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mRNA-Scaffolded Cytoplasmic Compartments

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Abstract

The cytoplasm of vertebrate cells is compartmentalized into the cytosol and several messenger RNA (mRNA)-scaffolded condensates, present at steady-state conditions and in the absence of stress. They include TIS granules and the FXR1 network and act as translation, folding, and signaling environments. Therefore, in addition to serving as templates for protein synthesis, mRNAs play essential roles in cytoplasmic organization. However, not all mRNAs function as condensate scaffolds. Whereas mRNAs with short and structured 3′ untranslated regions (UTRs) usually diffuse freely and localize to the cytosol, scaffold mRNAs are characterized by long and multivalent 3′ UTRs. Scaffold mRNAs are responsible for the characteristic irregular, network-like morphology of mesh-like condensates and play active, functional roles during protein biosynthesis. For example, mesh-like condensates act as folding environments for proteins with long intrinsically disordered regions, where multivalent 3′ UTRs act as cotranslational chaperones to prevent protein misfolding. The scaffold function of mRNAs is also important for post-translational processes, where the mRNA-mediated proximity of signaling factors promotes cellular signaling reactions. In this review, the discovery of cytoplasmic mRNA-scaffolded mesh-like compartments and their currently known assembly principles and biological roles are discussed.

Contents

INTRODUCTION

Compartmentalization underpins the spatial organization of cellular processes, allowing distinct biochemical reactions to proceed in parallel within the same cell (Holehouse & Alberti 2025). Cells are organized by membrane-bound organelles and membraneless assemblies, termed biomolecular condensates (Banani et al. 2017). Spherical condensates form when multivalent proteins exceed a certain protein concentration, resulting in dynamic assemblies (Banani et al. 2016, Li et al. 2012, Sanders et al. 2020). Condensates can also be mesh-like, as observed in TIS granules, the FXR1 network, and oskar granules (Bose et al. 2024, X. Chen et al. 2024, Horste et al. 2023, Ma & Mayr 2018, Ma et al. 2021, Seim et al. 2024). As the network-like morphology is caused by multivalent messenger RNAs (mRNAs) that act as condensate scaffolds, mesh-like condensates represent mRNA-scaffolded cytoplasmic compartments.

Although most condensates contain proteins and RNA, the role of RNA in condensate assembly is only beginning to emerge (Neugebauer 2015, Parker et al. 2025, Ripin & Parker 2023, Van Treeck & Parker 2018, Wei et al. 2024, Zhang et al. 2015). Best-studied are nuclear condensates, such as the nucleolus, paraspeckles, transcription condensates (Fox et al. 2018, Hirose et al. 2023, Hou & Kraus 2021, Mitrea et al. 2018, Naganuma et al. 2012, Pei et al. 2025, Quinodoz et al. 2025), and stress granules (Guillen-Boixet et al. 2020, Parker et al. 2025, Sanders et al. 2020, Yang et al. 2020). Here, the focus is on cytoplasmic mesh-like condensates, in which mRNA is essential for assembly.

In steady-state conditions, the cytoplasm of vertebrate cells is compartmentalized into the cytosol and several mRNA-scaffolded condensates that act as translation, folding, and signaling environments, exemplified by TIS granules and the FXR1 network (X. Chen et al. 2024, Horste et al. 2023, Luo et al. 2026, Ma & Mayr 2018, Ma et al. 2021). These mRNA-protein assemblies emerge from the cooperative interactions between multivalent RNA-binding proteins and multivalent mRNAs, which have several accessible sites for intermolecular RNA–RNA interactions (Ma et al. 2021).

In this review, the discovery of cytoplasmic condensate networks is outlined and their biological functions in post- and cotranslational processes are described. mRNA-scaffolded condensates act as platforms for signaling pathways and provide mRNA-dependent folding environments for difficult-to-fold proteins, where mRNA 3′ untranslated regions (UTRs) act as chaperones for the folding of proteins with long intrinsically disordered regions (IDRs). The defining feature of scaffold RNAs is multivalency. Therefore, the features that distinguish multivalent from low-valency mRNAs are described. As part of mesh-like condensates, mRNAs control cellular processes that go beyond their functions as templates for protein synthesis. As one of their functions is to control the activity of the encoded proteins, three major mechanisms by which protein activity can be regulated are outlined below.

THREE MAJOR MODES OF PROTEIN ACTIVITY REGULATION

Proteins execute nearly all cellular processes and are synthesized through mRNA translation. Protein activity is regulated at many levels, but the major regulatory modes are (*a*) abundance, (*b*) post-translational modifications, and (*c*) cotranslational folding. For proteins primarily

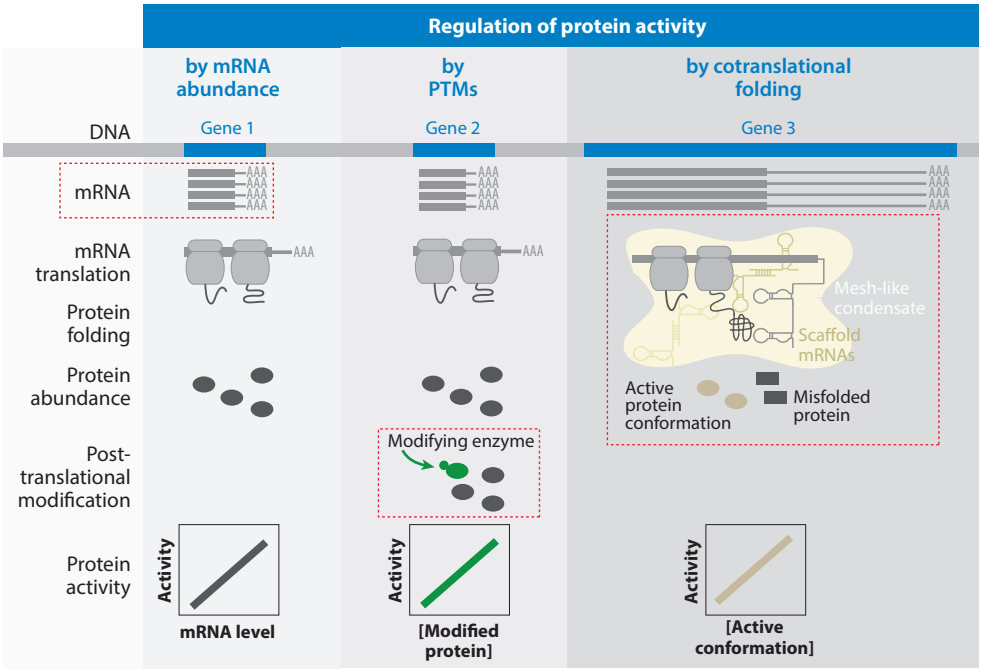

Figure 1

Different modes of protein activity regulation. The dashed red boxes indicate the major regulatory steps for protein activity. Abbreviations: mRNA, messenger RNA; PTMs, post-translational modifications.

regulated at the level of abundance, there is good correlation between mRNA levels, protein abundance, and cellular activity (**Figure 1**). The median size of human proteins is 440 amino acids. Abundance-regulated proteins usually are smaller than average, often contain few domains, and are predominantly translated in the cytosol (Horste et al. 2023, Litterman et al. 2019).

