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Cytoplasmic mRNPs revisited: Singletons and condensates

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Abstract
Cytoplasmic messenger ribonucleoprotein particles (mRNPs) represent the cellular transcriptome, and recent data have challenged our current understanding of their architecture, transport, and complexity before translation. Pre-translational mRNPs are composed of a single transcript, whereas P-bodies and stress granules are condensates. Both pre-translational mRNPs and actively translating mRNPs seem to adopt a linear rather than a closed-loop configuration. Moreover, assembly of pre-translational mRNPs in physical RNA regulons is an unlikely event, and co-regulated translation may occur locally following extracellular cues. We envisage a stochastic mRNP transport mechanism where translational repression of single mRNPs—in combination with microtubule-mediated cytoplasmic streaming and docking events—are prerequisites for local translation, rather than direct transport.

KEYWORDS
biomolecular condensates, mRNA transport, mRNP granules, P-bodies, RNA regulons, singletons, stress granules

INTRODUCTION
Since the early days of cell biology, basic dyes binding to the negatively charged phosphate backbone of RNA have been known to provide a purple and granular staining of the cytoplasm. Staining mainly reflects ribosomes bound to the endoplasmic reticulum, but the cytoplasm also contains numerous small cytoplasmic granules that are distinct from polysomes and larger bodies.

These small cytoplasmic granules are now recognized as mRNPs representing pre-translational units of the cellular transcriptome. As the name implies, these complexes consist of mRNAs and a plethora of RNA-binding proteins (RBPs), the latter often encompassing low-complexity sequences besides well characterized RNA-binding globular modules. The combination of multivalent RNA and protein in mRNPs is frequently encountered in biomolecular condensates, generated via liquid-liquid phase separation, and mRNPs may in principle form condensates at particular stages of their life cycle. There has been a tremendous interest in these membrane-less foci in recent years, and the most thoroughly examined cytoplasmic mRNP assemblies are P-bodies and stress granules.

The feasibility of forming RNP assemblies from mRNP units can be regarded as a physical manifestation of RNA regulons thus, providing an appealing scenario of functionally related mRNAs within the same particle, poised for subsequent co-regulated transport and translation at a subcytoplasmic locale. Regardless of the actual mRNP assembly, including P-bodies and stress granules, the common denominator is sequestered non-translating mRNAs. Therefore, an important question is the significance of RNP assemblies, in general, for mRNA recruitment to the translationally active pool and subsequent protein production.

Most of our knowledge regarding the nature of cytoplasmic mRNPs is derived from bulk biochemical characterizations, often using clever high-throughput approaches. It is only recently that...
the biochemistry has been supplemented with super-resolution and single-molecule fluorescence microscopy, so we now are able to examine the architecture of single mRNP units and address stoichiometric issues in vivo.\cite{10}

This essay will focus on our current understanding of pre-translational mRNP organization in terms of shape and dimensions in the somatic cytoplasm. Emphasis will be directed at mRNP transitory events between nuclear exit and association with the translational apparatus. In doing so, the essay will also touch upon the present status of the RNA regulon hypothesis, and provide our own perspective on how RBP-dependent stabilization of mRNA combined with cytoplasmic streaming and docking may lead to differential localization of mRNAs. A more general outline of the life of an mRNA can be found in the review by Singh et al.\cite{11}

**ARCHITECTURE AND DIMENSIONS OF CYTOPLASMIC mRNPs**

Cytoplasmic mRNPs can be divided into four distinct types, namely pre-translational mRNPs, translating mRNPs, and the multi-mRNP structures P-bodies, and stress granules. During normal physiological conditions, pre-translational/translating mRNPs and P-bodies are present in cells, whereas the formation of stress granules is induced by stress, for example, oxidative, metabolic, or various toxins. Figure 1 and Table 1 provide an overview of the interplay between cytoplasmic mRNPs and some information in terms of their properties.

Super-resolution microscopy has recently given a more detailed outline of pre-translational mRNPs. In contrast to the spherical appearance (“granular”) derived from conventional confocal microscopy, Structured Illumination Microscopy has shown that pre-translational mRNPs are branched and elongated structures,\cite{12} ranging from 100 to 1000 nm in size.\cite{13,14} The mRNPs are composed of a single mRNA and one or two handfuls of RBPs which is in fine agreement with their size. Pre-translational mRNPs are numerous, and each cell contains about 200,000 particles.\cite{12,15} Alternating patches of RBPs, such as YBX1, are scattered along the encoded transcript, whereas the cap-binding subunit CBP80 and the poly(A) binding PABPC1 locate at the ends (Figure 2). Translating mRNPs are, as the name implies, characterized by ribosomes and translation factors. P-bodies are, in contrast to the pre-translational mRNPs, spherical and believed to represent biomolecular condensates. In general, P-bodies are relatively small (100-500 nm), and this is puzzling considering that they are supposed to comprise multiple mRNAs and a large number of proteins.\cite{16-21}

