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Review

Mechanisms and Regulation of RNA Condensation in RNP Granule Formation

Devin Tauber,[1,4] Gabriel Tauber,[2,4] and Roy Parker[1,3,*]

Ribonucleoprotein (RNP) granules are RNA–protein assemblies that are involved in multiple aspects of RNA metabolism and are linked to memory, development, and disease. Some RNP granules form, in part, through the formation of intermolecular RNA–RNA interactions. *In vitro*, such *trans* RNA condensation occurs readily, suggesting that cells require mechanisms to modulate RNA-based condensation. We assess the mechanisms of RNA condensation and how cells modulate this phenomenon. We propose that cells control RNA condensation through ATP-dependent processes, static RNA buffering, and dynamic post-translational mechanisms. Moreover, perturbations in these mechanisms can be involved in disease. This reveals multiple cellular mechanisms of kinetic and thermodynamic control that maintain the proper distribution of RNA molecules between dispersed and condensed forms.

Highlights

Intermolecular RNA–RNA interactions contribute to the formation, content, and biophysical properties of many RNP granules.

Cells utilize both genetically programmed and promiscuous RNA–RNA interactions in RNP granules.

RNA–protein interactions modulate protein dynamics in RNP granules.

Cells have evolved mechanisms to regulate RNA condensation through RNA chaperones.

RNA chaperones can be influenced by post-translational modifications (PTMs).

Ribonucleoprotein Granules Are Built via a Summation of Multivalent Interactions

Eukaryotic cells contain a variety of **ribonucleoprotein (RNP) granules** (see Glossary). RNP granules are large non-membrane-bound assemblies of RNA and protein and are present in the nucleus and the cytosol. Examples of RNP granules include the nucleolus (the site of rRNA biogenesis), **stress granules** (**SGs**; which form from untranslating RNAs [1]), and neuronal granules (that are important for the transport and translation of synaptic mRNAs and synaptic plasticity [2]).

RNP granules are members of a growing class of biological assemblies referred to as **biomolecular condensates** (reviewed in [3]). Biomolecular condensates are non-membranous assemblies that form through multivalent interactions between their components. Condensates differ from traditional assemblies in that the diverse and multivalent nature of the interactions allows condensates to be variable in their assembly and size and lack any unique stoichiometry or stereospecificity.

RNP granules generally require a specific population of RNA for their formation and can be enriched for many RNAs. As examples, SGs and P-bodies (PBs) require a cytoplasmic population of untranslating RNAs, the nucleolus requires rRNA transcripts to maintain its organization [4], and nuclear paraspeckles require the *NEAT1* long noncoding (lnc)RNA [5]. RNP granules also compartmentalize specific RNA-binding proteins (RBPs). For instance, distinct RBPs accumulate in SGs and PBs, although they can also share some components [6–10].

RNP granules form from a summation of both protein–protein and **RNA–RNA interactions** between RNPs (Figure 1). Protein–protein interactions that promote RNP granule formation occur between RBPs bound to the RNA and can involve well-folded domains of RBPs [11]. For example, the G3BP1 protein can bind to mRNAs, and then through dimerization can increase the formation of SGs [12]. Many RNP granule proteins also contain **intrinsically disordered**

[1]Department of Biochemistry, University of Colorado Boulder, Boulder, CO 80308, USA
[2]Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305, USA
[3]Howard Hughes Medical Institute, University of Colorado Boulder, Boulder, CO 80308, USA
[4]These authors contributed equally.

*Correspondence:
roy.parker@colorado.edu (R. Parker).

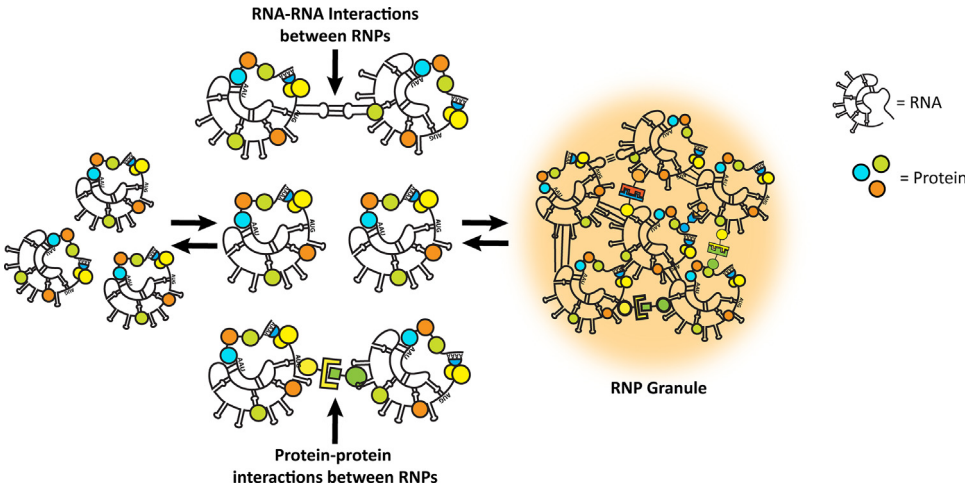

Figure 1. Ribonucleoprotein (RNP) Granules Form from a Diversity of Different Interactions. RNP granules form from a summation of multivalent protein–protein, RNA–RNA, and protein–RNA interactions that each imparts biochemical properties to define the characteristics of the granule. Different RNP granules most likely have different requirements for each type of interaction for the respective functions of the granule or for cellular regulation. In addition, each interaction type may be specific or promiscuous, as well as weak or strong, further contributing to defining an RNP granule.

regions (IDRs) that contribute to RNP granule assembly by forming specific interactions with well-folded domains, by providing additional RNA contacts, or by forming weak interactions with other IDRs (reviewed in [11]).

Three general lines of evidence argue that intermolecular interactions between RNAs can contribute to RNP granule formation, and that RNP granule formation is driven, at least in part, by **RNA self-assembly/condensation**. Specifically, and as detailed below, self-assembly of RNA *in vitro* occurs readily; biochemical changes that promote or inhibit RNA self-assembly *in vitro* correspondingly affect RNP granule formation in cells, and RBPs that inhibit RNA self-assembly *in vitro* also inhibit RNP granule formation in cells.

The robust self-assembly of RNA suggests that RNPs will coalesce into RNP granules whenever there is a sufficiently high local concentration of RNA sequences that can interact. Cells appear to have taken advantage of this biophysical phenomenon to build RNP granules with biological functions. For example, the formation of neuronal RNP granules can impact on synaptic plasticity [2], the formation of P-granules in *Caenorhabditis elegans* allows proper segregation of mRNAs during development [13], and the formation of stress granules correlates with increased survival during stress responses [14].