In addition to abundance, the activity of most proteins is controlled through covalent modifications, such as phosphorylation, acetylation, or ubiquitination, which are added after the protein has been fully synthesized. Post-translational modifications regulate the activation states of proteins and control interactor binding, protein localization, and their lifetimes (Duan & Walther 2015, Lee et al. 2023, Pohl & Dikic 2019) (**Figure 1**).

A third mode of protein activity regulation occurs during protein synthesis and is mediated by protein folding. Most proteins fold autonomously or with the help of protein chaperones into functional three-dimensional conformations (Hartl et al. 2011). Although protein folding is fundamental for our understanding of protein activity, it is still poorly understood. Nearly all folding studies over the past seven decades have focused on single-domain proteins that are smaller than 130 residues (Chen et al. 2008). Although these proteins are amenable to biophysical study, their sizes are not representative for human proteins, leaving unresolved the mechanisms that guide the folding of large, multidomain proteins (Braselmann et al. 2013, Han et al. 2007, Liu et al. 2019).

Recent evidence suggests that cotranslational folding and assembly into homo- or heteromeric complexes are widespread, indicating that 15% of proteins in bacteria and 40% of human proteins use concurrent folding and binding to generate stable proteins (Bertolini et al. 2021, Duncan & Mata 2011, Mallik et al. 2025, Schweke et al. 2024, Shiber et al. 2018, To et al. 2022). For cotranslational heterodimer assembly to take place, mRNAs encoding the different dimer subunits

must be proximal, a requirement that could be fulfilled by their colocalization within cytoplasmic compartments, including condensates (X. Chen et al. 2024, Horste et al. 2023, Ma & Mayr 2018).

Moreover, during protein synthesis, mRNAs are obligatorily colocalized with the nascent chains of their encoded proteins. This arrangement allows cotranslational processes to be regulated by mRNAs. mRNAs can regulate translation elongation speed, and their 3′ UTRs can control the recruitment of protein-binding partners (Berkovits & Mayr 2015; Gasparski et al. 2022, 2023) or directly act as RNA-mediated chaperones for IDRs (Luo et al. 2026). mRNA 3′ UTRs have recently emerged as regulatory hot spots that control where in the cytoplasm an mRNA is translated, even in nonpolarized cells (Horste et al. 2023). Moreover, by directing mRNA translation to mesh-like condensates, 3′ UTRs regulate the activity of the encoded proteins through protein folding (Luo et al. 2026) (**Figure 1**). However, we are only beginning to understand the mechanisms that turn genetic information in 3′ UTRs into cellular processes that control biological phenotypes. As 3′ UTRs are central for protein activity regulation, their features are described below.

HUMAN 3′ UTRs CONSTITUTE NEARLY HALF OF mRNA SEQUENCE

Transcriptome-Wide Mapping of mRNA 3′ UTRs

Although every mRNA molecule contains a 3′ UTR, the precise locations of human mRNA 3′ ends, and therefore 3′ UTR boundaries, were largely unknown until a decade ago. The development of mRNA 3′ end sequencing technologies enabled transcriptome-wide mapping of cleavage and polyadenylation sites, revealing the full landscape of 3′ UTRs for the first time (Gruber et al. 2014, Hoque et al. 2013, Lianoglou et al. 2013, Shepard et al. 2011). When averaged across the transcriptome, 3′ UTRs contain as many nucleotides as coding sequences; thus, roughly half of the human mRNA sequence resides in 3′ UTRs (Fansler et al. 2024).

Alternative 3′ UTR Isoform Expression Is Highly Cell Type–Specific

3′ end sequencing studies revealed that more than half of human and mouse genes undergo alternative cleavage and polyadenylation (APA), thus producing mRNA isoforms that share identical coding sequences but differ in their 3′ UTRs (Gruber et al. 2014, Hoque et al. 2013, Lianoglou et al. 2013, Shepard et al. 2011). More recently, analysis pipelines were developed that allow the simultaneous quantification of mRNA abundance and 3′ UTR isoform expression from single-cell RNA-sequencing data (Fansler et al. 2024). Applying this pipeline to more than 400 distinct primary cell types showed that most multi-UTR genes express two major 3′ UTR isoforms, with additional minor variants expressed at much lower levels. The relative expression ratios of short and long 3′ UTRs are highly cell type–specific (Lianoglou et al. 2013) and change gradually along differentiation trajectories, underscoring the tight regulation of APA (Fansler et al. 2024).

Importantly, during differentiation, a gene changes either the total transcript abundance or 3′ UTR isoform ratio, but rarely both (Brumbaugh et al. 2018, Gruber et al. 2014, Hoque et al. 2013, Li et al. 2021, Lianoglou et al. 2013, Nanavaty et al. 2020, Spies et al. 2013). This observation suggests that APA adds a second dimension to gene expression regulation. The first dimension corresponds to overall mRNA abundance, while the second reflects the relative usage of long versus short 3′ UTR isoforms. Both expression dimensions occur with comparable frequency, suggesting that changes in 3′ UTR length represent a regulatory mode as common as changes in mRNA abundance (Fansler et al. 2024).

Despite substantial advances in the transcriptome-wide understanding of 3′ UTR variability (Mitschka & Mayr 2022), the biological consequences of 3′ UTR length variation have remained largely unclear until recently. As mRNA isoforms with differences in 3′ UTR length encode

identical proteins, it was hypothesized that mRNA 3′ UTRs could influence the functions of the encoded proteins.

PROTEIN ACTIVITY CONTROL BY mRNA 3′ UTRs

3′ UTRs Can Control Protein Localization and Function of Membrane Proteins

To explore the functional significance of alternative 3′ UTRs, mRNAs encoding membrane proteins were investigated. In human cells, *CD47* mRNA has two 3′ UTR isoforms containing either 204 or 4,197 nucleotides that encode an identical protein. Strikingly, the 3′ UTR in the mRNA template controls the subcellular localization of the encoded protein (Berkovits & Mayr 2015). CD47 localizes to the plasma membrane when expressed from the long 3′ UTR–containing mRNA, whereas the protein mostly remains at the site of its synthesis, the endoplasmic reticulum (ER), when expressed from a short 3′ UTR–containing mRNA. The 3′ UTR–dependent difference in protein localization was also observed for additional membrane proteins, including integrin α1 and BAFF receptor (Berkovits & Mayr 2015). For CD47, the difference in protein localization dictates function. At the plasma membrane, CD47 serves as a "don't-eat-me" signal, which prevents macrophage-mediated phagocytosis and promotes immune escape (Jaiswal et al. 2009). As alternative 3′ UTRs determine where in the cell the CD47 protein accumulates, this discovery provided the first direct demonstration that 3′ UTRs can influence protein function by regulating protein localization independently from mRNA localization (Berkovits & Mayr 2015).

