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Review

Connecting the "dots": RNP granule network in health and disease

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ABSTRACT

All cells contain ribonucleoprotein (RNP) granules – large membraneless structures composed of RNA and proteins. Recent breakthroughs in RNP granule research have brought a new appreciation of their crucial role in organising virtually all cellular processes. Cells widely exploit the flexible, dynamic nature of RNP granules to adapt to a variety of functional states and the ever-changing environment. Constant exchange of molecules between the different RNP granules connects them into a network. This network controls basal cellular activities and is remodelled to enable efficient stress response. Alterations in RNP granule structure and regulation have been found to lead to fatal human diseases. The interconnectedness of RNP granules suggests that the RNP granule network as a whole becomes affected in disease states such as a representative neurodegenerative disease amyotrophic lateral sclerosis (ALS). In this review, we summarize available evidence on the communication between different RNP granules and on the RNP granule network disruption as a primary ALS pathomechanism.

1. Introduction: RNP granules are connected to form a molecular network

At any stage of their lifecycle, mRNAs are bound to a set of RNA-binding proteins (RBPs), to form RNP particles. These RNA-protein assemblies can form clusters and give rise to large membraneless compartments called RNP granules. RNP granule formation is considered to be a conserved cellular mechanism to concentrate specific factors and increase the efficiency of biochemical processes [1,2]. Multiple aspects of cellular RNA metabolism are controlled by RNP granules, including splicing, mRNA trafficking, silencing, translation, and RNA decay [3,4]. The dynamic nature of RNP granules allows for an exceptional level of functional flexibility that cannot be provided by membrane-bound organelles; for example, RNP granules are indispensable for rapid mounting of cellular stress response.

It is conceivable that in order to efficiently fulfil their functions, RNP granules need to maintain a connection with each other. While each RNP granule contains a certain core, or "signature", set of components that distinguishes it from other granule types, multiple proteins and RNAs are shared by different RNP granules. Each individual RNP granule therefore constitutes a part of a dynamic "continuum" – a cell-wide collaborative network of RNA- and protein-rich biomolecular condensates that organizes cellular processes in space and time. The structure of this network can be adjusted by changing the destination of biomolecules to match and support cellular functional states, be that a specific stress, developmental stage, or specialized function the cell has to fulfil. The concept of an RNP granule network is opposed to the perception of RNP granules being independent, autonomous entities.

Given the range of cellular roles, it comes as no surprise that abnormal composition and/or turnover of RNP granules underlie disease states. Being an integral part of a molecular network, an RNP granule that turned aberrant (e.g. because of a mutation in its core component) will inevitably "spread" an abnormal signal, leading to a global network dysregulation. Network-wide dysfunction of RNP granules likely plays a crucial role in major neurodegenerative diseases [5–7].

Here, we discuss published data supporting the concept of the interconnectedness of different, often spatially separated RNP granules. We also summarize available evidence for the collapse of the entire RNP granule network in a prototypical neurodegenerative disease amyotrophic lateral sclerosis (ALS).

2. RNP granule network is established and maintained via liquid-liquid phase separation

Mounting evidence suggests that liquid-liquid phase separation (LLPS) is the underlying force driving formation of the majority of RNP granules [3,4]. LLPS is a physicochemical phenomenon in which

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solutions of proteins and/or nucleic acids concentrate into a dense phase, demixed from the surrounding light phase. LLPS is enabled by weak interactions between multivalent proteins and RNA, and RNA-RNA interactions are also important for this process [8,9]. Similar to other cellular phase-separated assemblies, or membraneless organelles (MLOs), RNP granules exhibit liquid-like properties, i.e. assembly from smaller particles that fuse, ability to rapidly condense and dissolve, and to exchange components with the surrounding diluted phase. Protein components of RNP granules visualized with fluorescent tags quickly shuttle in and out of the structures forming a dynamic equilibrium with the surroundings, as revealed by fluorescence recovery after photobleaching (FRAP) experiments [2]. Despite their liquid-like properties, RNP granules are not homogeneous droplets, rather, they are structured entities formed through a highly coordinated process requiring ATP [10]. They often comprise a densely packed "core" (or multiple cores) that is held together through stronger interactions, and a more labile "shell" that exhibits more fluid-like properties [10,11]. Proteins with intrinsically disordered domains (IDRs) in their structure are pivotal for LLPS and hence biogenesis and maintenance of RNP granules [12].