We review here the properties of RNA self-assembly both *in vitro* and in cells, and the mechanisms that cells have developed to modulate RNA condensation for proper RNP function. We highlight that RNA condensation can be a spontaneous process, that cells regulate this process both kinetically and thermodynamically by way of **RNA chaperones**, and that cells can regulate RNA chaperone function through post-translational modifications (PTMs).

Biochemistry of RNA Condensation

RNAs can engage in intermolecular interactions that drive their self-assembly into condensates *in vitro*. For example, yeast total RNA extracts readily self-assemble into condensates under approximately physiological conditions *in vitro* [15]. UV crosslinking shows that self-assembled RNA

Glossary

Architectural lncRNA: the many lncRNAs, such as *NEAT1* and satellite RNAs, that scaffold the formation of nuclear bodies at their sites of transcription (reviewed in [29]).
Biomolecular condensates: membrane-less assemblies of biopolymers formed through multivalent interactions between constituent components. Such interactions promote the concentration of molecules into greater densities within the condensate. Examples include RNP granules, centrosomes, and heterochromatin (reviewed in [3,86]).
DEAD-box proteins (DBPs): ATP-dependent RNA-binding proteins that have high affinity for RNA when complexed with ATP, but low affinity following ATP hydrolysis and P_i release. Because they can disrupt RNA–RNA interactions through ATP-dependent RNA binding, they are often referred to as 'helicases', although (unlike canonical DNA helicases) they are typically nonprocessive and can only resolve duplexes of limited size (reviewed in [137]).
Excluded volume effect: polymer molecules in solution occupy a volume (the excluded volume), thereby reducing the effective volume available for other molecules to diffuse, and increasing their effective concentration. An increase in the excluded volume of polymers (e.g., crowding agents) increases intermolecular collisions and interactions.
Intrinsically disordered regions (IDRs): regions of protein sequence that do not adopt a defined secondary structure and instead exhibit conformational flexibility, and that can engage in promiscuous and dynamic interactions.
Kinetic RNA condensers: proteins that promote intermolecular RNA interactions by increasing the rate of *trans* RNA–RNA interaction formation, for example, by bringing RNAs into proximity (Figure 3B).
Kinetic RNA decondensers: proteins that reduce utilize dynamic binding to promote the dissociation of *cis* or *trans* RNA interactions and thereby accelerate RNA refolding (Figure 2B).
Proximity effect: the increase in the rate of a reaction because of increased probability that successful collisions between reactant molecules will occur. This is achieved by concentrating the

engages in multivalent interactions [16]. In addition, perturbations that promote intermolecular RNA–RNA interactions enhance RNA condensation. For instance, higher salt concentrations screen phosphate backbone repulsion interactions, and crowding agents strengthen the **excluded volume effect** to promote *trans* RNA–RNA interactions [15]. Thus, increasing salt or crowding facilitates increased RNA condensation *in vitro*. Further, multivalent cations such as polyamines and arginine-containing dipeptide repeat proteins are especially effective at promoting total RNA condensation *in vitro* [15,17–19]. RNA self-assembly is efficient and can occur at concentrations as low as 2 μg/ml across a range of conditions (corresponding to ~10^{-9}–10^{-7} M, depending on transcript length), that are lower than the 0.5–10 mg/ml concentrations (typically ~10^{-6}–10^{-4} M) used to demonstrate self-assembly of RBPs (reviewed in [20]), and well below the intracellular RNA concentration, although we note the caveat that the conditions used in these assays are often not directly comparable.

In addition to Watson–Crick base pairs, other types of molecular interactions can drive RNA condensation, including non-Watson–Crick base-pairing [21,22]. Hence, all homopolymer RNAs undergo condensation *in vitro* and can partition RNA oligonucleotides through non-Watson–Crick RNA–RNA interactions [15–17]. RNA molecules can also engage in intermolecular salt bridges, cation–π, and π–π interactions such as intermolecular helical stacking, which can be sufficient to condense short double-stranded RNA (dsRNA) oligonucleotides into liquid crystals [23,24].

The properties of an RNA condensate are determined by the strength of the underlying intermolecular RNA–RNA interactions. Although homopolymeric RNAs are not biological, they can be utilized as a simple model system to elucidate rules governing RNA assembly. For example, condensed polypyrimidines form more dynamic assemblies than polyA [15], which has increased capacity for π interactions. By contrast, polyG and mixtures of polyA and polyU condense into stable gels, presumably owing to stable G-quadruplex or Watson–Crick interactions [15,16].

Different specificities of RNA–RNA interactions can drive the homotypic clustering of RNA condensates *in vitro.* For example, polyU or polyA and polyC homopolymeric RNAs spontaneously segregate into distinct RNA assemblies that interact at their surfaces [16]. This suggests that specific patterns of RNA interactions can lead to spatial segregation of RNAs into distinct assemblies within cells, which is consistent with the observation that distinct mRNAs that partition into two different RNP granules in fungi also form distinct RNA condensates *in vitro* [18]. Particular RNA features such as repetitiveness, G-quadruplex formation, and ensemble diversity (the number of predicted structures of an RNA) may also promote RNA and RNP condensation [19,25–28]. The effects of ensemble diversity are predicted by the RNA folding problem (Box 1) in that RNAs with increased interaction diversity (a consequence of diversity in folding) are more likely to be trapped in an RNA condensate through promiscuous *trans* RNA–RNA interactions.

RNA–RNA Interactions Contribute to RNP Granule Formation

In addition to the ability of RNA to self-assemble *in vitro*, the possibility that intermolecular RNA–RNA interactions contribute to RNP granule formation was suggested by the requirement for RNA in RNP granule formation. For example, several nuclear RNP granules, such as histone locus bodies and paraspeckles, form at sites of high transcription of particular RNAs, where the local RNA concentrations are elevated (reviewed in [29]). Similarly, the formation of SGs and PBs depends on the cytosolic concentration of untranslating RNAs [30,31]. Increasing the pool of untranslating mRNAs, by decreasing translation initiation or by inhibiting RNA degradation, increases SG and PB formation, respectively [32–34]. Moreover, creating a high local concentration of RNA by injection or transfection promotes SG formation [35,36]. Conversely,

reactant surfaces that are available for interaction and by reducing the randomness of molecular orientations.

Ribonucleoprotein (RNP) granules: biomolecular condensates composed of interacting RNAs and proteins (Figure 1). RNP granules are involved in virtually every stage of RNA metabolism.