P-bodies contain several components participating in mRNA decay, but they are apparently not foci of mRNA turnover,\cite{17} so their physiological role is still uncertain. Each cell contains only a few P bodies, although the number and individual size may increase during stress.\cite{22,23} Stress granules are the largest particles, and during the late stages of stress a single cell may contain 30–50 granules of about 2 μm.\cite{24,25} Stress granules are dynamic structures that condense during the progression of the stress reaction and dissolve upon stress relief.\cite{26} By super-resolution microscopy, they look like raspberries,\cite{12} and they contain smaller mRNPs and 40S ribosomal subunits, in addition to G3BP, TIA-1 and other stress granule specific factors.\cite{27}

**REMODELING OF PRE TRANSLATIONAL mRNPs IN THE CYTOPLASMA**

Messenger RNPs experience an exchange of bound proteins throughout their existence. Nascent pre-mRNAs are co-translationally loaded onto the capping machinery and subsequently spliced and polyadenylated, before they exit the nuclear pore as a mature mRNA. Some RNA-binding proteins, or associated factors that bind to the nascent pre-mRNA, will escort the mRNA throughout its journey while others will bind in a sequential manner. Capped pre-mRNAs associate with the cap-binding heterodimer CBP20/CBP80 also known as the nuclear cap-binding complex (CBC). Subsequently, pre-mRNA introns are removed by the spliceosome, whereas some of the deposited exon-junction complex (EJC) proteins will remain bound to the mRNA when it appears in the cytoplasm (Figure 1B).

Hallmarks of the pre-translational mRNP entering the cytoplasm are the CBC at the 5′ terminus, heterotetrameric exon-junction complexes (eIF4A3, Magoh, Y14, and MLN51 or RNP51) deposited upstream of splice junctions, and the nuclear poly(A)-binding protein PABPN1 at the 3′ terminus.\cite{28,29} Binding of the latter to the poly(A)-tail may, at the early stage of cytoplasmic mRNP existence, be punctuated by the cytoplasmic PABPC1, which is also a nucleocytoplasmic shuttle protein (Figure 1B).\cite{30}

Our knowledge about the pre-translational mRNP in terms of compartmentation is limited, but the assumption is, maybe erroneously, that due to the absence of ribosomes it will be more compact than the translated species. This inference is based on analysis of mRNA in stress granules, exhibiting additional RNA-RNA interactions in the absence of ribosomes.\cite{31,32}

Pre-translational mRNPs containing EJC components, such as Magoh and eIF4A3, were found to behave like flexible rod-like structures or polymers.\cite{16} The study by Metkar et al. was based on tandem immunoprecipitation of RNA from pre-translational mRNPs through the EJC proteins Magoh and eIF4A3 and subsequent proximity ligation, termed RIPPLiT (RNA-immunoprecipitation and Proximity Ligation in Tandem—see Figure 3 for a method outline). It showed that mature mammalian mRNAs were compacted into flexible rod-like structures, irrespective of length, and without strong locus-specific structures. The low potential for higher-order mRNA structure is in line with earlier DMS and SHAPE probing studies in vivo.\cite{33,34} Moreover, no evidence of an interaction between the 5′ and 3′ termini was found. An added bonus of the RIPPLiT approach is the virtual absence of ligations products from intermolecular mRNA species, supporting the data from super-resolution microscopy and correlation spectroscopy.\cite{12} and strongly suggesting that pre-translational mRNAs do not form mRNP assemblies.

There are no measurements of the lifetime of a pre-translational mRNP. In spite of the fact that a pre-translational mRNP encompasses...
FIGURE 1  Schematic overview of the different cytoplasmic mRNP particles present in eukaryotic cells and their interrelationships. Protein exchange or remodeling steps are indicated between the different mRNP states: pre-translational mRNPs, active translating mRNPs, stress granules and P-bodies. (a) Mature messenger RNAs coated with RNA-binding proteins exit the nucleus through the nuclear pore. At this stage, three characteristic RNA-binding complexes are present in pre-translational mRNPs; namely CBP20/80 (forming the cap-binding complex CBC), the exon-junction complex (EJC), and PABPN1 (together with PABPC1). In the cytoplasm, pre-translational mRNPs are transported in a...
### TABLE 1 Main characteristics of pre-translational mRNPs, translating mRNPs, P-bodies and stress granules in mammalian cells