Conceivably, dynamic component trafficking is the process that underlies the association of RNP granules into a network. One of the most well-known examples of such trafficking is the exchange of mRNAs and RBPs between stress granules (SGs) and Processing bodies (P-bodies) [13]. Even essential protein components of a given RNP granule type, whose depletion dismantles it, are detected within other granule types. For example, the major Cajal body component coilin can be also recruited to the nucleolus [14], and a core paraspeckle component, DAZAP1, is found in arsenite-induced SGs [15] as well as in RNA transport granules [16]. Similarly, a P-body marker protein 4E-T is a nucleocytoplasmic shuttling protein recruited both to P-bodies in the cytoplasm and promyelocytic leukaemia (PML) bodies in the nucleus [17].

One important consideration for the concept of an RNP granule network is the finite number of multivalent protein molecules critical for the LLPS, and hence for the biogenesis and maintenance of RNP granules, – mainly RBPs and other IDR-containing proteins [18,19]. Due to the limited availability of these "building blocks", most of them are expected to be shared by RNP granules. As a result, assembly or enlargement of one RNP granule type often involves collapse or shrinkage of another. An example of such negative correlation is the impact of SG assembly on some nuclear bodies. SGs are exceptionally large structures that dominate the cytoplasmic landscape during stress. They recruit the bulk of RBPs and mRNAs, which leads to their depletion from other RNP granules. Affected RNP granules, e.g. splicing speckles [20] and Cajal bodies [21], display diminished number and/or smaller size.

In instances where the dynamic nature of phase-separated assemblies or their clearance mechanisms are perturbed, RNP granules have a tendency to solidify and persist, marking disease states, most prominently, common neurodegenerative diseases [5–7]. Cellular quality control mechanisms, such as chaperones and the autophagosome system, safeguard cellular homeostasis by timely and rapid disassembly of RNP granules [22]. In order to prevent these systems being overwhelmed, the number and size of the RNP granule network components must be kept within a manageable range. Opting for redistribution of available IDR-containing/multivalent proteins among RNP granules may therefore help avoid potentially dangerous overproduction of such LLPS-prone proteins. The cumulative volume of RNP granules increases during stress, and it is essential to reduce this volume rapidly and efficiently during the recovery phase. At present, it is difficult to estimate the maximum "phase separation capacity" of a cell; this parameter is presumably cell type- and cell state-specific. For instance, cells with a large cytoplasm, such as neurons, should be able to tolerate higher volumes of MLOs. However it is now clear that excessive RNP granule assembly and diminished rates of their disassembly can lead to the network overcrowding, resulting in cellular pathology of neurons, which are long-lived, non-dividing cells, and age-related neurodegenerative diseases. Aging cells are known experience a decline of proteostasis systems responsible for the timely RNP granule breakdown [23] and hence are characterized by decreased ability to manage these assemblies. Yet the molecular cascade of pathology is initiated by the formation of abnormal RNP granules prone to persist and accumulate, often due to a mutation in one of their components. For example, mutations in SG proteins, e.g. TIA1, hnRNP A1, FUS, generate SGs with abnormal properties; these structures may become a seeding ground for insoluble inclusion formation in ALS [24,25]. Formation of *de novo* RNP granules composed of mutant RNA and proteins would further destabilize the RNP granule network and phase separation balance. For example, mutant FUS protein not only incorporates into SGs and alters their properties but also forms a novel type of RNP granule when accumulated, and these granules can evolve into stable aggregates under certain conditions [26].

3. The diversity of physiological RNP granules

Physiological RNP granules can be subcategorized into cytoplasmic and nuclear granules; the latter are often called "nuclear bodies". Physiological RNP granule formation is a basic principle of cellular compartmentalization. Many of these granules support basal metabolic processes and are found in all types of mammalian cells, e.g. nucleolus and splicing speckles. Cells modulate the number, size and composition of these vital RNP granule types to adjust for their metabolic needs. Other RNP granules are cell type- or context-specific, where their presence (or absence) marks particular functional states. A well-known example is RNA transport granules found in neurons and involved in long-distance trafficking of mRNA in axons [27]. Other types of granules can be absent from specialized cells under normal conditions and only assemble in response to physiological cues, e.g. *de novo* paraspeckle assembly during embryonic stem cell patterning [28]. The majority, if not all, RNP granules are stress-responsive, meaning that their size, numbers, morphology, structure and composition change in stressed cells [29]. For example, nucleoli undergo significant structural remodelling and display stress-induced changes in their architecture and function [30], whereas paraspeckle numbers increase dramatically at the late stages of stress or during recovery [15].