RNA chaperone: analogous to protein chaperones, RNA chaperones combat improper interactions such as misfolding or aggregation, and promote proper RNA interactions. They can act kinetically, by altering the rate of transitioning between RNA conformers, or thermodynamically, by utilizing specific RNA binding to bias RNA folding and interaction equilibria to particular conformers (Box 1).

RNA entanglement catastrophe: the hypothesis that aberrant or toxic RNA aggregation is driven by spontaneous RNA condensation.

RNA–RNA interactions: RNAs can interact in *cis* (i.e., RNA folding) and in *trans* through both base-pairing and non-Watson–Crick interactions. The relatively similar energetics of a high number of potential interactions gives rise to the RNA folding problem (Box 1).

RNA self-assembly/condensation: collectively refer to the processes by which RNA in solution spontaneously forms a condensed assembly through RNA–RNA interactions. dsRNA liquid crystallization [24] and repeat RNA gelation [25] are examples of RNA self-assembly processes. An RNA aggregate is a nonspecific, stable RNA condensate.

Stress granules (SGs): cytosolic RNP granules formed from untranslating RNPs in response to cellular stressors such as heat shock, viral infection, proteotoxic stress, and oxidative agents, among others.

Thermodynamic RNA condensers: proteins that lower the ΔG of RNP granulation through their binding, for example by networking RNAs by contributing protein–protein interactions to granule formation (Figure 3A), shielding phosphate backbone repulsions, or by stabilizing *trans* RNA–RNA interactions.

Thermodynamic RNA decondensers: proteins that limit RNP granulation by high-affinity RNA binding to restrict the sites or conformations that are available for *trans* RNA–RNA interactions (Figure 2A).

Box 1. The RNA Folding Problem and RNA Chaperones

For a typical RNA transcript, there are many possible RNA–RNA interactions between transcript regions that would be of similar free energy (Figure IA). These interactions are often metastable and dissociate on very slow timescales. Thus, RNA possesses a rugged folding landscape where molecules have a relatively high probability of being kinetically trapped in a nonnative conformation, a phenomenon referred to as the RNA folding problem [138] (Figure IA). Put differently, the structure of an RNA is best understood as an ensemble of different RNA folds that are in (often very slow) equilibrium with each other [139]. Cells resolve the RNA folding problem through the actions of RNA chaperones [137,140–142] that can reduce energy barriers between conformations to resolve kinetic traps (Figure IA) or act thermodynamically to stabilize particular conformations through RNA binding (Figure IB). To direct RNA folding or to resolve kinetic traps, kinetic RNA chaperones promote various degrees of partial RNA folding or unfolding, often through dynamic or catalytic cycles of binding and unbinding [141–143], thus allowing the RNA to fold or to convert to a different conformation through a partially unfolded transition state via a strand-displacement mechanism (Figure IA and B). Thermodynamic RNA chaperones act by stabilizing or destabilizing particular structures, for example, by blocking interactions or by binding specific structures with high affinity (Figure IB).

Because *cis* and *trans* RNA interactions can influence each other [139], intermolecular RNA–RNA interactions contribute to the RNA folding problem, particularly in the context of RNP granules, which have elevated RNA concentrations and stabilize RNA–RNA interactions [16]. The compartmentalized conditions of condensates may also promote RNA ensemble redistribution [139]. Thus, one would predict that RNA chaperones similarly modulate the kinetics and thermodynamics of intermolecular RNA interactions and RNA self-assembly.

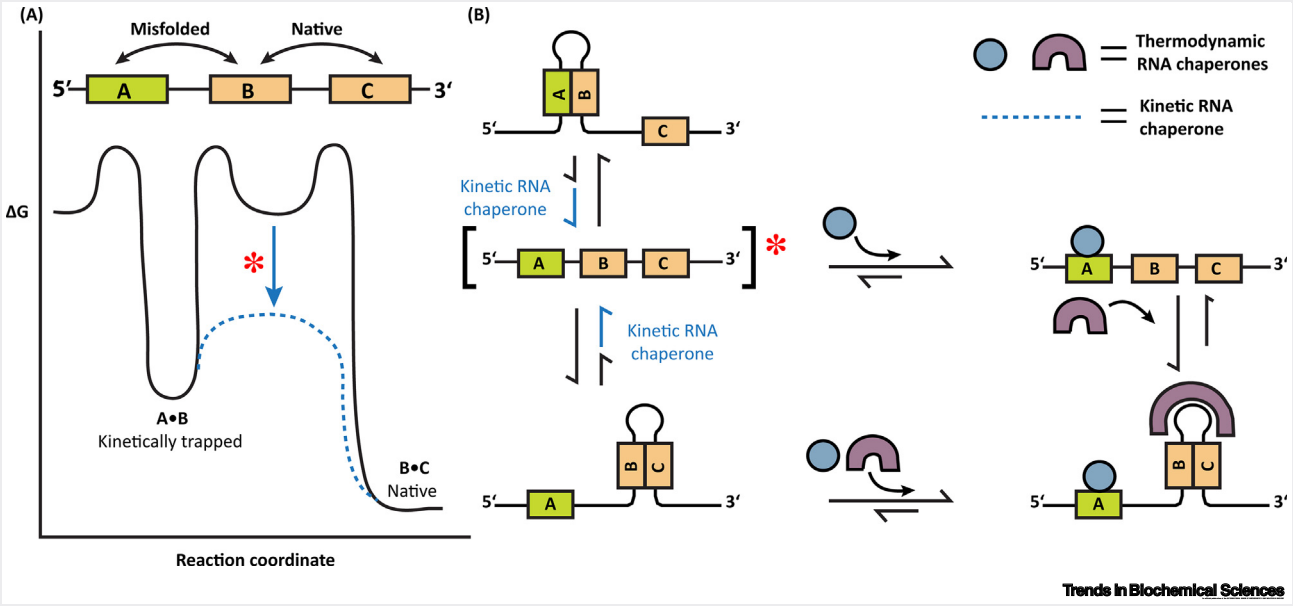

Figure I. The RNA Folding Problem and RNA Chaperones. Kinetic and thermodynamic RNA chaperones function to modulate RNA folding. (A) Energy diagram of kinetic RNA chaperone function. Compared with unassisted RNA melting and refolding (solid black curve), kinetic RNA chaperones utilize dynamic binding to destabilize structures and facilitate structural transitions by partial RNA unfolding and strand displacement, thereby lowering the activation energy of the structural transition (dashed blue curve). Depending on the structure and context, kinetic RNA chaperones may promote different degrees of unfolding. For example, DEAD-box proteins can completely melt small RNA duplexes [75,76,137]. (B) Reaction diagram of RNA chaperone function. (Left) Kinetic RNA chaperones accelerate RNA unfolding through dynamic binding. Although the bracketed structure is depicted as being completely unfolded for clarity, kinetic RNA chaperones can accelerate RNA refolding through partially unfolded intermediates. The energy of these complexes determines the activation energy (red asterisks). (Right) Thermodynamic RNA chaperones utilize high-affinity binding to reduce the free energy of an RNA structure. One means of doing so is by binding unstructured regions to prevent them from interacting (blue protein). A second means is to bind to and stabilize particular RNA structures (purple protein).

decreasing the untranslating mRNA concentration by trapping them on polysomes, or by widespread mRNA degradation, inhibits SG formation [30,37–40].

