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Review

Review

Mapping subcellular RNA localization with proximity labeling

Jiapeng Liu, Binglin Zhong, Shuojun Li, and Shuo Han*

Key Laboratory of RNA Innovation, Science and Engineering, Shanghai Institute of Biochemistry and Cell Biology, Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai 200031, China
*Correspondence address. Tel: +86-21-54921227; E-mail: shuohan@sibcb.ac.cn

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Abstract

The subcellular localization of RNA is critical to a variety of physiological and pathological processes. Dissecting the spatiotemporal regulation of the transcriptome is key to understanding cell function and fate. However, it remains challenging to effectively enrich and catalogue RNAs from various subcellular structures using traditional approaches. In recent years, proximity labeling has emerged as an alternative strategy for efficient isolation and purification of RNA from these intricate subcellular compartments. This review focuses on examining RNA-related proximity labeling tools and exploring their application in elucidating the spatiotemporal regulation of RNA at the subcellular level.

Key words RNA localization, proximity labeling, spatiotemporal regulation, subcellular compartments

Introduction

In eukaryotic cells, biomolecules are intricately organized and localized to specific cellular compartments, essential for their functional roles. Localized mRNA can be translated to produce many copies of proteins in response to local stimuli, providing an effective means to regulate gene expression with high spatial and temporal control. RNA localization represents a critical post-transcriptional regulatory mechanism that affects a multitude of RNA-associated biochemical processes, including folding, editing, splicing, degradation, translation, and interactions with protein binding partners [1]. These processes subsequently influence the functionality and destiny of the proteins encoded by the RNA.

Impact of RNA Localization on Cellular Function

The subcellular localization of RNA is fundamentally connected to its functional roles. Asymmetric RNA distribution facilitates localized protein translation [2], and the three-dimensional structuring of chromatin [3], and development [4,5]. The intracellular positioning of RNA crucially influences its storage, processing, translation, and degradation.

In the initial stages of embryogenesis, the establishment of the body axis is essential for development. This axis formation involves numerous genes, broadly classified into maternal and zygotic categories. During oogenesis, oocytes accumulate significant amounts of mRNAs, which are distinctly distributed within the oocyte, setting the stage for axis differentiation [6]. Specifically, in *Drosophila*, the maternal gene *gurken* is crucial for initiating anterior-posterior differences in the oocyte cytoplasm. The *gurken* mRNA localizes posterior to the nucleus, between the nucleus and the posterior follicular cells [7]. This localization promotes the directional organization of microtubules within the oocyte, enabling the transport of *oskar* mRNA to the oocyte's posterior cytoplasm. There, *oskar* mRNA is translated into Oskar protein, which stabilizes the microtubules and enhances the posterior accumulation of maternal gene products, setting the stage for pole cell formation.

In neurobiology, neurons are tasked with coordinating functions between the nucleus and distal components such as axons and dendrites, which can span distances greater than one meter. Additionally, neurons must dynamically modulate their proteome in response to fluctuating environmental conditions. It is widely acknowledged that the localized translation of mRNA within dendrites is both essential and widespread [2,8]. The mRNA of GluR1 and GluR2 is localized in dendrites and their localization is regulated by nerve cell activity [9]. These mRNAs are transported to axons *via* the axonal shaft, where they are locally translated. This not only conserves energy and time necessary for protein transport but also minimizes the risk of misdirected targeting. Furthermore, it

supports synaptic plasticity and the formation of dendritic spines. For example, the Fragile X mental retardation protein (FMRP) plays a pivotal role in regulating the translation of these localized RNAs in various cell types, including both neurons and fibroblasts [10].

In cell biology, cellular signaling networks must rapidly adapt their actin cytoskeleton to meet the demands of the complex environment. As one of the most discussed cases of polarized RNA distribution, β-actin mRNA is well-known for its enrichment at the cell's leading edge. It features a 54-nucleotide sequence in its 3′ untranslated region, known as a 'zip code', which is essential for the unique localization of β-actin mRNA independent of translation [11].

Thus, mapping the uneven distribution of RNA within cells is essential for understanding the molecular and cellular mechanisms underlying a variety of biological processes.