<table>
<thead>
<tr>
<th>Shape</th>
<th>Dimensions</th>
<th>Numbers/cell</th>
<th>Typical marker</th>
<th>Present in normal conditions (Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-translational mRNPs</strong></td>
<td>Linear[^1^]</td>
<td>500 nm (250–800 nm range[^12^])</td>
<td>90,000–150,000[^12^]</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>Linear[^10^,^70^]údo Circular[^66^,^67^]</td>
<td>MDN1 mRNA (18 413 nt) = 135 nm POLA1 mRNA (5486 nt) = 96 nm PRPF8 mRNA (7295 nt) = 92 nm</td>
<td>YES</td>
<td></td>
</tr>
<tr>
<td><strong>Translating mRNPs</strong></td>
<td>Linear[^10^,^70^]údo Circular[^66^,^67^]</td>
<td>~4-7 (unstressed conditions[^17^])</td>
<td>eIF4E[^10^,^100^]</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Stress granules</strong></td>
<td>Irregular sphere Dynamic shell-like structure surrounding a stable core[^27^]</td>
<td>~0.1-2 μm[^25^]</td>
<td>G3BP[^26^]</td>
<td>NO</td>
</tr>
</tbody>
</table>

[^2^]No distinction is made between numbers of pre-translational and translating mRNPs, due to great individual differences in their translation ratios.

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multivalent components prone to undergo liquid-liquid phase separation, clear-cut cases of RNP assemblies of pre-translational mRNPs have not been reported. Therefore, it is likely that pre-translational mRNPs are kept as single units by abundant RNA chaperones. A good candidate as an RNA chaperone is the ubiquitous and abundant YBX1, present at concentrations greater than 1 μM, that may ensure the singleton status of mRNPs.[^35^] YBX1 associates with RNA in a beads-on-a-string manner of low specificity.[^36^] In particular, high resolution atomic force microscopy of a model luciferase transcript of 3 kb complexed with YBX1 showed that a rod-like structure of about 150 nm was formed, suggesting a 6-fold compaction. YBX1 contains a single cold-shock domain, combined with a low-complexity C-terminal region containing clusters of positive charges.[^37^] Deletion of the C-terminal region was followed by an almost complete decompaction of the nucleoprotein complex to an extended linear filament. It was proposed that phosphorylations of the intrinsically disordered region in full-length YBX1 would facilitate remodeling from a repressed mRNA to a translationally active species.[^38^] In our own studies, the beads-on-a-string attachment of YBX1 to a single mRNA molecule was visualized by structured illumination microscopy of fixed cells (Figure 2), exhibiting no signs of higher-order mRNP assemblies. Another candidate as a general RNA chaperone is the ATP-dependent DEAD-box helicase eIF4A, which decreased RNA condensation in vitro and limited stress granule formation in live cells.[^32^] We conclude that pre-translational mRNPs appear to be single units absent from biomolecular condensates. A notable exception to this generalization is germ granules, in which stochastic seeding of single mRNPs is followed by multi-copy homotypic clustering in a sequence-independent manner.[^39,^40^]

**PRE-TRANSLATIONAL mRNP TRANSPORT**

Cytoplasmic pre-translational mRNPs may be recruited to the translational apparatus far from the nuclear pore. This is especially observed within polarized cells such as neurons, and models/hypotheses cytoskeleton-dependent manner until they reach a site of protein synthesis. The pioneer round of translation removes exon-junction complexes, and translation promotes PABPN1 exchange for PABPC1. CBC is exchanged for eIF4E at steady-state translation. Translational repression by “4E inhibitors” leads to non-translating mRNP sequestration in P-body condensates. If cells are stressed with an external agent such as arsenite, ribosomes are cleared from actively translating mRNPs, and mRNPs condense into stress granules (SGs). Messenger RNA conformation within P-bodies is unknown, but drawn in an open linear version here. (b) Outline of protein attachments as a function of translational status. (c) Schematic representation of the importin-α/importin-β (IMPA1/IMPβ) dependent mechanism that promotes replacement of the cap-binding complex (CBC), consisting of CBP20 and CBP80, by eIF4E.
Pre-translational mRNPs

P-bodies

Stress granules

**FIGURE 2**  Super-resolution fluorescence microscopy images acquired by Structured Illumination Microscopy of pre-translational mRNPs, P-bodies and stress granules in HeLa cells. **Upper panel**—Direct immunofluorescent staining of a linear pre-translational mRNP unit with three of its protein constituents: CBP80 (green), YBX1 (red), and PABPC1 (cyan). CBP80 is at the 5′ terminus of the mRNP (5′-cap), YBX1 is coating the mRNA body, and PABPC1 is binding to the poly(A) tail at the 3′ terminus. Antibodies against CBP80 (E-7, Santa Cruz Biotechnology), YBX1 (ab12148, Abcam) and PABPC1 (HPA067156, Atlas Antibodies) were directly conjugated to Alexa Fluor 488, 568 and 647 dyes, respectively. Scale bars = 5 μm (whole cell image) and 0.1 μm (zoomed-in image). **Lower panels**—Image of a whole cell with its P-bodies visualized by the presence of DCP1a-GFP and a zoomed-in view of one P-body (left). Indirect immunofluorescence staining of G3BP in a cell stressed with 1 mM sodium arsenite for 20 minutes showing stress granules in the cytoplasm and a detailed view of two stress granules (right). A mouse monoclonal anti-G3BP (ab56574, Abcam) primary antibody and a secondary antibody anti-mouse Alexa Fluor 488 were used. Scale bars = 5 μm (whole cell images) and 0.5 μm (zoomed-in images).