Additional evidence that RNA–RNA interactions can promote RNP granule assembly comes from multiple correlations between RNA condensation *in vitro* and RNP granule formation in cells. First, protein-free yeast total RNA condensation largely recapitulates the yeast SG transcriptome [15]. Second, RNAs that are prone to self-assembly as G-quadruplexes seed SG-like foci in both lysates and cells [26]. Third, RNA condensation *in vitro* is promoted by elevated ionic strength

and crowding [15], whereas SG assembly in cells is sensitive to osmolarity [35], which influences both. Similarly, the condensation of G-rich repeats found in some repeat-expansion RNAs is sensitive to NH_4OAc *in vitro* and in cells [25]. Finally, increased concentrations of monomeric RBPs that compete for intermolecular RNA–RNA interactions limit RNA condensation *in vitro* and SG assembly in cells [16,35].

Another line of evidence that *trans* RNA–RNA interactions contribute to RNP granule formation comes from high-throughput psoralen crosslinking studies that have identified heterotypic intermolecular duplexes between many eukaryotic mRNAs and/or long noncoding RNAs (lncRNAs). These studies have also identified homotypic RNA interactions, which may be intramolecular, intermolecular, or both. For example, the paraspeckle **architectural lncRNA** *NEAT1* demonstrates conserved long-range interactions between 5′ and 3′ domains *in vitro* and *in vivo* that are thought to be important for paraspeckle organization [41–43]. At the high local RNA concentrations of RNP granules (~1 mg/ml for the paraspeckle [20]), it is likely that some of these interactions are intermolecular. Consistent with RNA interactions promoting paraspeckle assembly, *NEAT1* RNA foci persist in cells even after the RBPs present on *NEAT1* transcripts are dispersed by 1,6-hexanediol treatment [44], and *NEAT1* demonstrates much slower dynamics in paraspeckles than key paraspeckle proteins [45]. Other lncRNAs, including architectural lncRNAs and the X-inactivation lncRNA *XIST*, similarly demonstrate specific RNA elements/interactions that are important for their proper localization and function [29,42,46–48]. Moreover, hundreds of different intermolecular mRNA–mRNA interactions have been identified by crosslinking experiments [42,46,49,50], and one anticipates that such interactions will be increased when RNAs are at elevated local concentrations in RNP granules.

Intermolecular RNA–RNA interactions can be either random or genetically programmed. Random interactions could occur simply because long RNAs that are often enriched in RNP granules have the potential for many sites of interaction simply through chance [20]. Thus, some RNP granules that compartmentalize long untranslating mRNAs, such as SGs [51] and bacterial RNA (BR)-bodies in *Caulobacter crescentus* [52,53], may simply assemble through relatively random interactions between long RNAs. Random interactions could also result from conformational diversity (and thus diversity of potential *trans* interactions), increasing the probability of kinetic traps. By contrast, some RNP granules assemble, at least in part, by genetically programmed RNA–RNA interactions that are likely tied to particular RNA folds. For example, during *Drosophila* oogenesis, defined base-pairing interactions between the *oskar* or *bicoid* 3′ untranslated regions (UTRs) target them to specific maternal mRNP granules [54,55]. Evolutionary pressure might also lead to the evolution of mRNA sequences and structures that limit their ability to base-pair with RNAs in a particular RNP granule. For instance, in *Ashbya gosypii*, *cis* RNA duplexes are suggested to limit the ability of the *CLN3* mRNA to interact with *BNI1* mRNA, and thereby allow the *CLN3* mRNA to be physically segregated from the *BNI1* mRNA in different RNP granules [18]. Finally, noncanonical RNA–RNA interactions may also contribute to RNA condensation in biological contexts. For example, the packaged genomes of several dsRNA viruses are dsRNA liquid crystalline condensates [56–59].

The available evidence suggests that RNAs generally fold in *cis* first, and then form intermolecular RNA–RNA interactions between the surface regions of each RNA that promote RNA condensation. Specifically, it is observed that mRNAs fold in *cis* into a compact structure as ribosomes run-off, and then later accumulate in RNP granules [37]. Moreover, estimates of the amount of proteins on mRNAs suggest that substantial regions of RNA are not coated with proteins, particularly when they are not being translated and are in the cytosol (Box 2). This suggests the formation of intermolecular RNA–RNA interactions generally does not compete with intramolecular interactions, but instead

Box 2. Building an mRNP

Like the granules they form when condensed, individual ribonucleoproteins (RNPs) are dynamic macromolecular assemblies of RNA and protein, the components of which are remodeled throughout the life of an RNA. Messenger RNPs (mRNPs) begin to form cotranscriptionally as the nascent pre-mRNA begins to fold and proteins, such as splicing factors and hnRNPs, bind to the RNA. Interestingly, the directional nature of cotranscriptional folding does not appear to affect the final RNA structure because structure-probing experiments show that refolded RNAs *in vitro* demonstrate similar structural features to untranslating cellular transcripts [144,145]. This is likely because of RBP binding and the actions of both kinetic and thermodynamic RNA chaperones that keep RNA structures more dynamic. This may also reflect the higher ensemble diversity of mRNAs compared with noncoding RNAs (ncRNAs) [71]. Within the nucleus, where RBP concentrations are much higher [71], mRNPs may adopt a more linear, extended conformation [146,147], consistent with the action of RBPs to reduce *cis* RNA folding. After nucleocytoplasmic export, the pioneer round of translation removes proteins coating the mRNA, such as the exon junction complex, and remodels the mRNP [148]. Cytosolic mRNA structures are further modulated by translation because ribosomes greatly decompact mRNAs [37,147]. One reason for the generally greater mRNA compaction in the cytosol than in the nucleus may be the estimated ~30-fold lower concentration of RBPs relative to RNA [71]. Following translational arrest, ribosomes run off and mRNAs compact in a 5′ to 3′ manner [36,147]. Owing to the proximity effect, *cis* interactions occur much faster than *trans* interactions, meaning that the *cis*-folding of the RNA determines what RNA surfaces are available to interact in *trans*. Consistent with this notion, mRNAs undergo compaction independently of their recruitment to SGs [36,147], implying that the *trans* RNA–RNA interactions recruiting them to SGs are with the folded mRNAs in the ensemble and do not necessarily compete with intramolecular structures.