The nucleus is densely packed with RNP granules, and this abundance is partially due to high RNA content in this compartment, as compared to the cytoplasm. A subset of nuclear bodies form around a single type of transcript (so-called "architectural RNA"), which serves as their scaffold, – most notably, paraspeckles and nuclear stress bodies (nSBs) built around long non-coding RNAs (lncRNAs) [31]. Other nuclear bodies, similar to cytoplasmic RNP granules, contain multiple RNA types with none of them assigned a clear structural role. In addition to the major nuclear bodies described herein, multiple minor and context-specific nuclear bodies exist; their characteristics have been reviewed elsewhere [32,33]. RNP granules are usually visualized by detection of a specific marker protein or RNA, e.g. coilin for Cajal bodies or NEAT1 RNA for paraspeckles. Characteristics of RNP granule types discussed in this review are summarized in Table 1, and their microscopic appearance is shown in Fig. 1.

It should be noted that the current review will focus on mammalian RNP granules and the connections between them. However, multiple types of RNP granules exist in organisms other than mammals, e.g. germ granules in *Drosophila*, or P granules in nematode; these also represent integral parts of the organism-specific RNP granule network.

4. Disease-linked RNP granules

A number of RNP granules are only found in diseased cells, and both nuclear and cytoplasmic disease-linked granules have been described. They are nucleated by either mutant RNA or protein and significantly perturb cellular metabolism via toxic gain of function, including

Table 1
Major types of physiological RNP granules.

RNP granule[a]	Shape, size and structure	Markers; other major components	Function	Main references
Nuclear bodies				
Nucleolus	Large, irregular shape, consisting of fibrillar centres and dense fibrillar components embedded in the granular component	Nucleolin, nucleophosmin, numerous other proteins, pre-rRNA transcripts	Transcription and processing of rRNA and assembly of ribosomal subunits; stress response; cell cycle progression; tRNA biogenesis and quality control	[30,146,147]
Cajal bodies (CBs)	Small, dot-like, frequently found associated or overlapping with Gems	coilin p80	Processing, assembly and maturation of snRNPs; telomerase biogenesis; ribosome biogenesis; recycling of spliceosome components	[59]
Gemini of Cajal bodies (Gems)	Small, dot-like, frequently found associated or overlapping with CBs	Small nuclear RNPs (snRNPs), SMN	snRNP maturation, storage or recycling	[148]
Splicing speckles	Large, irregularly shaped	SC35, lncRNA MALAT1, splicing factors - e.g. SR proteins, polyadenylated RNA	Sites for splicing factor storage and modification; harbour proteins involved in epigenetic regulation, chromatin organization, DNA repair and RNA modification	[110,149]
Paraspeckles	Spherical, occasionally elongated, with core-shell structure	lncRNA NEAT1, NONO and SFPQ proteins, chromatin remodelling factors, pri-miRNA machinery	Gene expression regulation through retention of transcription factors and certain RNAs; modulation of pri-miRNA processing	[142,150]
Promyelocytic leukaemia (PML) bodies	Small, round	PML (shell), inner core of proteins that can be sumoylated	Implicated in telomere lengthening; DNA replication; DNA damage response; nuclear protein quality control; oxidative stress response; post-translational protein modifications	[85]
Nuclear stress bodies (nSBs)	Varying shape and size	Satellite III (satIII) lncRNA; SAFB and HSF1 proteins	Platforms for splicing factor phosphorylation; contribute to gene expression regulation under stress	[48,98,101]
Cytoplasmic RNP granules				
Stress granules	Large, 1–5 μm in diameter; collection of compact cores embedded in a less dense shell	G3BP1, TIA1, PABP1, ataxin-2 and other RBPs; 40S ribosomal subunits; translation initiation factors	Stress signalling; selective protein synthesis; mRNA protection	[13,25,29,71,151]
Processing bodies (P-bodies, PBs)	Small dot-like	Dcp1a, decapping factors, deadenylation complex, exonucleases, RBPs involved in translational inhibition	RNA triage	[152,153]
Neuronal RNA transport granules	Heterogeneous class, varying size and shape	FMRP, Staufens, SYNCRIP, molecular motors	Repression of mRNAs during neuronal transport; local protein translation at the axonal terminals and dendrites	[27,74]

[a] Constitutive and stress-induced RNP granules are given in black and blue, respectively.

negative impact on physiological RNP granules.