regarding mechanistic details of mRNP transport and local translation have been proposed.\[^{41}\] Active mRNP transport via microtubules facilitated by direct coupling to motor proteins has been described.\[^{42,43}\] Microtubules are seen as key players of mRNP transport based on the effects of depolymerizing agents such as nocodazole, but drug-induced collapse of microtubules would have a profound effect on both direct motor-driven transport and an indirect mechanism via cytoplasmic streaming.

Cytoplasmic streaming can be generated by kinesin-1-dependent (KIF5B in vertebrates) microtubule sliding between anchored cortical microtubules and free cytoplasmic microtubules, as well as by kinesin-1 cargo transport.\[^{44}\] Defects in cytoplasmic streaming cause a more diffuse localization of Staufen-containing RNPs to the posterior pole of *Drosophila* oocytes, suggesting a connection between kinesin-mediated transport and streaming in localization of mRNPs. Moreover, sliding defects have profound effects on *Drosophila* nervous system development, since kinesin-1 knockouts exhibit synaptic dysfunction, locomotion defects, paralysis and eventual death. Strikingly, a kinesin-1 mutant, deficient in membrane-bound organelle transport but still able to perform microtubule sliding, could rescue neuronal axon outgrowth, reinforcing microtubule sliding as a key biological process.\[^{45}\] Similarly, KIF5B knock-down in rat hippocampal neurons caused a reduction of FMRP mRNPs as well as *CaMKIIα* and *Grin2b* mRNAs in dendrites.\[^{46}\]

Transport or movement of mRNPs is intermittent and stochastic in nature and has been described to be composed of discrete "runs" and "rests."\[^{47}\] Run phases represent directed movement, while rest phases represent very slow subdiffusive motion or a trapped particle behavior. In general, mRNPs spend more time in the rest phase (>80%) than in the run phase, and runs can be either anterograde or retrograde with the same probability. Although "runs" have been associated with active transport via microtubules by kinesins and dyneins, mRNPs transport behavior is different from motor-driven active transport, in which runs occupy most of the time, only being interrupted by short pauses. In addition, mRNPs tend to be more immobile with time (ageing), with fewer particles having run phases and
RNA tracking: TRICK is a single-molecule method that allows RNA tracking in combination with imaging of the first round of translation.\(^{[62]}\) The method requires the presence of MS2 loops in the 3' UTR, which are bound by the MCP protein fused to a red fluorescent protein (RFP). In addition, PP7 loops are inserted in the open reading frame of a reporter mRNA and are bound by a the PCP protein fused to a green fluorescent protein (GFP). If the mRNA has not been through the pioneer round of translation, both GFP-PCP and RFP-MCP are bound to the mRNA, yielding a yellow signal. When the mRNA gets translated, the ribosome unwinds the loops present in the open reading frame of the mRNA and displaces GFP-PCP. Therefore, the fluorescent signal from the translating mRNA will appear red.

RBP binding: APEX sequencing (RNA) or APEX-MS (protein) allows the identification of RNA and proteins, respectively, that are in close proximity (20 nm) to an APEX2-tagged protein (typically an RBP).\(^{[7,9]}\) With the addition of biotin-conjugated phenol and H\(_2\)O\(_2\), RNAs and proteins can be biotinylated and subsequently identified via streptavidin purification and high-throughput sequencing (RNA) or mass-spectrometry (protein), respectively.

RNA structure: RIPPLiT (RNA Immunoprecipitation and Proximity Ligation in Tandem) is a technique used to determine the 3D conformation of RNAs associated with specific proteins, for example, EJC core factors MAGOH and eIF4A3.\(^{[6]}\) Combining a double immunoprecipitation, with nuclease digestion and proximity ligation, chimeric reads can be analyzed and mapped using the bioinformatic tool ChimeraTie in order to extract information about 3D RNA conformations.

Microtubules could participate in indirect mRNP transport in an alternative manner by associating with membrane-bound organelles. An example of the latter scenario is the mechanism in which mRNP granules "hitchhike" on moving lysosomes during long-distance transport.\(^{[50]}\) The molecular tether is an RNA granule-associated phosphoinositide-binding protein annexin A11, in which the N-terminal low-complexity region enables interaction with the mRNP granule, and the C-terminal domain provides membrane attachment to the vehicle.