Overview of Methods to Study RNA Subcellular Localization

Traditional biochemical and genetic methods analyze the collective average expression of RNA molecules, neglecting intracellular heterogeneity. Over four decades ago, the introduction of fluorescence *in situ* hybridization (FISH) allowed for the analysis and elucidation of RNA regulatory patterns through fluorescence microscopy [12]. FISH has since advanced to incorporate multiple rounds of hybridization [13] and high-throughput imaging [14,15]; however, it still confronts challenges such as low throughput when compared to omics studies. Additionally, constrained by its technical principles, FISH is primarily suited for studying predefined RNAs of interest and is less practical for comprehensive investigations of RNAs involved in specific biological processes. When applied to live-cell RNA imaging, this technique often requires manipulation or modification of the target RNA, which may alter its native properties. In the study of subcellular RNA distribution using biochemical fractionation methods, cell lysis is an essential preliminary step. This process causes significant disruption and only allows for effective separation of membrane-bound organelles, often leading to incomplete purification [16].

In recent years, enzyme-mediated proximity labeling techniques have become increasingly prevalent for investigating RNA distribution within subcellular regions (Figure 1). By fusing enzymes with specific localization peptides or proteins to target a region of interest, these enzymes can catalytically convert exogenously supplied substrate probes into active intermediates that covalently label nearby RNA. Due to the limited lifespan of the probe's active intermediates, the enzyme can only catalyze the labeling reaction within a short radius, with signal intensity rapidly decreasing away from the enzyme. Consequently, this technique is particularly well-suited for studying complex biomolecular interactions inside living cells, especially for exploring interaction networks in membraneless organelles and around specific proteins.

Indirect Approaches for RNA Proximity Labeling

Proximity-specific ribosome profiling

Ingolia *et al.* [17] developed a ribosome-profiling strategy to study protein translation. The translating RNAs are partially masked by ribosomes. Under nuclease digestion, the RNA fragments protected by ribosomes can be subsequently reverse transcribed to DNA and identified by deep sequencing. Jan *et al.* [18] combined ribosome-profiling and proximity labeling strategy, developing proximity-