The biological importance of including UTRs in mRNA transcripts is further supported by gene therapy studies (Geng et al. 2017). Usher syndrome type III is characterized by progressive childhood hearing loss, caused by mutations in *CLRN1*, which encodes a hair cell plasma membrane protein. Therapeutic strategies to restore hearing are being tested in *Clrn1* knockout mice. Restoration of hearing failed when the therapeutic construct contained only the *Clrn1* coding sequence, but a construct that included both the 5′ and 3′ UTRs of *Clrn1* mRNA fully restored hearing in mice throughout their lifetimes (Geng et al. 2017). This profound rescue underscores the essential roles of UTRs in ensuring proper protein function and demonstrates their critical importance for the design of effective gene therapy strategies.

mRNA 3′ UTRs Are Necessary for Protein Complex Assembly of Their Encoded Proteins

For CD47 plasma membrane localization, the 3′ UTR effect was mediated by the RNA-binding protein HuR, which binds to the long but not the short *CD47* 3′ UTR (Berkovits & Mayr 2015). HuR is essential for facilitating a protein–protein interaction between CD47 and the multifunctional adaptor protein SET, which was previously described as a histone chaperone and phosphatase inhibitor (Bayarkhangai et al. 2018, Brennan et al. 2000). Although SET is abundant in cells, it becomes binding competent only when recruited via HuR to the site of *CD47* mRNA translation (Berkovits & Mayr 2015). The SET–CD47 interaction promotes efficient plasma membrane trafficking and immune escape, which are adopted by liver cancer cells with overexpressed alpha-fetoprotein (AFP). Physiologically, AFP protects the fetus from maternal immune attack. In liver cancer cells, AFP increases cytoplasmic HuR, leading to increased CD47 surface expression and the potent escape of cancer cells from immune surveillance (Pan et al. 2025).

The key regulatory role of the long *CD47* 3′ UTR is to facilitate a specific protein–protein interaction between the nascent, mRNA-encoded protein and a 3′ UTR–recruited interactor. This finding established a new paradigm: mRNA 3′ UTRs can control protein properties through

the cotranslational recruitment of binding partners necessary for proper protein maturation and function (Berkovits & Mayr 2015). The principle of 3′ UTR–dependent partner recruitment has since been observed in multiple systems, including trafficking of the β_2-adrenergic receptor, PD-L1, and tissue factor protein (Chatterton-Bartley et al. 2025, H.-H. Chen et al. 2024, Ma & Mayr 2018, Tholanikunnel et al. 2010).

mRNA 3′ UTRs Allow Rapid Stimulus-Dependent Protein Trafficking

It is possible that 3′ UTR–mediated trafficking of plasma membrane proteins has evolved to allow rapid stimulus-dependent trafficking (Chatterton-Bartley et al. 2025). For example, the *F3* mRNA encodes tissue factor, a membrane protein that regulates blood coagulation and inflammation. Its 3′ UTR contains an inverted Alu repeat, which is necessary for retention of the tissue factor protein in the Golgi apparatus. Without further regulation, the presence of the 3′ UTR causes low protein expression at the cell surface. Activation of PAR2, a G protein–coupled receptor involved in coagulation and inflammation, releases the Golgi apparatus–retained protein, thus leading to rapid plasma membrane localization of the tissue factor protein (Chatterton-Bartley et al. 2025). This study nicely revealed how elements in 3′ UTRs cooperate with cellular signaling events to control rapid changes in protein localization, which are especially relevant for blood coagulation or immune responses.

3′ UTR–Mediated Protein Complex Assembly Is Not Restricted to Membrane Proteins

3′ UTR–mediated protein complex assembly is evolutionarily conserved (Ashley et al. 2018, Lee & Mayr 2019, Pastuzyn et al. 2018). In yeast, 3′ UTRs facilitate the cotranslational recruitment of the signal recognition particle to membrane proteins (Chartron et al. 2016, Loya et al. 2008), promote the RPS28B–EDC3 interaction required for P-body formation (Fernandes & Buchan 2020), and mediate the 3′ UTR–dependent incorporation of ribosomal protein paralogs into ribosomes (Singh et al. 2024).

Together, these findings showed that 3′ UTRs encode far more than regulatory elements for mRNA abundance, localization, or stability. Instead, 3′ UTRs contain genetic instructions that influence the functional fate of the encoded protein, including its ability to assemble into higher-order complexes (Berkovits & Mayr 2015, Gasparski et al. 2023, Lee & Mayr 2019). The observation that alternative 3′ UTR isoforms dictate differential binding partner selection suggests a cell type–specific mechanism: By expressing distinct 3′ UTR isoforms, cells may selectively modulate protein interaction networks, thereby tailoring protein function to specific physiological contexts (Cheng et al. 2021). Moreover, even without changes in 3′ UTR isoform expression, 3′ UTR elements cooperate with signaling-dependent events to change protein trafficking pathways that require rapid rewiring of protein interaction networks during receptor trafficking and recycling (Chatterton-Bartley et al. 2025, Lee & Mayr 2019).

TIS GRANULES MEDIATE 3′ UTR EFFECTS ON PROTEIN FUNCTIONS

RNA-Binding Proteins Mediate the 3′ UTR Effects on Protein Function

3′ UTR–dependent CD47 protein regulation requires HuR, which is necessary, but not sufficient. Cooperating RNA-binding proteins were identified through a small-scale screen that identified minimal 3′ UTR sequences capable of recapitulating the 3′ UTR effect in CD47 trafficking (Ma & Mayr 2018, Mayr 2019). The top hit was a short region of the *TNF* 3′ UTR, containing six canonical AU-rich elements, previously characterized as an mRNA stability regulatory