When an mRNP reaches a distal destination, the assumption is that it has not yet undergone a pioneer round of translation and should exhibit the hallmarks of a pre-translational mRNP in terms of protein components. This has been examined in neuroblastoma cells, where both CBP80 and the EJC components elf4A3 and Y14 were found to be present in transport granules distinct from elf4E-containing granules and P-bodies.\(^{[51]}\) Moreover, the CBP80 transport type was more closely associated with microtubules, implying that exchange of the

**FIGURE 3** Described technologies in studies of RNA tracking, RBP binding, and RNA structure. RNA tracking: TRICK is a single-molecule method that allows RNA tracking in combination with imaging of the first round of translation.\(^{[62]}\) The method requires the presence of MS2 loops in the 3' UTR, which are bound by the MCP protein fused to a red fluorescent protein (RFP). In addition, PP7 loops are inserted in the open reading frame of a reporter mRNA and are bound by a the PCP protein fused to a green fluorescent protein (GFP). If the mRNA has not been through the pioneer round of translation, both GFP-PCP and RFP-MCP are bound to the mRNA, yielding a yellow signal. When the mRNA gets translated, the ribosome unwinds the loops present in the open reading frame of the mRNA and displaces GFP-PCP. Therefore, the fluorescent signal from the translating mRNA will appear red. RBP binding: APEX sequencing (RNA) or APEX-MS (protein) allows the identification of RNA and proteins, respectively, that are in close proximity (20 nm) to an APEX2-tagged protein (typically an RBP).\(^{[7,9]}\) With the addition of biotin-conjugated phenol and H\(_2\)O\(_2\), RNAs and proteins can be biotinylated and subsequently identified via streptavidin purification and high-throughput sequencing (RNA) or mass-spectrometry (protein), respectively. RNA structure: RIPPLiT (RNA Immunoprecipitation and Proximity Ligation in Tandem) is a technique used to determine the 3D conformation of RNAs associated with specific proteins, for example, EJC core factors MAGOH and eIF4A3.\(^{[6]}\) Combining a double immunoprecipitation, with nuclease digestion and proximity ligation, chimeric reads can be analyzed and mapped using the bioinformatic tool ChimeraTie in order to extract information about 3D RNA conformations.
FIGURE 4  mRNP transport model depicting how the lifetime of a pre-translational mRNP in combination with microtubule-mediated cytoplasmic streaming and docking would increase the likelihood of mRNA localization at distal sites. Distribution of mRNPs throughout the cytoplasm would follow stochastic movements governed by cytoplasmic streaming and docking events. Pre-translational mRNP lifetime is dictated by the potential of translation inhibition of an mRNA by RBPs, so a strong inhibition would correlate with an enriched presence at distal cell sites. An important assumption behind the model is that general mRNA turnover is associated with the actively translated pool on ribosomes.

PREPARING THE PRE-TRANSLATIONAL mRNP FOR STEADY-STATE TRANSLATION

Pre-translational mRNPs are subjected to a pioneer round of translation that serves both as a quality control step and as a means of recruiting factors needed for subsequent steady-state translation.\[52\]

During the pioneer round of translation, the CBC-capped mRNA is stripped of EJCs by the scanning ribosome.\[53\] It is often assumed that it is only the first ribosome which employs the CBC-capped mRNA as template, but polysome analyses have revealed that multiple rounds of translation may be CBC-directed at a low initiation frequency.\[54,55\] It should also be recalled that the scaffold protein CTIF (CBC-dependent translation initiation factor)—via the eIF3g subunit—participates in recruiting the 40S ribosomal subunit, whereas eIF4G is the platform protein at steady-state eIF4E-directed translation.\[55,56\] At some point, the CBC complex is substituted with the cytoplasmic cap-binding eIF4F complex, but when, where and how this exchange is taking place is uncertain. In fact, the remodeling may take place in a translation-independent manner before the pioneer round by removal of CBC via an importin interaction mechanism (Figure 1C), thereby exposing the cap to eIF4E binding.\[57\] Further extensive remodeling of the pre-translational mRNP may take place, depending on whether the substoichiometric EJC core protein MLN51 (also known as CASC3 or Barentsz) or the peripheral RNPS1 is the fourth EJC component. The former facilitates formation of monomeric EJC, whereas the latter provides SR-rich multimeric EJC assemblies.\[58\] An added complexity
to the exchange could be the dual role played by eIF4A3, that besides being a core component of the EJC also is able to interact directly with CTIF, and thus indirectly with CBP80.\[^{[59]}\] Besides CBC and EJCs, various shuttling hnRNPs, YBX1, and PABPC1 are invariably found in most cytoplasmic pre-translational mRNPs.\[^{[11]}\]