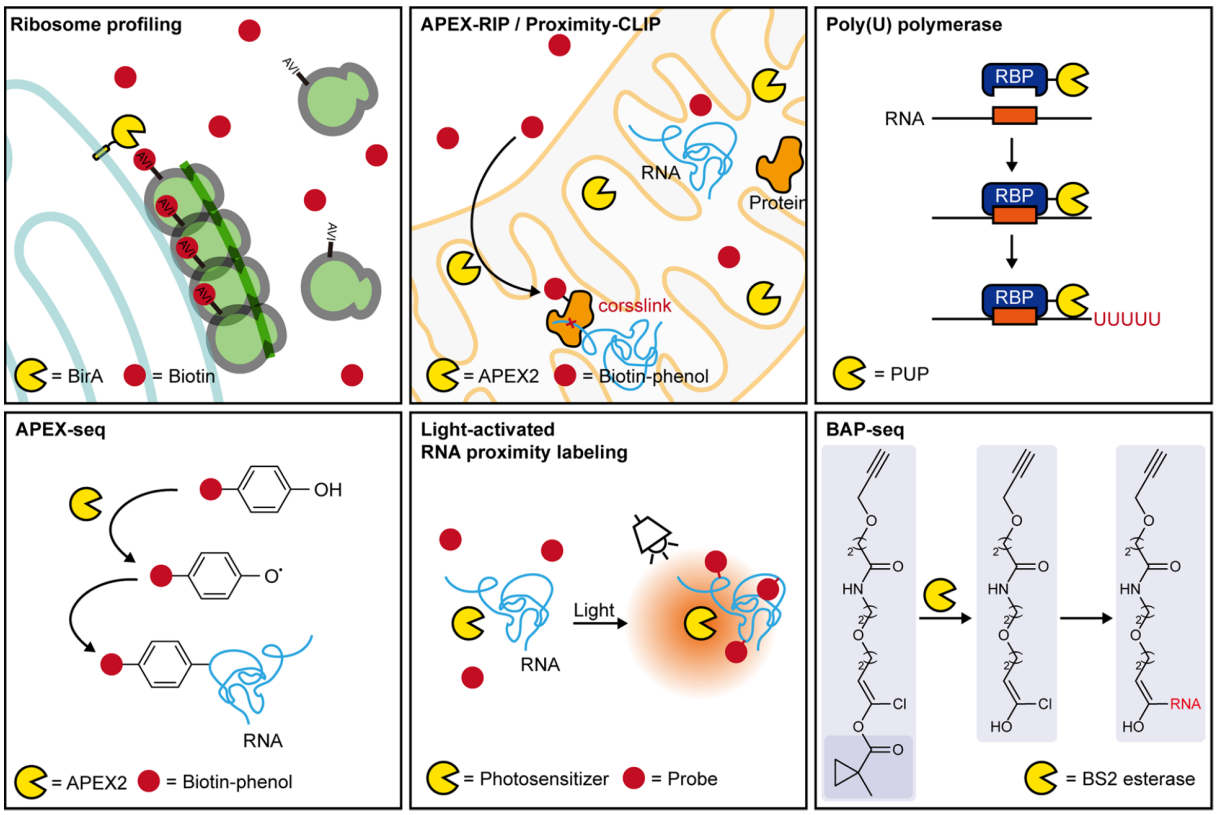

Figure 1. Schematic of the principles of the direct and indirect methods for proximity labeling RNA, including proximity-dependent ribosome profiling, APEX-RIP, APEX-CLIP, poly(U) tail labeling, APEX-seq, various photoactivated proximity labeling methods, and BAP-seq

specific ribosome profiling method to detect RNA translation at subcellular locations. They engineered yeast cells and HEK293T cells to express AviTag on ribosomes and BirA at specific subcellular locations. After cycloheximide treatment and biotin supplementation, the mRNA-ribosome complex is labeled by biotin. Subsequent ribosome profiling and deep sequencing identified translating mRNAs at specific regions. This tool was applied to profile translation at endoplasmic reticulum membrane (ERM) [18] and outer mitochondrial membrane (OMM) [19]. However, this ribosome-dependent method only identifies translating mRNAs, while functional non-coding RNAs are neglected. Additionally, this tool requires biotin starvation of cells prior to BirA labeling, which is tolerable in yeast but too toxic for mammalian cells. This requirement of biotin starvation by BirA-based proximity ribosome profiling was recently solved by using TurboID in combination with CLIP and ribosome profiling, which enabled simultaneous protein and RNA mapping in the postsynapse of primary cortical neurons [20].

APEX-RIP and proximity-CLIP

Given that RNA immunoprecipitation (RIP) is a classic method to profile RNA-protein interactions and proximity labeling is highly efficient in labeling nearby proteins, one idea to enrich RNAs within a subcellular region is to combine these two methods. Kaewsapsak *et al.* [21] developed the APEX-RIP method: proteins in a subcellular region are labeled by APEX2-mediated biotinylation, followed by quenching and formaldehyde cross-linking for enrichment by streptavidin beads. After digesting away the protein components, subcellular RNAs can be sequenced. However, due to chemical crosslinking between RNA and proteins, it is challenging to obtain RNA of high integrity, thereby compromising the quality of RNA sequencing data. Another tool, called proximity-CLIP [22], also labels subcellular proteins by APEX2 biotinylation. Instead of formaldehyde crosslinking, proximity-CLIP relies on UV for RNA-protein crosslinking. Before APEX2 proximity labeling, 4-thiouridine (s^4U) is added to the cell culture media to facilitate crosslinking. In contrast to APEX-RIP, which uses formaldehyde after 1 min incubation of quenching solution, UV can be applied during quenching, thus minimizing the temporal gap between protein biotinylation and RNA-protein crosslinking. Although proximity labeling combined with crosslinking provides new approaches to identify RNAs in subcellular regions, it faces one important caveat: APEX2-labeled proteins may diffuse away before crosslinking, reducing the specificity of these methods when it comes to non-membrane regions, including the ERM and OMM.

TurboID-RIP

The combination of biotin ligase TurboID and RIP-seq for mapping subcellular RNA localization was recently showcased in *Arabidopsis*. Liu *et al.* [23] fused TurboID to DECAPPING 1, a core element of processing bodies (PBs). After biotin supplementation and cellular stress induction, PB proteins are biotinylated by TurboID following PB formation. After application of formaldehyde solution to crosslink RNAs and proteins related to PBs, the RNAs were further enriched and sequenced. This method captured RNAs associated with PBs in plants.

