variables to improve the identification and prioritization of candidate binding sites. Models trained on large-scale eCLIP datasets may also assist in predicting how genetic variants could influence RBP–RNA interactions. Nevertheless, computational predictions remain hypothesis-generating and require experimental validation before being interpreted as evidence of functional or disease-relevant effects (1,2,8,22).

CLIP may contribute to biomarker discovery by identifying disease-associated changes in RBP occupancy and RNA-processing networks. However, a CLIP-derived binding event should not itself be considered a validated diagnostic or prognostic biomarker. Clinical translation requires independent patient cohorts, analytical and clinical validation, assessment of sensitivity and specificity, and demonstration of incremental value beyond established clinical or molecular measures (1,2).

Similarly, CLIP can help prioritize RBP–RNA interactions for therapeutic investigation but cannot establish therapeutic efficacy. Candidate interactions can subsequently be evaluated using approaches such as antisense oligonucleotides, small molecules, RNA-targeting strategies, or genetic perturbation. A strong translational framework should therefore integrate CLIP-defined molecular maps with functional validation, patient-derived models, and clinically relevant outcome measures (1,3).

Conclusion

CLIP technologies have transformed the study of RBP–RNA interactions by enabling transcriptome-wide mapping of RBP-associated RNAs and, depending on the platform, high-resolution identification of interaction sites. Their greatest contribution to disease biology arises when binding maps are integrated with functional and transcriptomic evidence linking RBP occupancy to alterations in RNA splicing, polyadenylation, localization, translation, or cellular phenotypes.

Evidence from amyotrophic lateral sclerosis and frontotemporal dementia, Fragile X syndrome, SMA, myotonic dystrophy, HD, SCAs, and AD illustrates both the mechanistic value and the analytical limitations of CLIP-based approaches. Neuroblastoma further demonstrates how CLIP-based RNA biology can inform neuro-oncological research when direct CLIP-derived evidence is clearly distinguished from complementary functional and molecular studies.

Overall, CLIP should be regarded as a powerful tool for defining RBP–RNA occupancy rather than as a standalone method for establishing functional causality. Future advances in low-input, single-cell, spatial, computational, and integrative multi-omics approaches may improve the biological resolution and translational utility of CLIP-derived interaction maps. However, functional validation, disease-relevant experimental models, and independent clinical validation will remain essential for determining the mechanistic and translational significance of identified RBP–RNA interactions.

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List of Abbreviations

AD	Alzheimer disease
ALS	amyotrophic lateral sclerosis
CLIP	crosslinking and immunoprecipitation
DM	myotonic dystrophy
eCLIP	enhanced CLIP
FMRP	fragile X mental retardation protein
FTD	frontotemporal dementia
FXS	fragile X syndrome
HD	Huntington disease
HITS-CLIP	high-throughput sequencing CLIP
iCLIP	individual-nucleotide resolution CLIP
irCLIP	infrared CLIP
MBNL	muscleblind-like
NONO	non-POU domain-containing octamer-binding protein
PAR-CLIP	photoactivatable-ribonucleoside-enhanced CLIP
RBP	RNA-binding protein

SCA	spinocerebellar ataxia
SMA	spinal muscular atrophy
SMN	survival motor neuron
UV	ultraviolet
uvCLAP	ultraviolet crosslinking and affinity purification

Conflict of interest

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Author contributions

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Khaliel Rafee Almutairi: Literature review and manuscript revision.

Abdullah Ayad Alaujan: Literature review, table preparation, and critical revision.

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All authors contributed to the manuscript, reviewed and approved the final version, and agreed to be accountable for the work.

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