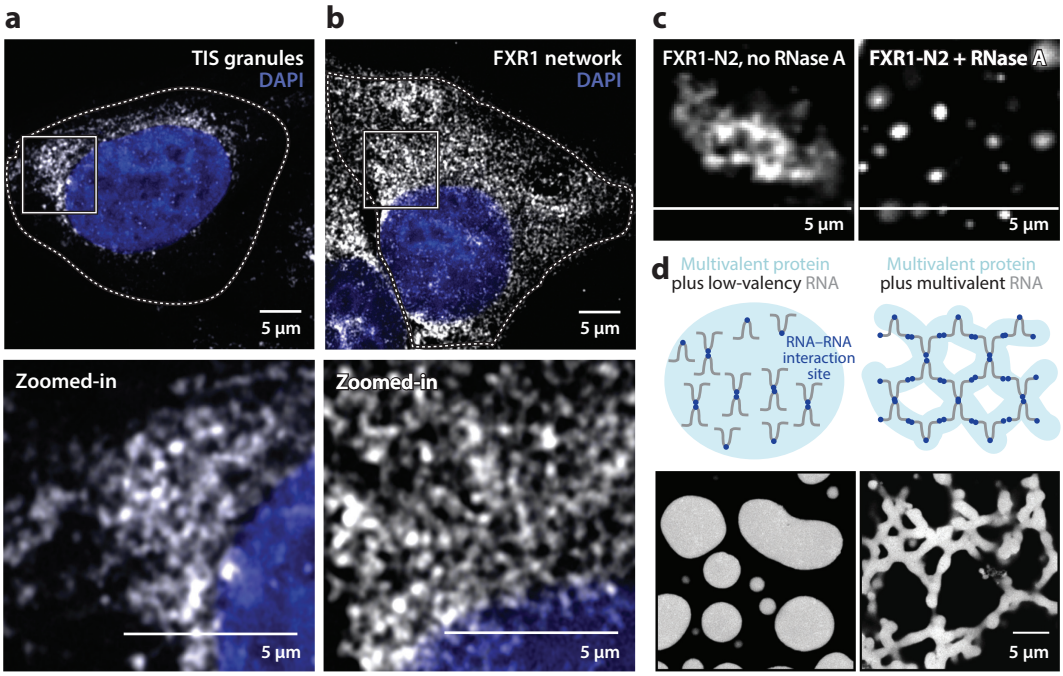

Figure 2

Multivalent messenger RNAs are the scaffolds of mesh-like condensates. (*a*,*b*) Immunofluorescence staining of (*a*) endogenous TIS11B in HeLa cells and (*b*) endogenous FXR1 in U2OS cells. DAPI was used to stain the nucleus. The cellular outlines are shown by the dashed lines. The squares denote the regions for the zoomed-in images shown below. (*c*) Mesh-like condensates were generated by expression of the N-terminal 399 amino acids of human FXR1 (FXR1-N2). RNase A treatment disrupts the network connections, indicating that they are formed by RNA. (*d*) Schematic of intermolecular RNA–RNA interactions of low-valency or multivalent RNAs. Multivalent proteins are shown in light blue, RNA in gray, and RNA–RNA interaction sites in dark blue. The schematic serves as a model for in vitro–generated condensates, shown below, that are formed by mixing of low-valency RNAs together with a multivalent protein, which results in droplet-like condensates (*left*), or mixing of multivalent RNAs with multivalent proteins, which results in mesh-like condensates, where RNA acts as a condensate scaffold (*right*). Abbreviation: DAPI, 4′,6-diamidino-2-phenylindole.

element (Kontoyiannis et al. 1999). However, when flanked by short engineered sequences that maintain element accessibility, the resulting 56-nucleotide-long 3′ UTR directed efficient CD47 cell surface localization, thus strongly overcoming its slightly destabilizing effect (Ma & Mayr 2018).

RNA pull-down experiments revealed that only two RNA-binding proteins—HuR and TIS11B—bound specifically to the engineered sequence. High-resolution imaging of endogenous TIS11B demonstrated that it assembles into perinuclear, mesh-like structures termed TIS granules (Ma & Mayr 2018) (**Figure 2*a***). TIS granules enrich mRNAs with AU-rich elements and exclude mRNAs with GC-rich 3′ UTRs, and form in steady-state, unstressed conditions. TIS11B is essential for TIS granule formation, as its knockdown abolishes the local mesh-like morphology of TIS granule–enriched mRNAs (Ma & Mayr 2018).

Importantly, the 3′ UTR effect on CD47 protein localization requires TIS granules and not only the presence of the TIS11B protein. This result indicates that mesh-like condensates, formed by TIS11B and its bound mRNAs, are necessary to turn 3′ UTR information into specific cellular outcomes, such as a cotranslational protein interaction, which controls CD47 protein activity at the cell surface (Ma & Mayr 2018).

Identification of TIS Granule–Enriched mRNAs

To elucidate broader biological functions of TIS granules, the enriched mRNAs were identified. TIS granules are surrounded by the cytosol—the soluble part of the cytoplasm—and are tightly associated with the rough ER (Ma & Mayr 2018). The mRNA composition of TIS granules, the ER surface, and the cytosol was determined through a combination of biochemical fractionation and fluorescent particle sorting (Cui et al. 2012, Hubstenberger et al. 2017), followed by transcriptome-wide profiling and validation by single-molecule RNA fluorescence in situ hybridization (RNA FISH) (Horste et al. 2023). This study revealed that distinct mRNA subsets are enriched in each cytoplasmic compartment. Although TIS granules are tightly associated with the ER, they are not enriched in membrane protein–encoding mRNAs. Consistent with earlier observations, these mRNAs mostly localize to the ER not associated with TIS granules (Horste et al. 2023, Jan et al. 2014).

Since roughly 70% of cellular mRNAs encode proteins lacking transmembrane domains, this group was analyzed in greater detail. Half of these mRNAs were enriched in one of the investigated compartments—TIS granules, the ER surface, or the cytosol. Independent studies using orthogonal methods—including APEX-seq, multiplexed error-robust fluorescence in situ hybridization (MERFISH), localization of RNA (LoRNA), and RNA-SPRITE—identified similar groups of membrane-, condensate-, and cytosol-enriched mRNAs. Despite the use of different cell types, the datasets correlate strongly, which underscores the robustness of cytoplasmic transcriptome organization (Becker et al. 2025, Fazal et al. 2019, Horste et al. 2023, Villanueva et al. 2024, Xia et al. 2019).

Interestingly, the mRNAs enriched in distinct cytoplasmic compartments differ in the functional classes of their encoded proteins. Cytosolic mRNAs have short and GC-rich 3′ UTRs and encode small proteins, often involved in mRNA processing, splicing, or translation. By contrast, mRNAs encoding large, highly abundant proteins, such as cytoskeletal binding proteins or chromatin regulators, are enriched on the ER surface and associated with TIA/TIAL1. The so-called TIA-ER region appears to be an additional mesh-like compartment, but a detailed characterization has not yet been performed (Horste et al. 2023, Luo et al. 2026).

Although CLIP datasets from 170 RNA-binding proteins were analyzed to elucidate the molecular basis of compartment-specific localization, it turned out that in addition to the binding patterns of a few RNA-binding proteins, intrinsic mRNA architectural features—including mRNA length, coding sequence length, exon number, and average coding sequence exon length—were necessary to explain the observed subcytoplasmic mRNA localization patterns (Horste et al. 2023). Together, these results demonstrate that mRNA localization within the cytoplasm is highly organized and nonrandom. The spatial segregation of mRNAs arises from an interplay between *cis*-encoded mRNA features and *trans*-acting RNA-binding proteins, which collectively define a combinatorial code to control cytoplasmic mRNA localization and demarcate distinct subcytoplasmic environments for translation (Becker et al. 2025, Horste et al. 2023).

Low-Abundance Proteins Are Translated in TIS Granules

TIS11B, the TIS granule scaffold protein, is encoded by the *ZFP36L1* gene, which has an atypical exon architecture (Horste et al. 2023). Its coding sequence contains only two exons, one of which spans nearly 1,000 nucleotides—a remarkable deviation from the average coding exon length of 150 nucleotides (He et al. 2023, Sanjeev et al. 2025, Uzonyi et al. 2023). mRNAs with such atypical exon architectures are enriched in TIS granules. As many transcription factor genes have such architectures, it may explain the substantial overrepresentation of transcription factor–encoding mRNAs in TIS granules (Horste et al. 2023).

Characteristic of TIS granule–enriched mRNAs is the low cellular abundance of their encoded proteins. Low protein abundance correlated with low mRNA levels, and notably, the lower transcript levels arise primarily from low transcriptional output rather than accelerated degradation. Consistent with this observation, SunTag translation reporter assays demonstrated that low-abundance mRNAs are actively translated within TIS granules (Horste et al. 2023, Yan et al. 2016). It was proposed that TIS granule localization protects these mRNAs from degradation, thereby promoting the production of proteins that are otherwise transcribed at low levels (Horste et al. 2023).