Direct Approaches for RNA Proximity Labeling

Poly(U) polymerase

Poly(U) polymerase (PUP) from *Caenorhabditis elegans* mediates uridylation at the 3′ end of RNAs. Lapointe *et al.* [24] used PUP to RNA-binding proteins, inducing poly(U)-tails to proximal RNAs. They expressed PUP fused with Puf3p, a yeast RNA-binding protein, to label and identify the RNA-binding partners of Puf3p. To identify endogenous substrates of Puf3p, RNAs near Puf3p-PUP are tagged by a poly(U)-tail, followed by total RNA extraction from yeast. Within the extracted total RNA, the labeled RNAs with poly(U) tails are specifically amplified by U-selected primers and sequenced. Using the strategy of poly(U) tagging, Medina-Munoz *et al.* [25] further developed a method to label RNAs in subcellular locations. To promote binding and labeling efficiency, the PUP protein was linked to the RNA-recognition motif of poly(A)-binding protein. They localized PUP to ERM (facing the cytosol) by fusing PUP to the C-terminus of Sec63p, and to mitochondrial outer membrane through Om45p. This method was demonstrated to work in both yeast and human cells.

APEX-seq

The engineered peroxidase APEX2 has been shown to efficiently label proximal proteins, with high spatial and temporal resolution. Fazal *et al.* [26] found that horseradish peroxidase (HRP) could utilize biotin-phenol (BP) to label tRNAs *in vitro*, and further confirmed that cytosolic APEX2 catalyzed BP labeling of RNAs. Mechanistic studies then revealed that BP probes were added to guanine. Inspired by the fact that peroxidases mediate direct RNA labeling, they developed APEX-seq to map RNAs at subcellular regions, including the nucleolus, nucleus, nuclear lamina, nuclear pore, cytosol, ERM and OMM. Padrón *et al.* [27] applied APEX-seq to identify RNAs proximal to stress granules. These two reports both used BP as the substrate probe. Zhou *et al.* [28] tested several substrates, including previously used BP and newly developed biotin-naphthylamine, biotin-aniline (Btn-An/BA) and biotin-hydroxybenzamide, for APEX2-mediated proximity labeling of protein, RNA, and DNA, respectively. They found that BP has the highest protein labeling efficiency, while BA probe labels RNA with higher yield. The specificity was confirmed by RT-qPCR detection of subcellular RNA markers. In proximity-CLIP, s^4U labeling is designed to enhance crosslinking efficiency. Huang *et al.* [29] explored how s^4U labeling influences APEX-seq reaction. They performed a set of experiments to demonstrate that s^4U incorporation enhances APEX2-mediated RNA biotinylation by BP and BA. Furthermore, Li *et al.* [30] developed metabolically incorporating electron-rich ribonucleoside (MERR) APEX-seq. They examined the effect of electron-rich ribonucleosides incorporated in RNA of APEX-seq, demonstrating that both s^4U and 6-thioguanosine (s^6G) promote labeling efficiency of APEX2, using BP or BA as probes. APEX-seq methods were developed and tested in mammalian cells. However, when it comes to yeast, the cell wall presents a challenge for exogenous BP probe due to its poor permeability. Li *et al.* [31] developed a new substrate, clickable alkyne-phenol (AP), for coupling proximity labeling with Copper (I)-Catalyzed Alkyne-Azide Cycloaddition (CuAAC) click reaction methods. Compared to BP, AP is a smaller molecule with considerable permeability, enabling it to pass the cell wall for application in yeast. AP is suitable for both protein and RNA labeling. After proximity labeling of proteins or RNAs with AP, azide-biotin conjugated by CuAAC reaction enables subsequent enrichment and identification. Proximity labeling enzymes, including APEX2, were originally designed to be genetically

encoded tools. Based on APEX2, Yap *et al.* [32] developed an *in situ* method called hybridization-proximity (HyPro) to label specific RNA-proximal RNAs and proteins in fixed and permeabilized cells. They applied digoxigenin (DIG)-labeled DNA probes to target specific RNA sequence, and purified DIG-binding protein-APEX2 fusion protein to label proteins and RNAs near the RNA sequence. In contrast to APEX-seq, which is applicable to living cells, HyPro works with fixed samples. However, dependence on genetic expression of the APEX2 enzyme limits its application to clinical samples or tissue slices. HyPro, on the other hand, takes advantage of *in situ* proximity labeling and is applicable to these fixed samples to explore molecular interactions.