4.1. Nuclear repeat-containing RNA foci

A prominent class of disease-linked RNP granules are nuclear foci containing microsatellite repeat-containing RNA as their primary component. Disease-linked repeat expansions of 3–6 nucleotides most commonly map to non-coding gene regions and can vary in length (~20 to several thousand repeats). Transcription of these regions results in massive accumulation of abnormal sense and sometimes antisense RNAs presenting as round foci when visualized by RNA in situ hybridization [34]. Formation of such RNP granules is an exclusive feature of neurological diseases, of which neuromuscular conditions is the most common type, including Huntington's disease (*HTT*), myotonic dystrophy (*DMPK*), Fragile X syndrome (*FMR1*), spinocerebellar ataxia (*ATXN* genes), and subtypes of ALS (*C9ORF72*) [35]. For example, intronic hexanucleotide repeat GGGGCC (G4C2) expansion in the *C9ORF72* gene is the most frequent cause of familial ALS [36]. Patients with an expanded allele can have several hundred and even thousand repeats; this is in contrast to 2–20 repeats in healthy individuals. Nuclear foci composed of repeat-containing RNA are believed to exert toxicity by abnormal retention of RBPs, most notably, splicing factors [37]. Sequestration and loss of function for various RNAs as a toxicity mechanism, when these RNAs become sequestered into the foci, also cannot be ruled out.

4.2. Abnormal cytoplasmic RNP granules

We and others found that mutant forms of the ALS-linked protein Fused in Sarcoma (FUS) are capable of forming *de novo* (i.e. not seen in cells expressing only normal protein) cytoplasmic RNP granules [26,38,39]. The majority of *FUS* mutations affect the protein's nuclear localization signal (NLS), such that mutant proteins exhibit impaired nuclear targeting and cytoplasmic mislocalization [40,41]. Formation of FUS immunoreactive cytoplasmic inclusions in affected neurons is the pathological hallmark of ALS-FUS [42]. Mutant FUS accumulated in the cytoplasm of cultured cells spontaneously forms small RNP granules when its level reaches a certain threshold; these granules coalesce into larger assemblies in stressed cells [26,43]. It is possible that these assemblies serve as precursors of insoluble inclusions in FUS proteinopathies [26,43,44].

Mutations in the NLS disrupting FUS nuclear import and dramatically changing its subcellular location seem to be unique to this RBP. Another ALS-linked RBP, TDP-43, is almost completely shifted to the cytoplasm from its normal nuclear location in motor neurons of ALS patients [42]. However, formation of similar *de novo* RNP granules by TDP-43 has not been reported in cell models; instead wild-type or mutant TDP-43 targeted to the cytoplasm often forms aggresome-like structures [45]. Whether or not ALS-linked variants of other RBPs are able to form *de novo* cytoplasmic RNP granules akin to those assembled by mutant FUS remains to be addressed.

Cytoplasmic foci nucleated by repeat-containing RNA have been described as well and may also play a role in ALS with *C9ORF72* expansions, spinocerebellar ataxias and myotonic dystrophy subtypes, although these structures are much less studied than their nuclear counterparts [34].