An important question (reviewed in [71]) concerns to what extent an mRNA is coated with protein. Notably, many RBPs bind to RNA dynamically, meaning that the mRNP composition is continually changing. Both footprinting studies [149,150] and $CsCl_2$ buoyant density gradient measurements lead to estimates that 50–80% of the mass of nuclear mRNPs is protein, although because of lower RBP concentrations and higher RNA compaction in the cytosol, cytosolic mRNPs probably have lower protein compositions [71]. Although these are rough estimates, they suggest that much or most of the mRNA in a cytosolic mRNP is not bound by protein and is free to engage in *cis* or *trans* interactions [71].

takes place between regions of the folded RNA that remain relatively unfolded and available for interactions in *trans*. In this manner, such intermolecular interactions between RNAs will carry less of an entropic penalty in comparison with those that directly compete for *cis*-folding interactions. Thus, RNA self-assembly can be understood as another aspect of the RNA folding problem (Box 1), which is caused by the diversity of RNA conformers present in a particular RNA, and that requires RNA chaperones to resolve.

RNP Granule Dynamics

Before discussing how cells modulate RNA/RNP condensation, it is important to consider the dynamics of RNP granules. The dynamics of any component of an RNP granule are largely dictated by two factors [60]. First, a higher valency of any protein/RNA for other components of the granule leads to a slower exchange rate as a result of increased avidity [60]. This provides an explanation for why RNAs, which are generally much bigger than proteins, and would be expected to have multiple interactions with other components of RNP granules, generally display slower dynamics in RNP granules and *in vitro* condensates than proteins (*in vitro* [15,16,61–64] as well as *in vivo* [45,65–67]; Table 1 and references therein). Strikingly, mRNPs that are centrally located in RNP granules can be rigidly positioned and very static in the granule, consistent with these RNPs having many trapping interactions [65]. By contrast, RNPs interacting with the surface of RNP granules can be very dynamic, consistent with these RNPs only forming few transient interactions with the granule [65,66].

For RBPs, a second key principle is that a tighter affinity of the component for RNA leads to a slower exchange rate. For instance, RNA affinity strongly correlates with the dynamics of RNA-binding peptides in RNP condensates *in vitro* [19]. In cells, this is observed in the relative differences of exchange rate, as assessed by FRAP (fluorescence recovery after photobleaching), for many RNP granule components (Table 1). This suggests that, for many components of RNP granules, their exchange rate represents their off-rate from RNA. Thus, appending an

Table 1. Classification of SG Proteins as RNA Chaperones[a]

Class	Protein	Mobile fraction	Do SGs form when overexpressed?	Can SGs still form when knocked down/out?	Cellular concentration [151]
Kinetic condenser	G3BP1/2	High [91]	Yes [152]	No [91]	3000 nM
	UBAP2L	High [153]	Yes [93]	No [153]	500 nM
	TIA1	High [91]	Yes [92]	Reduced [91]	1400 nM
	TIAR	High [91]	Yes [92]	Reduced [91]	175 nM
	CAPRIN	High [154]	Yes [92]	No [91]	620 nM
Thermodynamic condenser	FMRP	Low [154]	Yes [154]	Slightly reduced [155]	121 nM
	ATAXIN2	Low [2]	Yes [2]	Slightly reduced [156]	114 nM
	PUMILIO	Low [154]	Yes [154]	Slightly reduced [85]	126 nM
	CBEP	Low [157]	Yes [158]	N/A	34 nM
Thermodynamic decondenser	YB1	Low [91]	No [35]	Yes [91]	4640 nM
	HUR	Low [91]	No [35]	Yes [91]	2300 nM
	PABP	Low [159]	No [7]	N/A	4000 nM
	STAUFEN	Low [161]	No [162]	Yes [162]	161 nM
Kinetic decondenser	DHX36	High [68]	No [68]	Yes [133]	170.3 nM
	eIF4A	N/A	No [16]	Yes [16]	11 066 nM
	DDX19	N/A	No [16]	N/A	1312 nM
Client/unknown proteins	ZBP1	Low [91]	No [160]	Yes [91]	950 nM
	FUS	High [154]	Yes [154]	Yes [14]	975 nM
	hnRNPA1	High [63]	N/A	Yes [171]	975 nM
	TTP	High [7]	Yes [7]	N/A	N/A
	HDAC6	N/A	N/A	Reduced [163]	14.4 nM
	DDX3X	High [164]	Yes [165]	Slightly reduced [165]	2372.3 nM
	YTHDF1/3	N/A	N/A	Reduced [166]	372.6 nM
	YTHDF2	High [167]	N/A	Slightly reduced [166]	447.5 nM
	EWS	N/A	N/A	Yes [168]	536.4 nM
	TAF15	N/A	N/A	Yes [168]	1675.2 nM
	SMN	N/A	Yes [169]	Slightly reduced [170]	126 nM
	TDP43	High [154]	Yes [154]	N/A	840 nM

[a]Mobile fraction: high if >50%; N/A, not available.

additional RNA-binding domain to G3BP reduces its dynamics in SGs [39]. The trend that higher numbers of RNA-binding domains reduce G3BP dynamics is preserved when the G3BP RNA-binding domain is replaced by varying numbers of KH or Zn-finger RNA-binding domains [39]. Catalytic reactions or PTMs can also alter the exchange rates by changing the affinity of binding. For example, mutations in DHX36 that cannot hydrolyze ATP, and therefore are trapped in a high-affinity state for RNA, show a slower exchange rate from SGs than the wild-type (WT) protein [68].

Modulation of RNA Condensation

The cell has multiple mechanisms for controlling the condensation of RNA. In the simplest case, RNA condensation can be affected by modulating local RNA concentrations through transcription, transport, and decay. Alternatively, *cis* and *trans* RNA–RNA interactions are actively and directly disrupted by energy-consuming processes such as translation, which decompacts mRNPs and prevents SG formation [30,37]. In principle, RNA modifications may also alter the

interaction landscape. For example, ADAR-mediated adenine-to-inosine editing of RNAs destabilizes duplexes and thereby could limit the formation of dsRNAs within cells [69]. Finally, as detailed below, RNA interactions are modulated by RNA chaperones (Box 1), including **DEAD-box proteins (DBPs),** to both promote or inhibit RNA condensation.