Translation decreases the amount of EJC factors (eIF4A3 and UPF3X) bound to mRNA, implying that displacement is translation-dependent.\[^{[57]}\] In contrast, there is no difference in the amount of mRNA that can be immunoprecipitated by CBP80 or eIF4E under translating and non-translating conditions, implying that translation is not a requirement for the CBC-eIF4E exchange. Instead, the latter is facilitated by the binding of the nuclear import receptor IMP\(^\beta\) to the adaptor protein IMP\(^\alpha\) in complex with CBP80.\[^{[60]}\] Therefore, replacement of CBC by eIF4E is not dependent on the pioneer round of translation, and it has been suggested that replacement is a combination of IMP\(^\beta\) binding to IMP\(^\alpha\)-CBP80 via CBP20 and the high level of eIF4E in the cytoplasm (Figure 1C).\[^{[61]}\] In addition to EJC removal, translation increases the removal of PABPN1 and/or increases the binding of PABPC1, which results in the replacement of PABPN1 by PABPC1.\[^{[57]}\]

A single-molecule imaging technique, termed TRICK for “translating RNA imaging by coat protein knock-off”, has been developed to address the progress by the pioneer ribosome in real time.\[^{[62]}\] In brief, a reporter is tethered by green fluorescent protein in its coding region via a PP7-PCP interaction and by red fluorescent protein in its 3’UTR via the well-known MS2-MCP system (see Figure 3 for a method outline). The idea is that a pre-translational mRNA will exhibit a yellow signal, whereas a translated mRNA appears red. A broad spectrum in the timing of translation initiation was reported. In the case of a human cell line, it occurred within minutes after nuclear exit, whereas in the Drosophila oocyte oskar mRNA translation was postponed until it reached the posterior pole.\[^{[10]}\]

The pioneer round of translation represents a crucial step for mRNA fate, because it is a quality control step acting via the nonsense-mediated decay pathway.\[^{[52]}\] In addition, mRNPs are remodeled extensively to engage in protein translation, and mRNP components and ribosome unwinding of the mRNA induce changes in mRNA conformation. Conformational changes resulting in 5′-3′ proximity might be the consequence of mRNP factor exchange (e.g., eIF4E-PABPC1 interaction via eIF4G), regulating processes such as ribosome recycling.\[^{[63]}\]

**TRANSLATING mRNPS—CIRCULAR OR LINEAR?**

Since pre-translational mRNPs seem to be linear, an important question is whether this is also the case for translating mRNPs. Neither CBP80 nor CTIF encompasses a binding motif for either PABPN1 or PABPC1, so a priori there is no reason to expect that pre-translational mRNPs should exhibit a pseudo-circular conformation. However, translating mRNPs interact with the platform protein eIF4G, that has binding sites for both the cap-binding eIF4E subunit and PABPC1, giving rise to the paradigm of a pseudo-circular architecture. The first evidence of mRNA pseudo-circularization (closed-loop model) came from electron micrographs of polysomes.\[^{[64]}\] Subsequently, a series of in vitro and in vivo approaches have further supported the closed-loop model.\[^{[65–67]}\] Although favored for decades, this model has recently been challenged.

3D maps of dense mammalian polysomes have been modeled from the distribution of neighboring ribosomes in intact mammalian cells. This 3D modeling predicts a dense helical organization of ribosomes with almost four ribosomes per turn, with the small subunits pointing towards the inside of the helix, and the peptide exit tunnels facing towards the cytosol—a configuration that is remarkably similar to isolated bacterial polysomes.\[^{[69]}\] This model would not be compatible with the closed-loop model, because the mRNA termini would be separated by the helical structure of the polysome.

In the study by Adivarahan et al., three mRNAs (>5 kb) were examined by single-molecule fluorescent in situ hybridization with probes directed towards the two termini and the middle of the message.\[^{[10]}\] It was shown that the examined mRNAs were mainly present as translating species in an extended conformation, and polysome collapse by either puromycin or homoharringtonine treatment resulted in a more compact mRNP by a factor 3-4. In other words, ribosome occupancy facilitates an open mRNP state.

In a similar approach also examining large mRNAs, Khong and Parker observed that a translated mRNA exhibited about a 20-fold compaction that appeared length-dependent (using 0.29 nm as the extended contour length of one nucleotide).\[^{[70]}\] Arsenite treatment increased the compaction by a factor 2 in an ATP-independent manner, and the mRNA termini in the latter situation appeared closer, regardless of accumulation within stress granules.

Therefore, both models are supported by in vivo evidence, and their co-existence could have different ramifications. One possibility is that certain mRNAs tend to circularize while others do not, and this could be dependent on mRNP factor exchange, ribosome occupancy, or RNA secondary structure, leading to different translation efficiencies. Another possibility is that mRNP circularization is transient and predominant during the first few eIF4E-mediated rounds, subsequently leading to a linear conformation characteristic of high-density polysomes.