5. Connections between RNP granules

Before the development of approaches for RNP granule purification,

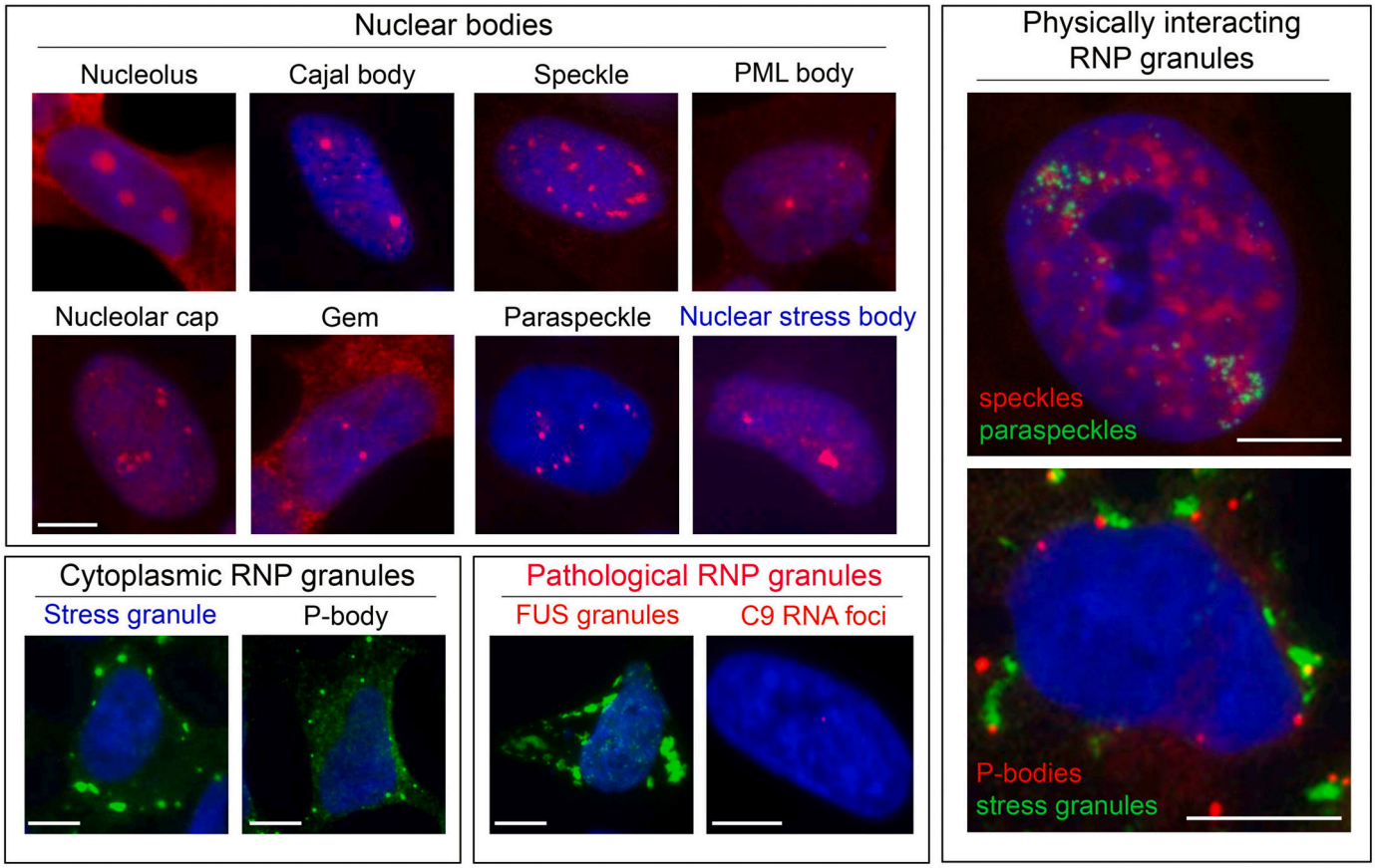

Fig. 1. Localization and morphology of major RNP granules, as detected by fluorescent microscopy of respective protein and RNA markers.
Major physiological and pathological RNP granules are shown. Two pairs of RNP granules known to localize in close proximity and interact with each other (SGs/P-bodes and speckles/paraspeckles) are also shown.
Markers used to detect RNP granules are as follows: nucleolus (ethidium bromide); nucleolar cap (CREST); Cajal body (colin p80); Gem (SMN); speckle (MALAT1); paraspeckle (NEAT1); PML body (PML); nuclear stress body (TDP-43); stress granule (G3BP1); P-body (Dcp1a); FUS granules (FUS); *C9ORF72* RNA foci (C9ORF72). Nucleus is visualized with DAPI in all panels. Scale bars, 5 μm.
All images are for neuroblastoma SH-SY5Y cells, except the images for speckle/paraspeckle co-localization and RNA foci, which were taken in fibroblasts. Image for RNA foci is reproduced with permission from [126].
Black and blue fonts denote constitutive and stress-induced RNP granules, respectively; pathological RNP granules are in red font.

their individual protein components were discovered one by one and often serendipitously. Others were identified based on the interaction with known components or using imaging-based screening. For example, the majority of SG proteins currently used as SG markers were identified using the candidate approach [46]. For RNP granules nucleated by a single type of transcript – paraspeckles and nSBs – RNA-centric approaches were successfully applied, e.g. capture hybridization analysis of RNA targets (CHART) or chromatin isolation by RNA purification (CHIRP) coupled with mass spectrometry analysis [47,48]. Recently, affinity purification and proximity-based labelling approaches have been utilized to comprehensively characterize the composition of SGs and P-bodies [10,49–52]. Systematic cataloguing of RNP granule proteins has unveiled striking compositional overlaps between different RNP granule types and pointed to an extensive component exchange within the RNP granule network. Below, we provide an overview of proteome overlaps for the major RNP granules, highlighting the cross-granule proteins whose function in more than one granule type is supported by functional studies. All proteomes discussed and their comparisons are available in Table S1.

5.1. Crosstalk between nuclear bodies

It can be inferred that a certain basal, "housekeeping" level of communication is in place for the majority of nuclear bodies under normal conditions, e.g. the flow of snRNPs from Cajal bodies to speckles. Upon stimulation, including stress, this crosstalk is overhauled and tuned to meet the needs of a new cellular state.

5.1.1. Nucleolus

The nucleolus can be purified with relative ease, which enabled its analysis early on, and since then, the nucleolar proteome has been expanded significantly, to include over 1300 proteins [53,54]. The bulk of nucleolar proteins are involved in rRNA processing, ribosome biogenesis and transcription. However, a recent spatiotemporal analysis has reported that up to 87% nucleolar proteins are additionally found in other cellular compartments, including ~50% within subnuclear structures, – presumably, other nuclear bodies [53].