Mechanisms to Limit RNA Condensation

Proteins can act in at least two distinct manners to limit RNA condensation (Figure 2). In one case, **thermodynamic RNA decondensers** (Figure 2A) use high-affinity RNA binding to bind to RNAs and thereby compete for *trans* RNA–RNA interactions, thus lowering the energy of the dispersed state of RNAs. Thermodynamic RNA decondensers are generally abundant RNA chaperones which, when overexpressed, deter RNP granule formation (Table 1). For example, overexpression of YB-1 or HuR limits the formation of SGs in response to arsenite treatment [35]. Because these are high-affinity RBPs, when they are bound to mRNAs in RNP granules they exhibit slow rates of exchange and have low mobile fractions in granules. Several common SG-associated RBPs demonstrate these properties (Table 1). Similarly, in bacteria, the protein CspA promotes the formation of RNA structures, which can be in *cis* or *trans*, during the cold shock response [70]. Like YB-1, CspA is abundant, with an intracellular concentration of ~30 μM, and has high affinity for RNA [70]. The role of abundant RBPs in limiting RNA condensation may explain why RBPs are at a substantially higher concentration in the nucleus relative to RNA, thereby limiting the condensation of nascent transcripts [71].

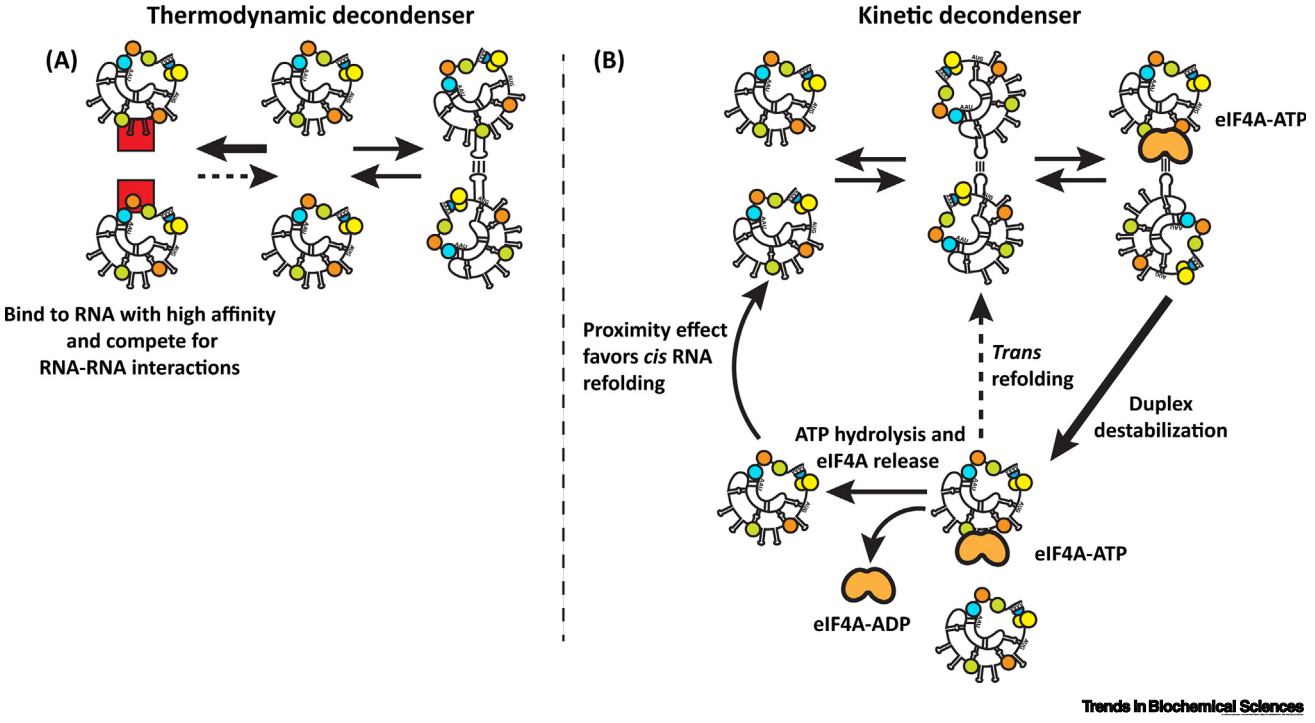

Figure 2. Mechanisms by Which RNA Chaperones Limit RNA Condensation. RNA chaperones have multiple mechanisms to limit RNA condensation. (A) Thermodynamic decondensers (red) use high-affinity RNA binding to compete for RNA–RNA interaction sites so as to limit RNA condensation. Thermodynamic decondensers therefore lower the valency of RNA and prevent RNA conformational changes by locking RNA conformers in Δ*G* wells. (B) Kinetic dencondensers prevent RNA condensation by destabilizing *trans* RNA–RNA interactions, therefore lowering the activation energy barrier between *trans* interacting RNAs and dispersed states. Kinetic decondensers could also promote *cis* RNA refolding, thereby reducing the valency of a given RNA. Because kinetic RNA decondensers such as DEAD-box proteins destabilize RNA secondary structure by RNA binding and not by ATP hydrolysis, the key difference between a thermodynamic and kinetic RNA decondenser is the relative off-rate for RNA, with kinetic decondensers binding dynamically. Kinetic decondensers such as eIF4A (orange) can function as thermodynamic decondensers in the absence of ATP hydrolysis [16].

In a second manner, **kinetic RNA decondensers**, such as some DBPs, can act to lower the activation energy barriers between folded and unfolded RNA conformations, and decrease the valency of RNAs by resolving *trans* interactions and/or promoting *cis* RNA refolding (Figure 2B). For example, the translation initiation factor eIF4A functions to limit RNA condensation both *in vitro* and in cells [16]. In this mechanism, eIF4A and other related proteins, such as DDX19A/DBP5 [16,39,72], lower the activation energies between folded RNA and unfolded RNA secondary structure through ATP-dependent RNA binding, and this disrupts several nucleotides in the structured RNA. By decreasing the stability of RNA–RNA interactions, this increases the rate at which the given RNA can unfold or undergo conformational transitions. In the absence of ATP hydrolysis, eIF4A acts as a thermodynamic decondenser by competing for *trans* RNA–RNA interactions. However, ATP hydrolysis and P_i release promotes eIF4A dissociation [73,74] and allows RNA refolding either in *cis* or in *trans*, with the former being favored by the **proximity effect**. ATP hydrolysis and P_i release also frees the eIF4A protein to engage in additional cycles of RNA structural rearrangements [73–77], which facilitates more effective RNA decondensation [16]. This indicates that the key difference between a kinetic and thermodynamic decondenser is the off-rate for RNA. Other DBPs likely function to limit RNA condensation. For example, knocking down UAP56/DDX39B, which is related to eIF4A and consists of the conserved DBP core domain [78], increases nuclear speckle size and traps mRNAs in speckles [79,80].