THE FXR1 NETWORK IS AN mRNA-SCAFFOLDED CYTOPLASMIC COMPARTMENT

mRNAs Scaffold the FXR1 Network

A small-scale, high-resolution imaging screen of abundant cytoplasmic RNA-binding proteins uncovered the FXR1 network (X. Chen et al. 2024) (**Figure 2*b***). The FXR1 network forms an extensive, cytoplasm-spanning mRNA-protein assembly, generated by the RNA-binding protein FXR1 together with its bound mRNAs. FXR1 is among the most highly expressed RNA-binding proteins across human cell types (Han et al. 2020). FXR1 binds to the 3′ UTRs of more than 1,000 mRNAs, with a marked preference for exceptionally long 3′ UTRs—typically three- to sixfold longer than those of mRNAs not bound by FXR1 (X. Chen et al. 2024).

mRNAs are large, self-assembling macromolecules (Van Treeck et al. 2018) that need to be organized by RNA-binding proteins to prevent aggregation and ensure translational competence. In the coding sequence, the exon-junction complex, which assembles upstream of exon–intron junctions, packages mRNAs (Singh et al. 2015). Since most 3′ UTRs lack introns, it remains unclear how these regions are organized. Given that the median human 3′ UTR length is approximately 1,300 nucleotides (Fansler et al. 2024), shorter 3′ UTRs may not require dedicated packaging factors. In contrast, the binding of FXR1 to very long 3′ UTRs may serve as a structural mechanism for organizing these extended mRNA regions (X. Chen et al. 2024).

Importantly, short-term RNase A treatment turns the cellular FXR1 network into discrete droplets, revealing that the connections between individual droplets consist of RNA and suggest that mRNAs with long 3′ UTRs act as an FXR1 condensate scaffold (X. Chen et al. 2024) (**Figure 2*c***). These findings are supported by insights gained from an assembly-deficient FXR1 mutant, which was instrumental for dissecting the molecular assembly mechanism of the FXR1 network.

Surprisingly, the endogenous FXR1 network is disrupted through a single point mutation, which prevents FXR1 dimerization and higher-order assembly. FXR1 iCLIP analysis of wild type and an assembly-deficient mutant identified two classes of bound mRNAs: scaffold and client transcripts. Even in the absence of ultraviolet cross-linking, scaffold mRNAs are tightly bound to assembled FXR1, indicating that they bind with high affinity. In contrast, client mRNAs only associate with the FXR1 network through cross-linking (X. Chen et al. 2024). Although in vitro FXR1 has a low intrinsic affinity to RNA (Athar & Joseph 2020), the large number of binding motifs potentially present in long 3′ UTRs, which are typical for scaffold mRNAs, may overcome the low intrinsic affinity through the presence of many binding sites.

The FXR1 Network Concentrates Proteins with Similar Domains as FXR1

To identify proteins enriched in the FXR1 network, proteins associated with wild-type or assembly-deficient FXR1 were determined through proteomic analyses. These experiments identified numerous RNA-binding proteins, subunits of intermediate filaments,

cytoskeleton-associated proteins, and kinases. These proteins bind specifically to assembled FXR1, but not to unassembled monomers, and are enriched for Tudor, coiled-coil, and RGG domains, mirroring the protein domains present in FXR1 itself (X. Chen et al. 2024).

In the current model of assembly, FXR1 homodimerizes through its coiled-coil domains. FXR1 dimers bind to mRNAs with long 3′ UTRs, acting as molecular scaffolds. The 3′ UTR scaffolds enable the proximity of additional FXR1 molecules, increase their local concentration, and drive phase separation. FXR1 contains RNA-binding domains and multiple protein–protein interaction domains, which mediate extensive interactions between FXR1 molecules as well as between FXR1 and proteins with binding motifs similar to FXR1 (Adinolfi et al. 2003, Alex & Lee 2005, Handa et al. 2006, Hu et al. 2015, Mason & Arndt 2004). While mRNAs provide the underlying scaffold, the surface of the FXR1 network is decorated with multivalent protein-binding sites, forming a large-scale cytoplasmic framework for protein organization (X. Chen et al. 2024).

The FXR1 Network Enables the Proximity of Kinases and Their Substrates

Although FXR1 is an RNA-binding protein, the FXR1 network exerts biological functions that go beyond RNA metabolism. Quantitative proteomics comparing control and FXR1-depleted cells revealed no major changes in global protein abundance (X. Chen et al. 2024), indicating that the FXR1 network does not primarily regulate translation or stability. Instead, the proteins encoded by FXR1-bound mRNAs showed strong overrepresentation of signaling-related proteins, including GDP-binding proteins, ubiquitin enzymes, phosphatases, and kinases. Remarkably, nearly all components of the RhoA signaling pathway, which controls actomyosin remodeling, represent FXR1 targets (X. Chen et al. 2024).

RhoA-dependent actomyosin remodeling governs cell adhesion, migration, and morphology (Hall 1998, Riento & Ridley 2003) and can be monitored through surface receptor–stimulated stress fiber formation. The assembly-deficient FXR1 mutant revealed that signaling-induced actomyosin remodeling requires not merely the FXR1 protein but specifically the assembled FXR1 network (X. Chen et al. 2024). These findings provide the first evidence that a cytoplasmic signaling pathway depends on an intact mRNA-scaffolded condensate network for proper signal transduction. Mechanistically, the proximity between the ROCK kinase and one of its substrates is established by the FXR1 network. Both the kinase and substrate harbor coiled-coil domains, which enrich them within the FXR1 network, thus enabling efficient phosphorylation through spatial proximity (X. Chen et al. 2024). Given that many FXR1-bound mRNAs encode signaling molecules, additional cytoplasmic pathways may similarly exploit the FXR1 network as a signaling scaffold.

Together, these findings define the FXR1 network as a cytoplasmic, mRNA-scaffolded condensate that organizes signaling factors through multivalent RNA- and protein-mediated interactions. By engendering the proximity of kinases, substrates, and adaptors, the FXR1 network functions as a molecular platform for signal transmission, extending the paradigm of RNA-based cellular organization to the field of the regulation of cytoplasmic signaling.

Is Impaired Signaling Involved in Fragile X Syndrome?

In vertebrates, FXR1 has two close homologs—FXR2 and FMR1. While FXR1 is ubiquitously expressed across tissues, the expression of FXR2 and FMR1 is more restricted (Han et al. 2020). Whereas loss of FXR1 results in embryonic lethality in mice and humans (Lek et al. 2016), loss-of-function mutations in FMR1 cause fragile X syndrome (FXS), a disorder of inherited intellectual disability and autism (Richter & Zhao 2021).

Similar to FXR1, FMR1 forms large, cytoplasm-spanning condensate networks together with its bound mRNAs (X. Chen et al. 2024). This structural and biochemical similarity suggests that

condensate-mediated signaling could contribute to FXS pathogenesis. This idea is supported by previous findings of reduced cAMP signaling in patients and animal models of FXS. Moreover, restoration of signaling through phosphodiesterase inhibitors alleviated patients' symptoms (Berry-Kravis et al. 2021, Choi et al. 2016, Kanellopoulos et al. 2012). Mesh-like condensates could indeed be involved in FXS pathogenesis, as most components of the cAMP cascade are FXR1 targets.

MULTIVALENT mRNAS ARE THE SCAFFOLDS OF MESH-LIKE CONDENSATES

Multivalent mRNAs Form Intermolecular RNA–RNA Interactions

Initially, it was thought that biomolecular condensates are spherical, a morphology attributed to the surface tension inherent to liquid-like phase-separated droplets (Hyman et al. 2014). Consequently, the irregular, mesh-like architecture of TIS granules raised the question of whether they are classical condensates.