Although Cajal bodies/Gems have not been purified and proteomically profiled, they are known to be highly enriched in small nuclear RNPs (snRNPs) and their associated proteins. Initially termed "nucleolar accessory bodies", Cajal bodies are frequently found at the nucleolar periphery and share a number of protein and RNA components with nucleoli. In particular, Cajal bodies were found to contain typical nucleolar proteins, such as fibrillarin, nucleolin, Nopp140 and dyskerin, whereas established Cajal body components coilin, CRM1 and SUMO1 are also detected within nucleoli [14]. This tight connection is in line with the joint functions of these RNP granules in processing and/or assembly of snRNPs and small nucleolar RNPs (snoRNPs) [14,30].

The primary PML body component, PML protein, accumulates in the fibrillar and granular regions of the nucleolus under the conditions of proteasome inhibition, alongside other PML body proteins such as SUMO1 and Sp100 [55].

Transcriptional inhibition leads to the segregation of many nucleolar proteins as well as proteins resident in Cajal bodies (coilin), PML bodies (PML) and paraspeckles (SFPQ, hnRNP K, PSPC1, NONO) into so-called 'nucleolar caps' on the surface of the nucleolus, whereas proteins resident in splicing speckles are excluded from these cap-like structures [56]. In fact, the ability to redistribute to nucleolar caps can be used as one of the criteria for protein classification as a paraspeckle component [57].

5.1.2. Splicing speckles and paraspeckles

Speckles, previously referred to as "interchromatin granule clusters", represent a collection of smaller granules, which were biochemically purified with subsequent analysis of their protein composition [58]. Recently, the spectrum of speckle-recruited proteins has been expanded using CHART coupled with mass spectrometry analysis of proteins bound to MALAT1, a reliable marker of speckles [47]. Speckles and paraspeckles are often found next to each other (see Fig. 1). In the same study, CHART was used to profile RBPs bound to NEAT1 and hence associated with paraspeckles. Overall, this study found that paraspeckles and splicing speckles share 25 proteins accounting for 83.3% and 67.6% of their proteomes, respectively.

Speckles are also in constant component exchange with Cajal bodies. Once snRNPs are generated, modified and matured in Cajal bodies, they relocate to speckles for further assembly steps, before undergoing the final assembly into the spliceosome on nascent mRNAs [59,60].

Most recently, a connection between speckles and nSBs has been reported [48]. A significant overlap between the nSB proteome and that of speckles was revealed: 63 out of 224 nSB proteins (28.1%) were found to localize to splicing speckles. Predictably, 59 nSB proteins (26.3%) were also paraspeckle components, and 41 proteins (18.3%) were shared among nSBs, splicing speckles and paraspeckles. Multiple SR protein family members are known to reside in speckles under basal conditions, and the above study found that they translocate to nSBs during thermal stress. Moreover, the speckle-localized CDC like kinase 1 (CDK1) was found to be recruited to nSBs to phosphorylate SR proteins, thereby promoting intron retention and contributing to gene expression regulation [48].

Subunits of the SWI/SNF chromatin-remodelling complex, e.g. BRG1, BRM and BAF155, are shared by paraspeckles and nSBs and are important for the integrity of both nuclear bodies [61]. These proteins maintain interactions between essential paraspeckle proteins such as SFPQ and RBM14 and facilitate paraspeckle biogenesis. Another established paraspeckle component, TDP-43, is also enriched in thermal stress-induced nSBs [62]. Since paraspeckles and nSBs are triggered by the same stressors [63], the requirement of SWI/SNF subunits for the biogenesis of both nuclear bodies seems somewhat counter-intuitive. However, nSBs form early during stress (e.g. 1-h arsenite treatment) [63], whereas paraspeckle assembly is a late event during stress (3 h into recovery from arsenite) [15]. Thus the processes of nSB and paraspeckle assembly are temporally separated, ensuring little or no competition for the SWI/SNF assembly factors between the two nuclear bodies. Furthermore, since TDP-43 is a negative regulator of paraspeckle formation [64], its recruitment to nSBs may alleviate its inhibitory effect on paraspeckles and contribute to paraspeckle hyper-assembly during the recovery phase.