Mechanisms to Promote RNA Condensation

RNA chaperones also act in at least two distinct manners to promote RNA condensation (Figure 3). **Thermodynamic RNA condensers** would reduce the ΔG of RNP condensation through RNA binding. Potential mechanisms include binding intermolecular RNA structures with high affinity, shielding phosphate backbone repulsions to stabilize RNA–RNA interactions,

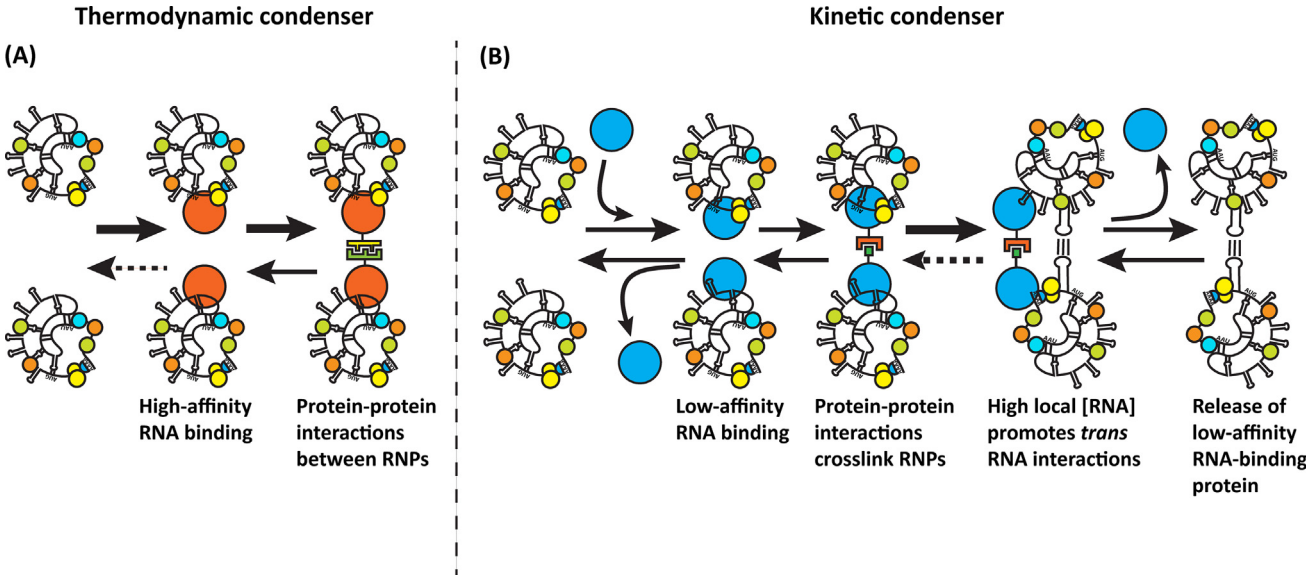

Figure 3. Mechanisms by Which RNA Chaperones Promote RNA Condensation. RNA chaperones can promote RNA condensation through kinetic and thermodynamic modes. (A) Thermodynamic condensers increase the valency of RNA by contributing stable protein–protein interactions to a ribonucleoprotein (RNP). In addition, high-affinity RNA binding results in an RNA–protein co-condensate that reduces the exchange of RNP granule components. (B) Kinetic condensers raise the rate of forming *trans* RNA–RNA interactions, for example, by imparting a proximity effect between RNAs. Such kinetic condensers utilize protein–protein interactions to dimerize, coupled with tandem RNA binding that positions RNAs for interactions in *trans*. Because kinetic condensers increase the frequency of properly orientated collisions between RNA molecules for *trans* interactions, kinetic condensers generate rate enhancements by increasing the pre-exponential factor of the Arrhenius equation. Coupled with a high off-rate for RNA, kinetic condensers can recycle on dispersed RNPs. Therefore, the key difference between a kinetic and thermodynamic RNA condenser is the relative off-rate for RNA binding.

and binding exposed RNA with high affinity and crosslinking individual mRNPs through protein–protein interactions (Figure 3A). Thermodynamic condensers should generally have a low mobile fraction in RNP granules owing to their high affinity for RNA, and produce granules when overexpressed, thus promoting RNP granule formation. In general, thermodynamic condensers should be at low intracellular concentrations to prevent irreversible RNP complex formation. Examples of thermodynamic condensers are seen in yeast PBs where Dcp2, Pat1, and Edc3 all have low mobile fractions, exchange slowly, and promote PB formation [81–83]. Because RNA binding can be specific, thermodynamic condensers might be important for the compartmentalization of specific RNAs into specialized granules such as neuronal or germ granules. For example, FMRP, Pumilio, and Ataxin-2 are slow-exchanging, multivalent RBPs with relatively low intracellular concentrations (Table 1). These proteins may aid in the formation or integrity of neuronal granules [2,84,85]. In addition, RNA and thermodynamic condensers may undergo cooperative phase transitions [19,86].

In a second mechanism, we predict that some RNA chaperones will act as **kinetic RNA condensers** that accelerate the rate of forming a *trans* RNA–RNA interaction. One mechanism to accomplish this effect is to unwind intramolecular structures to allow intermolecular interactions to occur. Alternatively, RNA condensation could be promoted by utilizing dynamic protein multimerization to bring RNAs into close proximity and thereby promote the formation of intermolecular RNA–RNA interactions (Figure 3B). This mechanism is similar to how the Hfq complex in bacteria promotes interactions between small regulatory RNAs and target mRNAs [87], and to how the mammalian hnRNP A2/B1 proteins promote *trans* interactions between the lncRNA *HOTAIR* and its mRNA targets by dimerizing to bring RNA molecules into proximity in an antiparallel orientation [88–90]. Such a proximity effect would in turn accelerate the formation of intermolecular RNA–RNA interactions, thus allowing cooperativity between RNA–RNA and protein–protein interactions to promote RNP condensation.