Light-activated RNA proximity labeling

Based on the principle of proximity labeling, which involves generating highly reactive intermediates to covalently tag proximal molecules, Wang *et al.* [33] developed a light-activated RNA proximity labeling tool called CAP-seq. They applied genetically encoded, subcellularly localized mini Singlet Oxygen Generator (miniSOG) to generate reactive oxygen species, which oxidizes the guanosine of RNAs. They selected a suitable probe, propargyl amine (PA), for nucleophilic attack of the oxidized guanine, thus labeling RNAs near miniSOG. Click chemistry reaction was then performed to add the biotin group to PA probe to enable fluorescence imaging and RNA enrichment. The specificity was evaluated by testing in the well-established mitochondrial matrix transcriptome model. Compared to the APEX-seq protocol that requires about 30 min incubation of probe substrate, the CAP-seq method only needs 10 min incubation of PA probe due to its higher membrane permeability. However, as described in CAP-seq protocol, 20 min illumination is used for proximity labeling reaction. This time gap may harm the temporal resolution of CAP-seq when applied to open regions, including OMM and ERM, and highly dynamic biological processes. In addition, Hananya *et al.* [34] applied another genetically encoded photosensitizer, the LOV domain to label proteins with BA. They reported that RNA might also be labeled by this method, but the detailed mechanism and the spatial specificity of LOV-mediated RNA labeling remain uncharacterized. Apart from photosensitizer proteins, photoactivatable small molecules are developed to label RNAs. Li *et al.* [35] found that under blue light illumination, eosin generated singlet oxygen to oxidize guanines, which were further labeled by amino-PEG2-biotin. This reaction worked both *in vitro* and inside living cells. To apply this labeling reaction in specific regions, they targeted HaloTag to different subcellular regions and treated cultured cells with dibromofluorescein (DBF), a HaloTag ligand derived from eosin. After incubation with PA probe and illumination, RNAs near HaloTag were labeled with alkyne and ready for click chemistry reaction, enrichment and detection. In a subsequent study, they found that HaloTag-DBF also labeled proteins with PA probe [36]. Engel *et al.* [37] developed the Halo-seq method, using this DBF-based labeling reaction to study subcellular transcriptome. Recently, fluorogen activating protein (FAP) was used for RNA proximity labeling. Li *et al.* [38] developed FAP-seq using malachite green (MG) as photosensitizer, which is inactive until it binds to FAP. Like Halo-seq, FAP-seq also uses the clickable PA probe, but with an alternative wavelength for illumination: MG is activated by red light ($\sim$660 nm), which provides deeper tissue penetration.

BAP-seq

Recently, Pani *et al.* [39] introduced the BAP-seq method. They developed a new proximity labeling reaction: the small molecule probe used in this method is enol-ester; after hydrolysis of this "masked acylating reagent" by subcellularly localized esterase (BS2), the alkyne-conjugated enol group is released and labels proximal RNAs. For proximity labeling reaction, the BAP-seq method only needs 3 min pre-incubation of the probe solution. It will be exciting to see the future application of this new RNA proximity labeling technique to a broad range of biological systems and questions.

Current Applications of RNA Proximity Labeling

In cell biology, density gradient centrifugation is frequently used to isolate subcellular compartments with enclosed membranous structures, such as nuclei, mitochondria, synaptic vesicles, and exosomes. Additionally, this approach facilitates the purification of RNA from these organelles [40]. However, conventional mechanical centrifugation techniques are inadequate for enriching RNA from non-membrane-bound subcellular spaces, including those formed from phase separation, the mitochondrial outer membrane, and other intracellular membrane structures. Consequently, there is a need for alternative methods that are compatible with live cells and provide high spatiotemporal resolution for studying these distinct subcellular regions. Proximity labeling, which involves chemically tagging biomolecules *in situ*, has emerged as a particularly promising method for addressing these challenges.