5.2. Interaction between cytoplasmic RNP granules

5.2.1. Stress granules and P-bodies

The physical link between the two has long been known, since P-bodies are found attached to, or docked with SGs [65] (see Fig. 1). Comparison of published SG and P-body proteomes [10,49–52] showed that 101 proteins are shared between the two structures, which accounts for 51.8% and 12.5% of P-body and SG proteomes, respectively. Both RNP granules recruit proteins traditionally used as markers for either type of granule, e.g. SG markers Staufen2 and ELAVL1, or P-body markers Pat1b, Dcp2 and Upf1/2. Translationally stalled mRNAs are also present in both RNP granules [65]. Although SGs and P-bodies co-exist in the cytoplasm during stress, stress-specific protein component redistribution between the two does take place. For example, heat shock induces translocation of eIF4E, Xrn1, TTP, APOBEC3G, MOV10, Ago2, and YBX1 proteins from P-bodies to SGs [65,66]. Likewise, a P-body component nuclear RNA export factor NXF7 translocates to SGs upon arsenite stress [67]. An Sm-like protein RAP55 also shuttles between SGs and P-bodies to promote targeting of irreversibly damaged mRNA from SGs to P-bodies for degradation [68]. TTP protein travels between the two granules in association with AU-rich element (ARE)-containing mRNPs in a transportin-dependent manner [69]. Live imaging of arsenite-treated cells revealed that PCBP2, a facilitator of IRES-mediated translation, shuttles between P-bodies and SGs, suggesting its role in the stress-induced rearrangement of mRNPs [70]. Originally it was postulated that translationally stalled mRNAs cycle between P-bodies, SGs and polysomes enabling mRNA triage [71] however, recent single-molecule imaging studies have revealed that only few mRNAs directly move between SGs and P-bodies [72].

5.2.2. Stress granules and RNA transport granules

RNA transport granules represent a very heterogeneous group; their compositional profile largely depends on the purification approach [27]. Proteomic analysis of large RNP particles purified from mouse or rat brain tissue established the major components of neuronal RNA transport granules [16,73]. Out of 79 proteins recorded in the two datasets, 67 proteins (76%) are also found in the SG proteome, among which are the two core SG proteins G3BP1 and PABP1. Several typical RNA transport granule proteins, e.g. Staufen1, FMRP and Pumilio 2, contribute to SG maintenance, such that their depletion impacts SG integrity and dynamics [74]. A recent study reported that the de-ubiquitylating enzyme OTUD4 is a novel RNA transport granule component [75]. Upon heat shock or oxidative stress, it is recruited to SGs and its loss results in smaller but more numerous SGs. Similar to SGs, RNA transport granules are found docked with P-bodies in rat hippocampal neurons [76], and these two granule types share at least 15 proteins [16,51,73].

5.3. Nuclear-cytoplasmic connections

Several lines of evidence support the existence of a regulatory and functional relationship among virtually all RNP granules, including those separated by the nuclear membrane. Some RNP granule components exhibit dual nucleo-cytoplasmic distribution and reside in RNP granules in both subcellular compartments. Others localize to one compartment/granule type under basal conditions but drastically change their location and RNP granule residency in stressed cells. Many stress states are characterized by the assembly of large cytoplasmic MLOs, SGs, coupled with radical reshuffling of the basal RNP granule connections and intensified component flux between granules. In some instances, component relocation does not visibly affect the RNP granule (i.e. its size and numbers remain unchanged); in other cases, it leads to dramatic changes, e.g. complete dissolution of one RNP granule with concomitant enlargement or multiplication of the other. Examples of interaction and component exchange between RNP granules separated by the nuclear membrane are given below and in Fig. 2.

5.3.1. Stress granules and nucleolus

The most recent nucleolar proteome [53] includes 81 SG components, accounting for 6.1% and 10.0% of the nucleolar and SG proteomes, respectively. Unsurprisingly, the majority of shared proteins (64 out of 81) are located outside of the dense fibrillar center, which