In vitro phase separation assays revealed the cause of the underlying mesh-like morphology. To generate liquid-like condensates, a chimeric multivalent RNA-binding protein with the TIS11B RNA-binding domain and the FUS IDR was used. Ma et al. (2021) tested individually in vitro–transcribed 3′ UTRs from nearly 50 different mRNAs for changes in condensate morphology. This systematic approach revealed two distinct classes of RNA: one that maintained the spherical morphology of the droplets and another that transformed them into mesh-like condensates (Ma et al. 2021). In both condensate types, the protein component rapidly recovered its fluorescence after photobleaching, indicating a dynamic protein phase. However, fluorescence recovery after photobleaching (FRAP) analysis of fluorescently labeled RNA revealed a striking difference: While RNA recovered rapidly in droplet-forming mixtures, RNA within mesh-like condensates showed no recovery, suggesting that the RNA itself functions as a scaffold or skeletal framework for the network (Ma et al. 2021). These findings indicated that in spherical condensates, RNA acts as client, whereas in mesh-like condensates RNA acts as condensate scaffold.

Since then, additional mesh-like or irregularly shaped condensates have been observed, including the FXR1 network, L-bodies from *Xenopus laevis* oocytes, oskar granules from fly oocytes, *CLN3*-scaffolded Whi3 condensates from *Ashbya gossypii*, and mRNA-protein assemblies detected at focal adhesions (Boraas et al. 2021, Bose et al. 2024, X. Chen et al. 2024, Neil et al. 2021, Seim et al. 2024, Smith et al. 2020, Zhang et al. 2015), further highlighting the prevalence of this architectural principle.

A central question arising from these findings was, What RNA features drive the formation of mesh-like condensates? Conventional RNA properties were excluded as explanatory factors, including RNA length, GC content, and binding site number for the scaffold protein (Ma et al. 2021). Although all tested long and AU-rich RNAs induced network-like condensates, RNA length and high AU content were not necessary requirements for the formation of mesh-like condensates. Instead, in vitro and in vivo reconstitution assays demonstrated that intermolecular RNA–RNA interactions are key. Droplet-forming RNAs could be converted into network-forming RNAs by the introduction of at least three accessible RNA–RNA interaction sites. Such RNAs, capable of forming multiple intermolecular connections, were termed multivalent RNAs, whereas RNAs without or with only a few intermolecular binding sites have a low valency. These findings established that spherical droplets arise from interactions between multivalent proteins and low-valency RNAs, whereas mesh-like condensates emerge when both proteins and RNAs are multivalent (Ma et al. 2021) (**Figure 2*d***).

Structural Flexibility Characterizes Multivalent RNAs

RNA multivalency can be determined experimentally, through phase separation assays or native gel electrophoresis, where multivalent RNAs migrate as high–molecular weight assemblies rather than as a discrete band (Bose et al. 2024, Ma et al. 2021, Wei et al. 2024). RNA structure predictions revealed that low-valency RNAs form strong intramolecular base pairs, whereas multivalent RNAs exhibited fewer Watson–Crick base pairing events, resulting in more flexible and extended conformations (Ma et al. 2021). To quantify these differences, we calculated the ensemble diversity (ED) for each RNA—a metric for the number of predicted structural conformations of an RNA, which correlates inversely with the degree of base pairing (Lorenz et al. 2011). Because ED scales with RNA length, a normalized ED (NED) value was introduced. Experimentally, NED proved a reliable predictor of RNA behavior: RNAs with high NED values formed mesh-like condensates, while those with low NED values formed droplets (Ma et al. 2021).

These results firmly established that weak intramolecular structure and high ED underlie RNA multivalency, which is a parameter that describes the extent of accessible binding sites of an RNA. Moreover, multivalent mRNAs are the architectural basis of cytoplasmic, mesh-like condensates, where they become enriched through intermolecular RNA–RNA interactions. Intriguingly, further characterization found that the most multivalent human mRNAs are those whose 3′ UTR sequences are extensively conserved (Luo et al. 2026).

mRNAs with Highly Conserved 3′ UTRs Are Multivalent

One strategy to identify 3′ UTR functions is to investigate mRNAs with alternative 3′ UTRs. These studies revealed that the translation of proteins from mRNAs with short or long 3′ UTRs can be responsible for distinct cellular phenotypes (An et al. 2008, Bae et al. 2020, Berkovits & Mayr 2015, Chen et al. 2018, Kwon et al. 2022, LaBella et al. 2020, Lee & Mayr 2019, Mitschka & Mayr 2022).

An alternative strategy for the identification of functionally relevant 3′ UTRs is comparative sequence conservation analysis (Luo et al. 2026). PhyloP conservation scores derived from alignments of 100 vertebrate species—including mammals, birds, reptiles, and fish—stratify human mRNAs into three groups (Luo et al. 2026, Pollard et al. 2010, Siepel et al. 2005): mRNAs with nonconserved (NC) 3′ UTRs lacking conserved nucleotides, highly conserved (HC) 3′ UTRs containing at least 150 conserved bases with PhyloP scores greater than two, and an intermediate group. Across the entire human genome, only 0.7% of bases meet the criteria used to define HC 3′ UTRs, but they nevertheless account for 15% of human 3′ UTRs (Luo et al. 2026).

With respect to their RNA features, HC and NC 3′ UTRs differ not only in evolutionary conservation but also in nucleotide composition, predicted secondary structures, and regulatory properties (Litterman et al. 2019, Luo et al. 2026). NC 3′ UTRs evolve quickly, are short and GC-rich, have low NED values, and form stable intramolecular structures (Litterman et al. 2019, Spitale et al. 2015). By contrast, HC 3′ UTRs are significantly longer and AU-rich and have high NED values and weaker intramolecular structures (Litterman et al. 2019, Luo et al. 2026). Therefore, HC 3′ UTRs have all known features currently associated with RNA multivalency, whereas NC 3′ UTRs represent low-valency RNAs. Intriguingly, in cells, mRNAs with HC 3′ UTRs are strongly enriched in mesh-like condensates, whereas mRNAs with NC 3′ UTRs predominantly localize to the cytosol (Luo et al. 2026).

In addition to differences in subcytoplasmic localization, massively parallel reporter assays showed that GC-rich 3′ UTRs repress mRNA and protein abundance, whereas AU-rich 3′ UTRs lack the repressive activities (Courel et al. 2019, Litterman et al. 2019), leading to the question, By which mechanism do AU-rich conserved 3′ UTRs control cellular processes?

CONSERVED 3′ UTRs CONTROL COTRANSLATIONAL PROTEIN FOLDING

mRNAs with Highly Conserved 3′ UTRs Encode Proteins with Long Intrinsically Disordered Regions

mRNAs with HC or NC 3′ UTRs strongly differ in the types of proteins they encode. mRNAs with HC 3′ UTRs mostly encode proteins with long IDRs, substantially enriched for transcription factors and chromatin regulators (Cermakova & Hodges 2023, Luo et al. 2026). Proteins encoded by mRNAs with NC 3′ UTRs are enriched for metabolic or immune regulatory function (Litterman et al. 2019).

To assess potential roles of HC 3′ UTRs, we expressed proteins with long IDRs from mRNA templates with or without their native HC 3′ UTRs (Luo et al. 2026). For the transcription regulators tested, inclusion of the 3′ UTRs did not alter steady-state protein levels or subcellular localization, yet it profoundly affected the functional activity of the expressed proteins. For example, expression of MYC from the 3′ UTR–containing mRNA altered the transcription of twice as many target genes compared with the MYC protein expressed from a 3′ UTR–lacking template (Luo et al. 2026). We set out to identify mechanisms by which 3′ UTRs—which are not part of proteins—change protein activity in the nucleus.