The above principles make predictions about the behavior of kinetic condensers in RNP granules. Kinetic condensers are predicted to have a high mobile fraction in RNP granules because their ability to efficiently and promiscuously condense RNAs relies on having a high off-rate to act in multiple cycles; the key difference between a kinetic and thermodynamic condenser is the off-rate for RNA. Kinetic condensers are also expected to promote RNP granule formation when overexpressed, reduce it when deleted, and be present at a high intracellular concentration to overcome decondensing machineries. Interestingly, several RBPs in SGs (Table 1), such as G3BP1, UBAP2L, and TIA-1, meet these criteria because they are highly dynamic in SGs, create SGs when overexpressed, and reduce SG formation when depleted [91–94]. Importantly, the formation of SGs requires both RNA binding and dimerization of these proteins [92–94], but increasing G3BP homodimerization potential does not modify its exchange rate in SGs [12], suggesting that protein–protein interactions do not control G3BP exchange. By contrast, increasing the RNA-binding capacity of G3BP reduces its mobility in SGs, suggesting that WT G3BP has a high off-rate for RNA compared with slow-exchanging, tighter-binding proteins [39]. Along similar lines, overexpression of mutant TIA-1 lacking its RNA-binding domain represses SG formation [31], consistent with mutant TIA-1 acting as a competitive inhibitor of WT TIA-1 interactions that promote the kinetic condensation of RNA. Therefore, we hypothesize that these proteins may function as kinetic condensers of RNA instead of strictly being direct thermodynamic SG scaffolds.

Putative condensers of RNA have also been identified in other granules. For example, the intrinsically disordered protein SERF1a promotes the annealing of RNA strands in *trans*, as well as the incorporation of RNA into polyarginine/RNA condensates *in vitro* and *ex vivo* nucleoli [95]. In addition, in *Drosophila* the protein Exu is important for proper *oskar* and *bicoid* localization to RNP

granules [96–98]. Exu binds to structured elements in the *bicoid* 3′ UTR, and Exu RNA binding and dimerization are essential for *bicoid* proper localization [99], which is facilitated in part through homotypic *trans* RNA–RNA interactions between *bicoid* 3′ UTRs [55,100]. This is consistent with a model where Exu dimerization promotes *trans* base-pairing between *bicoid* 3′ UTRs.

DBPs Can Modulate RNP Condensation in Multiple Manners

DBPs can also promote RNP granule assembly through additional interactions and the regulation of their ATPase activity. The tail domains can regulate the RNA-binding and ATPase activities of DBPs [101–104], or engage in protein–protein interactions [103,105,106] that modulate DBP function and facilitate RNP assembly [80]. Thus, in the ATP-bound state, a DBP bound to RNA and interacting with other proteins can promote assembly of the RNP granule [80,106]. Subsequently, ATP hydrolysis allows release from the RNA, and potentially can contribute to transitions or disassembly of the RNP assembly [80,105–107]. For example, the DDX3X/Ded1 protein has N- and C-terminal domains that interact with eIF4E, eIF4G, and itself to promote SG assembly in the ATP-bound state, and when ATP is hydrolyzed contributes to the release of RNAs from SGs [80,107–109]. This can explain how ATPase mutations, including those in neurodevelopmental disorder and medulloblastoma patients, in the DBPs Ded1/DDX3X [80,110,111] and Dhh1 [106] lead to the trapping of RNAs in SGs and PBs, respectively.

RBP Post-translational Regulation of RNP Condensation

Proteins modulating the condensation of RNPs are subject to multiple PTMs that allow their activity to be regulated (reviewed in [11]). For example, upon arsenite stress, essential SG proteins such as G3BP and UBAP2L undergo arginine demethylation of residues in their RGG RNA-binding domains, which promotes SG assembly [93,112]. Methylation of arginine residues can disrupt interactions between arginine and delocalized π systems such as in nucleobases [113], where methylation is predicted to interfere with RNA binding. Thus, demethylation might increase RNA binding of these factors, promoting RNA condensation. Additional modifications that can affect RNP granule formation include glycosylation [114], acetylation [115,116], phosphorylation [39,40,117], and SUMOlyation [118]. Interestingly, eIF4A1 is phosphorylated at T164 in *Arabidopsis* following osmotic or hypoxic stress [119], and mass spectrometry studies have confirmed phosphorylation events at the corresponding residue in human eIF4A1 (T158; www.uniprot.org/uniprot/P60842). Phospho-T164 prevents RNA binding [119], and therefore should inhibit the ability of eIF4A to limit RNA condensation. Taken together, we anticipate that a dense network of post-translational protein modifications modulates the function of proteins influencing RNP granule formation.

Dysregulation of RNA Condensation and Disease

Perturbations in the cellular machinery for regulating RNA condensation may be involved in human disease. For example, many viruses, including noroviruses, Dengue virus, and the MERS coronavirus, whose replication is reduced by SGs, subvert the formation of SGs [120–122]. In another possibility, failure in the RNA-decondensing machinery of the cell could cause aberrant RNA condensation, potentially causing an **RNA entanglement catastrophe** [16]. For instance, mutations affecting the ATPase activities of DDX3X [111,123], DDX6 [124], and DHX30 [125] coincide with aberrant RNP condensation and neurodevelopmental disorders. In addition, mutations affecting the DDX3X ATPase are involved in some types of medulloblastoma where cells demonstrate constitutive SG-like aggregates [110,126]. Interestingly, loss-of-function mutations in the DBP regulator Gle1 result in a fatal degenerative motor neuron disease called lethal congenital contracture syndrome (LCCS), which may result from aberrant ribostasis [127,128]. Moreover, one mechanism of toxicity of repeat-expansion RNAs appears to be the formation of RNA aggregates through RNA–RNA interactions [25], which can sequester RBPs, thereby altering RNA processing in

pathogenic manners [129–132]. Notably, expression of repeat-expansion RNAs, or promoting RNP granule formation by knockdown of DHX36, can activate the dsRNA-sensing eIF2α kinase PKR and the integrated stress response [133–136]. This raises the possibility that aberrant RNA condensation triggers a cellular response, which in some cases may lead to toxic effects.

Concluding Remarks

RNP granules are emerging as active compartments that are important for the maintenance of RNP homeostasis as well as being sites of high local RNA concentration. Thus, it is important for the cell to have a variety of regulatory modalities to control the effective RNA concentration and intermolecular RNA–RNA interactions, to thus promote RNA condensation when desired, and to limit it in other contexts. Future work to understand the diversity of intermolecular interactions between RNPs (see Outstanding Questions) and how cells regulate those interactions should be fruitful to increase our understanding of ribotoxicity, RNP granules, and the regulation of ribostasis.

Outstanding Questions

What is the nature of the *trans* RNA–RNA interactions that contribute to RNP granulation?

How have cells selected for programmed or promiscuous *trans* RNA–RNA interactions?

Why do mutations in RNA chaperones lead to disease?

How are RNA chaperones regulated in a spatiotemporal manner, and how does dysregulation of this process result in disease?

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