**mRNP ASSEMBLIES**

Since both RNA and RNA-binding proteins exhibit multivalent interaction possibilities, the combination is prone to forming higher-order RNP assemblies by default. In addition, modular RNA-binding proteins often contain intrinsically disordered regions that provide further transient interactions, facilitating formation of biomolecular condensates via liquid-liquid phase separation. This topic has been examined extensively with a focus on cytoplasmic RNP assemblies such as stress granules and the enigmatic P-bodies.

Following various forms of stress, bulk translation initiation is inhibited and, as a consequence, non-translating mRNAs together
with associated RNA-binding proteins condense into stress granules. Messenger RNAs must be completely free from translating ribosomes before entering stress granules, and mRNAs with longer coding regions are recruited slower than those with shorter ones.\[70]\] There is a small proportion of mRNAs (1-2\%) interacting with stress granules that are still associated with polysomes but the interaction is transient,\[71]\] reinforcing the need for mRNAs to exit translation completely before entering a stable association with stress granules. Ascorbate peroxidase (APEX) proximity labelling (see Figure 3 for a method outline) of the well-known stress granule protein G3BP1 revealed that although stress granules seem to form de novo upon stress, less than half of its protein interactors are stress-dependent, implying that the network of protein interactions exists before stress.\[72,81]\]

Another membrane-less granule type, present at homeostasis and distinct from stress granules, is P-bodies.\[72,73]\] P-bodies were initially characterized as compartments of mRNA decay, because many proteins involved in mRNA turnover are present in these foci. However, the current thinking is that they sequester translationally repressed mRNAs encoding regulatory functions.\[74,75]\] Three proteins are important for mammalian P-body assembly and maintenance, namely DDX6, 4E-T, and LSM14A,\[75]\] that provide interfaces with each other.\[76]\] Moreover, they are also closely associated with the CCR4-NOT deadenylase and decapping complexes,\[77,78]\] and 4E-T protects the deadenylated target mRNAs from degradation via inhibition of decapping within the condensate.\[79,80]\] This reservoir function is supported by recent data revealing that cytoplasmic mRNA decay pathways are widely linked to the translational apparatus in both yeast\[81,82]\] and mammalian embryonic stem cells.\[83]\]

Whether pre-translational mRNPs or only poorly translating mRNPs can be targeted to P-bodies is controversial and worth discussing. Several studies have shown that inhibition of translation elongation with, for example, cycloheximide inhibits the formation of P-bodies,\[84,85]\] which indicates that P-bodies form from the actively translating mRNA pool. On the other hand, proteomic studies of P-bodies have identified not only eIF4E and its inhibitor 4E-T, but also EJC components such as eIF4A3 and Magoh as well as CBP80, that are markers for pre-translational mRNPs.\[17]\] 4E-T binding to eIF4E is required for eIF4E recruitment to P-bodies, and 4E-T depletion also affects localization of the decapping factor DCP1a and the DDX6 homologue Me31B,\[84]\] suggesting compromised P-body formation. Importantly, elf4E and 4E-T have been confirmed to be present in P-bodies by immunocytochemistry, whereas the presence of the EJC core component elf4A3 and CBP80 have not been reported. On balance, the current evidence suggests that P-body recruitment of mRNAs takes place from the poorly translating pool of mRNAs rather than the pre-translational mRNP pool (Figure 1).

The summation of numerous protein-protein, RNA-protein, and RNA-RNA interactions appears to be the driving force in forming mRNP assemblies.\[86]\] Moreover, each assembly is not merely a dynamic entity generated by multiple transient interactions, but seems to consist of a stable high-valency core surrounded by a shell of loosely associated low-valency client mRNPs.\[71,87]\] During stress, P-bodies increase in size and exhibit a transcriptome similar to the stress granule transcriptome, reflecting the general translation repression.\[18]\]

**MESSENGER RNA REGULONS**

The monocistronic nature of eukaryotic mRNAs has led to the post-transcriptional RNA regulon hypothesis, stating that mRNAs encoding functionally related proteins are coordinately expressed via attachment of a regulatory RNA-binding protein.\[5]\] With the preponderance of intrinsically disordered regions among RNA-binding proteins and their inherent ability to undergo phase transition,\[88,89]\] the hypothesis has gained momentum. This is mainly due to an appealing scenario where the RNA regulon is embedded within a biomolecular condensate. While the ability of RNA-binding proteins to undergo liquid-liquid phase separation is evident, their role in the assembly of multi-RNA particles in physical regulons is unclear. This is mainly due to a scarcity of microscopy studies examining colocalizations of putative regulon mRNAs.