Mitochondrial matrix transcriptome

Since the mitochondrion is enclosed by two layers of membrane, mitochondrial matrix components are easily purified and examined by fractionation methods. The transcriptome of mitochondrial matrix is widely used as a model for testing the specificity of new proximity labeling tools. For instance, during the development of APEX-RIP, it was found that crosslinking followed by labeling enriched several off-target, cytosolic RNAs when APEX was localized to the mitochondrial matrix, while reversing the order of the two steps-labeling first and then crosslink-reduced the level of those off-target RNAs and dramatically improved the enrichment of known mitochondrial matrix RNAs [21]. In subsequent methods, including APEX-seq [26] and CAP-seq [33], mitochondrial matrix RNAs are also evaluated by RT-qPCR as indicators of labeling specificity.

Endoplasmic reticulum membrane transcriptome

Proximity labeling has also been used to characterize the transcriptomic content of open subcellular regions, such as the cytosolic side of ERM. mRNAs that encode secretory pathway proteins are localized near ERM. RNAs in this region are difficult to enrich by fractionation methods. Proximity-specific ribosome profiling was applied in yeast cells to identify translating ERM mRNAs [17]. The indirect APEX-RIP method was tested in HEK293T cells. While an ER surface resident ERM-APEX construct failed to show significant enrichment of target RNAs [21], targeting the enzyme HRP to inside the ER lumen enabled specific labeling and crosslinking of RNAs on the ER surface. In APEX-seq, ERM-APEX was also tested. RT-qPCR detected enrichment of several secretory gene mRNAs, while APEX-RIP failed, indicating a better performance of APEX-seq at open ERM region [26]. MERR APEX-seq [30],

CAP-seq [33], FAP-seq [38] and PUP-ER [25] methods also show considerable ERM RNA enrichment results according to GO analysis.

Outer mitochondrial membrane transcriptome

Apart from ERM, the OMM is another open region that has been studied by RNA proximity labeling. By expressing BirA fused to Om45, proximity-specific ribosome profiling was used to study RNAs near OMM in yeast [19]. In the enriched RNAs, 87% were annotated to be associated with mitochondria. The most enriched genes encode inner mitochondrial membrane proteins, indicating that most of the ribosome bound mRNAs near mitochondria encode inner mitochondrial membrane proteins, while only small portions of outer membrane, intermembrane space, and matrix proteins were enriched. In a separate study, BirA was fused to Tom20, an OMM protein, for proximity-specific ribosome profiling in HEK293T cells [41]. They utilized this system to explore changes in mRNA translation upon *Tom70* knockdown and RNA-binding protein *CLUH* knockdown. Fazal *et al.* [26] used the APEX-seq method to further explore mitochondrial mRNA translation patterns in HEK293T cells. They targeted APEX2 to OMM through MAVS sequence for OMM RNA labeling and enrichment. CHX treatment, which stops translation without disrupting the ribosome nascent chain complex, increased enrichment of a set of mitochondrial-related RNAs, indicating a ribosome-dependent pathway for RNA targeting. Another set of RNAs was enriched after puromycin treatment, which dissociates ribosomes and mRNA, suggesting an alternative, ribosome-independent pathway. They further explored the sequence features that determine the different pathways by investigating the enriched sequence motifs.

Plasma membrane transcriptome

Benhalevy *et al.* [22] used proximity-CLIP method to profile RNAs at cell-cell junction site. They expressed Connexin43-APEX2 to label proteins near the junction site and crosslinked proximal RNAs to biotinylated proteins. The top 19 localized mRNAs encode membrane-localized proteins, transcription factors, and several regulators. They also found a CUG motif specific to enriched RNAs. However, how RNAs localize to the cell-cell interface remains unknown. Similarly, APEX-seq and CAP-seq have been applied to profile plasma membrane-associated RNAs [42]. More recently, several studies reported the intriguing existence of glycosylated RNAs on the cell surface. Cell surface RNAs were labeled directly by HRP-catalyzed biotin aniline tagging [43]. As streptavidin-HRP was targeted to the cell surface using biotin-conjugated lectin, proximity labeling reactions were therefore restricted to extracellular glycosylation sites.