Highly Conserved 3′ UTRs Control the Cellular Activity of Intrinsically Disordered Region–Containing Proteins

3′ UTR–dependent regulation of protein activity was also observed for JMJD3, encoded by the *KDM6B* mRNA, and it was chosen for detailed analysis. As JMJD3 removes the histone modification H3K27me3 from chromatin (Agger et al. 2007, Xiang et al. 2007), its cellular activity can be measured by a reduction of H3K27me3 levels. JMJD3 expression from a 3′ UTR–containing mRNA generated an active protein, whereas its expression from a 3′ UTR–lacking template yielded a protein with strongly reduced demethylase activity, despite comparable protein abundance, protein localization, and chromatin occupancy (Luo et al. 2026). These results indicate that the *KDM6B* 3′ UTR controls JMJD3's cellular activity independently from protein levels.

Long Intrinsically Disordered Regions Contain Local Hydrophobic Clusters that Participate in Cotranslational Protein Folding

Structurally, JMJD3 comprises a C-terminal JmjC domain, which mediates demethylase activity, and an N-terminal IDR of more than 1,100 amino acids. IDR deletion generates an active protein, whose activity is 3′ UTR–independent, pointing to a functional interplay between the IDR in the protein and the 3′ UTR in the mRNA (Luo et al. 2026).

Compared with folded domains, IDRs contain fewer hydrophobic residues, which prevents them from adopting stable structures in isolation (Davey 2019, Wright & Dyson 2009). However, hydrophobic residues are not absent from IDRs. Long IDRs with more than 250 amino acids often contain short tracts with clustered hydrophobic amino acids. The number of these so-called hydrophobic clusters correlates with IDR length, and they subdivide long IDRs into smaller segments (Luo et al. 2026). Whereas the intervening IDR segments behave like classical IDRs, existing as dynamic ensembles of interconverting conformations (Holehouse & Kragelund 2024), the hydrophobic clusters in the IDR participate in cotranslational protein folding (Luo et al. 2026), likely driven by the need to be shielded from solvent exposure during protein biogenesis (Balchin et al. 2016, Bardwell & Jakob 2012).

Highly Conserved 3′ UTRs Act as Cotranslational Intrinsically Disordered Region Chaperones

Consistent with the idea of the structural plasticity of IDRs, the hydrophobic clusters in the JMJD3 IDR can either bind to each other, thus forming IDR–IDR interactions, or bind to the structured domain. Importantly, the presence of the *KDM6B* 3′ UTR in the mRNA template controls the binding pattern of the IDR during protein synthesis, as was observed by cross-linking mass spectrometry of the cellular JMJD3 protein. In the absence of the 3′ UTR during protein folding, the long IDR interferes with the folding of the structured domain, thus causing protein misfolding and reduced activity. In contrast, 3′ UTR–IDR interaction promotes proper protein folding, indicating that mRNA 3′ UTRs act as cotranslational chaperones for proteins with IDRs (Luo et al. 2026).

As the hydrophobic clusters in the IDR are involved in protein misfolding, their mutation is sufficient for the full cellular activity of the JMJD3 protein, even when the protein is generated from a 3′ UTR–deficient mRNA. Conversely, the cotranslational effect of the 3′ UTR is mediated by positively charged patches in the JMJD3 IDR, which function as RNA-binding regions, indicating that direct RNA-protein binding is required for 3′ UTRs to act as cotranslational IDR chaperones (Luo et al. 2026). Importantly, not all 3′ UTRs have IDR chaperone activity. 3′ UTR swapping experiments revealed that all multivalent 3′ UTRs, capable of localizing their mRNAs to mesh-like cytoplasmic condensates, had IDR chaperone activity. In contrast, low-valency 3′ UTRs were unable to enrich mRNAs in these condensates and were incapable of acting as protein chaperones. Together, these results suggest that mRNA-scaffolded condensates act as protein folding environments that promote the formation of biologically active conformations of IDR-containing proteins by providing locally enriched mRNAs with chaperone activity (Luo et al. 2026).

Not All Cotranslational Processes Require Scaffold mRNAs

Although cotranslational processes have evolved as major regulatory pathways for protein activity and the assembly of large multiprotein complexes (Bernardini et al. 2023, Bertolini et al. 2021, Lautier et al. 2021, Seidel et al. 2022, Shiber et al. 2018), not all cotranslational events are 3′ UTR–dependent (Kamenova et al. 2019, Philippe et al. 2026). In particular, mRNAs with NC 3′ UTRs poorly colocalize, although their encoded proteins assemble through nascent chain–nascent chain interactions (Philippe et al. 2026). It appears that 3′ UTRs are not involved in the cotranslational assembly of fully structured domains with large interaction surfaces (Mena et al. 2018, 2020; Padovani et al. 2022; Safieddine et al. 2026), supporting the idea that 3′ UTRs may be particularly important for the biogenesis of IDR-containing proteins.

mRNAs Act as Post-Translational Chaperones to Prevent Protein Aggregation

In addition to acting as a cotranslational chaperone for IDRs, RNA also acts as a post-translational chaperone to prevent the aggregation of recombinant proteins (Choi et al. 2008, Docter et al. 2016, Litberg & Horowitz 2024, Litberg et al. 2023, Park et al. 2024). Intriguingly, in these experiments, RNA was a more potent chaperone than proteins on a per-weight basis (Docter et al. 2016).

Also in cells, RNA widely binds to IDRs (Castello et al. 2016, Oksuz et al. 2023), solubilizes condensates (Maharana et al. 2018, Zhang et al. 2015), and dissolves pathological aggregates in mice. Intriguingly, the injection of specific short RNAs derived from 3′ UTRs can reverse FUS or TDP-43 aggregation in motor neurons, even after aggregates had been formed (Copley et al. 2024, Guo et al. 2026). FUS and TDP-43 are RNA-binding proteins mutated in several neurodegenerative diseases and aggregate upon mislocalization to the cytoplasm. Interestingly, the RNAs with disaggregase activity were derived from conserved 3′ UTRs (Bhardwaj et al. 2013,

Guo et al. 2026, Sun et al. 2014), further supporting the idea that mRNA 3′ UTRs have evolved sequences to prevent the aggregation or misfolding of the encoded proteins.

HOW mRNA-SCAFFOLDED CONDENSATES TURN GENETIC INFORMATION INTO CELLULAR FUNCTIONS

In addition to encoding the sequences of proteins, mRNAs have emerged as regulators of protein activity by acting as IDR chaperones, as scaffolds for cotranslational protein complex assembly, as the skeletons of mesh-like condensates, and as protein-independent mediators of biological phenotypes (Berkovits & Mayr 2015, X. Chen et al. 2024, Jenny et al. 2006, Luo et al. 2026, Ma & Mayr 2018, Seim et al. 2024). RNA multivalency is the major determinant for mRNA enrichment in mesh-like condensates (**Figure 3*a***). The following mechanisms may allow mesh-like condensates to mediate mRNA effects on protein function.