In an smFISH approach using cultured rat hippocampal neurons, eight dendritic mRNAs—some with a common targeting element—were examined pairwise.\[90]\] None of the mRNAs was found to colocalize, since “nearest neighbor” distances exhibited means in the 800-1300 nm range, even when the analysis was applied to different molecules of the same mRNA species. Therefore, the conclusion was that two or more mRNA molecules do not occur together in a common RNP granule, but travel singly and independently to dendrites.

Since pre-translational mRNPs represent mRNA singletons, a possibility for the physical manifestation of RNA regulons is that mRNAs are co-regulated at the translation stage. In yeast, assembly of many macromolecular complexes happens at the translation stage, where it is a requirement that one subunit has undergone translation prior to the synthesis of a partner subunit.\[91]\] Similarly, specific mRNAs encoding different translation factors have been described to localize in foci that are different from P-bodies and stress granules. In contrast to the latter, these foci are sites of active translation, and translation itself is a prerequisite for the mRNA subset to enter these so-called translation factories.\[92,93]\]

Besides the general co-translational ER association of mRNAs encoding protein components participating in the secretory pathway, additional scenarios exist. The Zn-finger protein TIS11B is an example of an RNA-binding protein that is able to form tubule-like membrane-less organelles—called TIS granules—intertwined with the endoplasmic reticulum.\[94]\] The twist here is that an mRNA such as CD47 mRNA, containing alternative 3'UTRs, will behave differently in terms of peri-translational protein complex formation on ER, depending on the presence or absence of HuR attachment to the 3'UTR. Both forms of CD47 mRNA will synthesize nascent protein on the ER surface, but the isoform with the

\[90,92,93\]
longer 3'UTR generates CD47 that will appear in the plasma membrane via protein-protein interaction with SET recruited by HuR. The latter interaction takes place in an ER subdomain, a membrane-less "nurturing niche", generated by TIS11B.195

Since the prerequisite for co-regulated mRNAs is attachment of a defining RNA-binding protein, RNA modification could also lead to coordinated expression. N6-methylation of adenosine in mRNA is a widespread post-transcriptional modification carried out by the core heterodimer METTL3/METTL14, and certain RNA-binding proteins (typically YTH-domain "readers") exhibit higher affinity towards the methylated mRNA than its corresponding unmethylated form.96-97 During human erythropoiesis, about 300 m6A-containing mRNAs were selectively translated, among which were found critical regulators of hematopoiesis and erythropoiesis.198 This study suggests that m6A marks may promote expression of physiologically related mRNAs, but it does not provide evidence of the level of coordination. However, cytoplasmic YTH-domain proteins are able to undergo liquid-liquid phase separation facilitated by polymethylated RNA in vitro and may be present in stress granules, thereby providing a mode of coordination.199

Taken together, coordinated mRNA assembly in RNA regulons is an unlikely event in the case of pre-translational mRNPs. Instead, coordination in terms of ensuring proper colocalization and stoichiometry of proteins is likely to take place during mRNP docking and subsequent translational recruitment.

CONCLUSIONS AND PROSPECTS

The "granule" concept has evolved in parallel with the improved resolution of microscopes. So RNP complexes, visualized as spheres decades ago, are not necessarily so today. The previous assumption that "transport granules" are granular in shape may be a misnomer, and what is actually observed is the movement of single elongated mRNP units. To distinguish them from the larger assemblies, we propose that smaller cytoplasmic granules should be designated mRNP singletons rather than granules.

We have witnessed considerable progress in terms of the importance of local translation for cellular biology. However, we are still lacking a molecular mechanistic understanding of how some mRNPs are repressed, whereas others are translated straightaway upon nuclear exit. Is the exchange of the cap-binding factors the cause of translational recruitment, and if so, what is the mechanism, since it is unlikely to be merely a consequence of mass action? Especially the timing behind the RBP-directed transition from a pre-translational mRNP to a translating species is an unanswered challenge.

There is a steady flow of reports suggesting the participation of biomolecular condensates in many cellular activities, and this has been especially pronounced within the field of RNA biology. Many studies have been performed in vitro, but apart from membrane-less organelles such as P-bodies, stress granules and germ granules, few studies of endogenous mRNPs are available. Although condensation seems to be the default fate, various RNA chaperones could participate in the in vivo situation, ensuring that single mRNPs are the units of communication rather than RNP granules embodying multiple mRNAs. In order to enable on-site protein synthesis of functionally related mRNAs, we envisage that coordinating RBPs mediate graded translational repression and docking opportunities via cytoplasmic streaming, rather than direct mRNA transport. In this stochastic model, pre-translational mRNP "longevity" is at the crux of local translation.

CONFLICT OF INTEREST

The authors declare no conflict of interest

DATA AVAILABILITY STATEMENT

There are no data available.

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