Stress granule transcriptome

One key feature of APEX-seq is its high temporal resolution because it takes just 1 mine to label RNAs, making it ideal for studying the highly dynamic stress granules (SGs) [27,44]. Shen *et al.* [45] applied the APEX-seq method to study the SG RNA component. They focused on RNA helicases DDX3X and DDX3Y encoded by the X and Y chromosome respectively. The two proteins are related to SG formation. They labeled DDX3X/DDX3Y-positive SGs with APEX2 fusion proteins, and found that DDX3X and DDX3Y targeted different mRNAs, which partially account for their different properties. Ren *et al.* [46] studied SG transcriptome using the CAP-seq method. They chose G3BP1, a well-known core component of SGs, for generating miniSOG fusion in HEK293T cells. Light-activated proximity labeling using miniSOG was subsequently carried out to identify *bona fide* SG RNA components. Using this platform, they revealed different SG transcriptomes induced by arsenite and sorbitol, respectively, and explored the sequence features of SG RNAs. Furthermore, taking advantage of the high temporal resolution offered by CAP-seq, they tracked the RNA dynamics during the assembly and disassembly process of SGs, and showed that some mRNAs were still associated with G3BP1 after stress recovery. This study nicely demonstrated the potential of proximity tools in SG research.

Outlook and Future Opportunities

To date, numerous studies have explored labeling and analyzing RNA in living cells *in vitro*. However, unlike the proximity labeling techniques used in proteomic studies, *in vivo* RNA labeling remains elusive. This entails the specific marking of RNA within different organs and tissues of animals under various physiological or pathological conditions. Achieving *in vivo* RNA labeling is crucial for conducting analyses that closely mimic real-life environments, thus revealing the functional relevance of its spatial regulation. Yet, challenges such as probe delivery remain as major hurdles. Furthermore, the absence of an optimal or standardized probe for RNA labeling adds to the complexity, as different modifications yield varied labeling efficiency and preference. Consequently, ongoing efforts are focused on developing optimizing probes for achieving proximity labeling in living tissue and organisms.

While a significant number of studies have been conducted in understanding the spatial regulation of the transcriptome in mammalian cells, as discussed above, the application of these tools to plants represents another interesting but challenging future direction. The plant cell transcriptome not only exhibits intricate spatial pattern at the subcellular level, similar to mammalian cells; but more importantly, plant cells are uniquely equipped with specialized structures, such as plasmodesmata, for intercellular RNA exchange. Biotin ligase-based proximity labeling has played a key role in mapping proteomic interactions with spatial and temporal precision, shedding light on the complex signaling pathways vital for plant adaptation and survival. However, biotin ligases do not label RNAs, and the scarcity of RNA proximity-labeling methods for plants presents a missed opportunity for tool development. Developing alternative approaches that are applicable for plants would greatly benefit our understanding of the communication and regulatory mechanisms operating between plant cells. This holistic understanding not only advances fundamental plant biology but also holds practical implications for agricultural and biotechnological endeavors aimed at enhancing crop productivity and resilience to extreme environmental conditions.

Current methods for studying the spatial regulation of RNA in plants mainly include grafting, gene silencing, and sequencing of specific tissue sap, which suffer from experimental complexity, long cycles, and low spatial resolution [47–49]. Therefore, developing a proximity labeling system with high spatiotemporal resolution is key to understanding the dynamic transfer of RNAs between plant cells, the molecular mechanisms of RNA movement, and the fate of these RNAs. Indeed, there is a lack of effective methods for the enrichment of RNA within the intercellular space of plasmodesma-

ta. The development of proximity labeling technology holds promise for providing novel strategies to elucidate the RNA spatial omics of plasmodesmata in plant cells. These advances in tool development might also have implications for elucidating the communication and regulatory mechanisms under various stress conditions. Furthermore, the study of transcriptome contents of other subcellular structures, such as plant plastids and mitochondria, could also hugely benefit from such tools. By applying similar techniques to other organelles, we can broaden our understanding of the composition and function of these organelles in plants. Therefore, a comprehensive understanding of the intricate interactions and regulatory networks among various subcellular structures, offers a different perspective to uncover deeper mechanisms in plant biology.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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