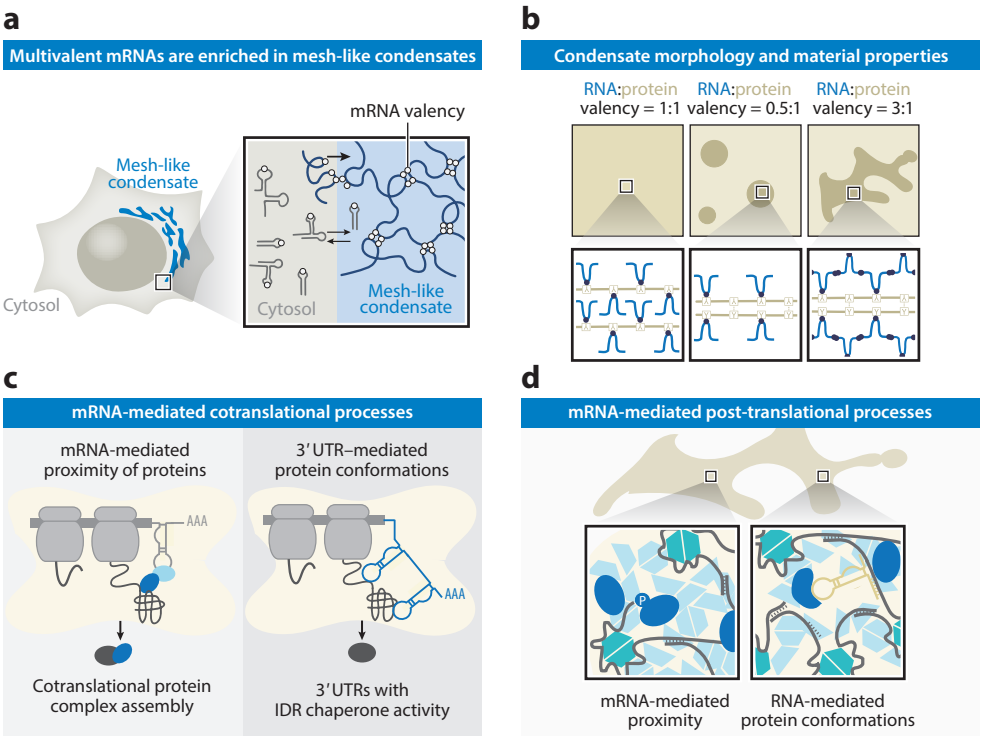

Figure 3

Mechanisms by which mesh-like condensates turn genetic information in mRNAs into cellular reactions and phenotypes. (*a*) Multivalent mRNAs (*blue*) use intermolecular RNA–RNA interactions to become enriched in mesh-like condensates, whereas low-valency mRNAs (*gray*) diffuse freely within the cytoplasm. Panel adapted with permission from Luo et al. (2026). (*b*) The valency ratio of RNA (*blue*) to protein (*brown*) determines condensate morphology and material properties. RNA–RNA interaction sites are shown in dark blue, and protein interaction sites are marked with Ys. (*c*) Mesh-like condensates control cotranslational processes, including protein folding. Translation of multivalent mRNAs in mesh-like condensates (*yellow*) is shown. (*d*) Mesh-like condensates control post-translational processes. The mRNA-mediated proximity of signaling factors enables the addition of post-translational modifications and signaling. Post-translational RNA–protein interactions prevent protein aggregation. Panel adapted with permission from X. Chen et al. (2024). Abbreviations: IDR, intrinsically disordered region; mRNA, messenger RNA; UTR, untranslated region.

Material Condensate Properties Control Cellular Phenotypes

A compelling example describing how mRNAs control biological phenotypes independently from protein sequence was recently shown by Amy Gladfelter's lab. The *CLN3* mRNA acts as a scaffold of Whi3 condensates in the fungus *A. gossypii* (Seim et al. 2024, Zhang et al. 2015). Synonymous point mutations were introduced into the *CLN3* coding sequence. These mutations did not alter the amino acid sequence but changed the physical properties of the RNA by altering its RNA conformational diversity. These mutations changed the morphology of the Whi3 condensates, the accessibility of Whi3-binding sites within the condensates, the condensate recruitment of the *CLN3* mRNA, and the material properties of the condensates in vitro and in vivo (Seim et al. 2024).

Most importantly, decreasing the ED of the *CLN3* coding sequence, an mRNA that encodes an important cell cycle regulator, increased progression through the cell cycle and enhanced the growth rate of the fungus (Seim et al. 2024). These findings indicate that coding sequences of mRNAs not only contain genetic information for the protein sequence but also contain physical information. RNA physical information is genetically encoded in the mRNA sequence and controls cell physiological processes through the extent of RNA–RNA interactions and the material properties of mRNA-scaffolded condensates (Seim et al. 2024) (**Figure 3*b***).

mRNA-Scaffolded Condensates Control Cotranslational Protein Folding

Multivalent 3′ UTRs control mRNA localization to condensates. During translation in the condensates, the enriched multivalent 3′ UTRs can act as RNA-based chaperones to prevent protein misfolding (Luo et al. 2026). In addition, protein interactors of nascent chains can become condensate enriched, either by hitchhiking on the translating mRNA (Ma & Mayr 2018) or through protein–protein interactions with condensate-enriched proteins (**Figure 3*c***). Condensate-mediated proximity allows cotranslational protein complex assembly or, through the recruitment of modifying enzymes, could change the interaction partners, final localization, or protein activity of the newly synthesized proteins.

mRNA-Scaffolded Condensates Control Post-Translational Processes Through Proximity

Mesh-like condensates provide mRNA-rich and RNA-accessible environments that potentially allow RNAs to act as post-translational chaperones, thus altering protein conformation (Copley et al. 2024, Guo et al. 2026). Moreover, they allow the proximity between condensate components to foster the formation of protein–protein interactions or post-translational modifications (**Figure 3*d***). Proteins can become condensate enriched through their protein domains, as exemplified in the FXR1 network (X. Chen et al. 2024).

FUTURE DIRECTIONS

As mRNAs emerge as having biological roles that go beyond their well-studied function as templates for protein synthesis (X. Chen et al. 2024, Hachet & Ephrussi 2004, Jenny et al. 2006, Luo et al. 2026, Seim et al. 2024), gene knockouts should be characterized at higher resolution to distinguish phenotypes caused by protein disruption, 3′ UTR deletion, impaired mRNA processing, or altered mRNA physical features.

It will be important to gain a molecular understanding of the processes that occur in individual mRNA-scaffolded condensates. Can the contributions of mRNA, RNA-binding proteins, and other enriched proteins be disentangled or are they interdependent? The latter is suggested

by structural studies involving RNAs and IDRs, showing that the binding of RNA to an RNA-binding protein nearly always changes the structure of the RNA and of the RNA-binding protein (Phan et al. 2011, Sashital et al. 2011, Williamson 2000).

RNA valency is an important characteristic of the RNA physical information, but it is currently poorly understood. RNA multivalency is independent of GC content and was observed in G-rich sequences associated with G-quadruplexes and C-rich as well as AU-rich RNA sequences (Isiktas et al. 2022, Litberg & Horowitz 2024, Litberg et al. 2023, Ma et al. 2021, Seim et al. 2024, Wei et al. 2024). Moreover, multivalency can be tuned, either by modulating the affinity of the binding sites or through conformational plasticity (Bevilacqua et al. 2022, Bose et al. 2024, Poudyal et al. 2021, Seim et al. 2024). As multivalent RNAs can be engineered and genetically encoded (Huang et al. 2017, Sorrentino et al. 2024), a better understanding of RNA multivalency may be achieved through synthetic biology approaches.

DISCLOSURE STATEMENT

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