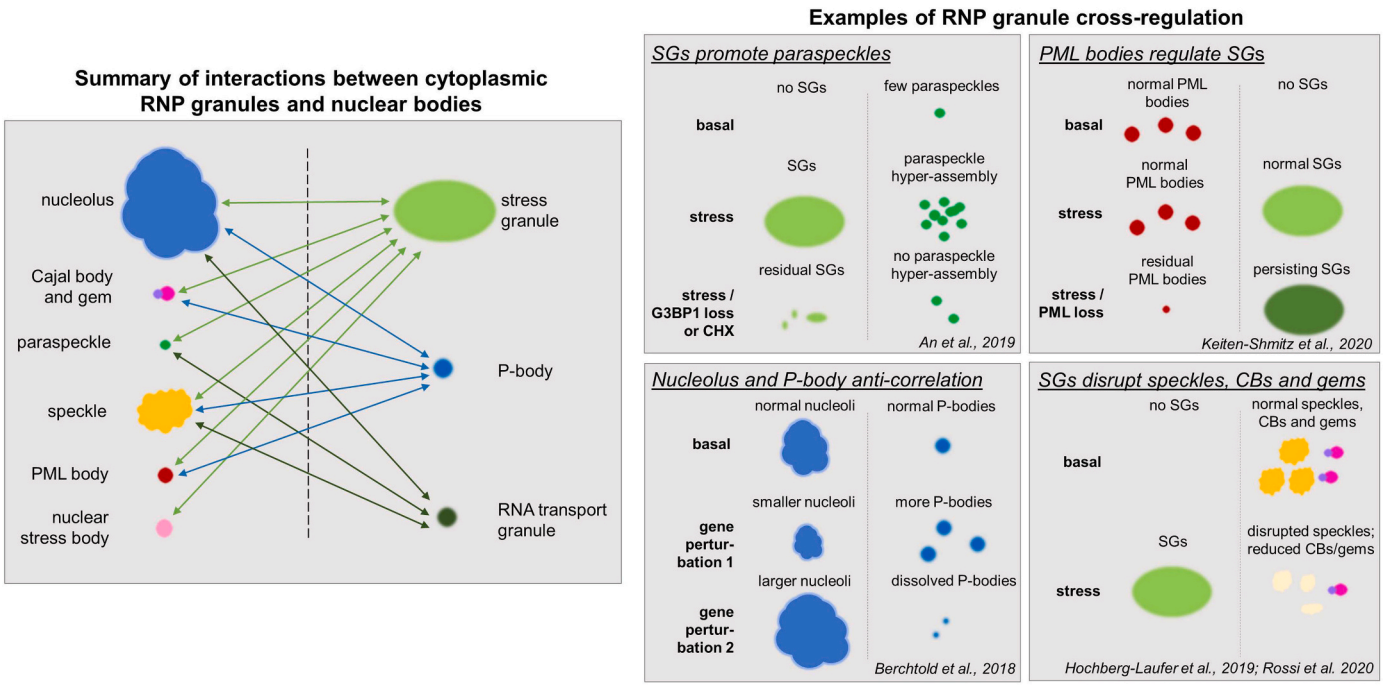

Fig. 2. Crosstalk between spatially separated RNP granules.
Left, component exchange between nuclear bodies and cytoplasmic RNP granules.
Right, examples of cellular contexts when one RNP granule controls or significantly modulates another RNP granule. CB, Cajal body; CHX, cycloheximide.

seemingly makes them more accessible and mobile. Both SGs and nucleoli recruit a number of factors involved in translation (small ribosomal subunit proteins, eIF2α and eIF3A), RNA splicing factors (hnRNP K, hnRNP A1, Sam68, SRSF3) and RNA helicases (DDX1, DDX2, DDX3), alongside other proteins important for RNA metabolism and protein post-translational modifications [77]. Four conserved components of H/ACA snoRNP complexes resident in nucleoli and Cajal bodies – dyskerin, Nhp2, Nop10 and Gar1 – are recruited to SGs and P-bodies [78]. Stress Granule and Nucleolar Protein (SGNP, aka SPATS2L-like) is another protein that localizes both to nucleoli and SGs, as its name suggests. Under oxidative stress, SGNP leaves the nucleolus and translocates to the cytoplasm, where it incorporates into SGs. This change causes a dramatic 5.8S rRNA increase in the nucleolus, therefore recruitment of SGNP into SGs modulates 5.8S rRNA processing or export during stress [79].

5.3.2. *Stress granules, paraspeckles and speckles*

Recently, our group described an unexpectedly tight connection between SGs and paraspeckles, in which paraspeckle assembly is dependent on the formation of microscopically visible SGs [15]. Comparison of paraspeckle and SG proteomes revealed a significant overlap between the two (~50% of paraspeckle proteins are also SG components). Subsequent cellular experiments established an important role for SGs in enabling paraspeckle hyper-assembly at the late stages of stress, whereby pharmacological or genetic inhibition of SG formation prevented efficient paraspeckle formation. Mechanistic studies suggested that sequestration of negative regulators of paraspeckles into SGs may be one of the molecular mechanisms behind this effect [15,80].

SGs significantly modulate the integrity of speckles under certain conditions, which is achieved through RNA rather than protein redistribution [20]. Polyadenylated mRNA, the primary speckle component, is sequestered into SGs in cells treated with an adenosine analogue, tubercidin; this causes speckle disappearance and release of speckle marker proteins such as SRSF2 into the nucleoplasm [20]. Speckles and SGs also share multiple protein components; over a quarter of reported speckle proteins (39/152) are present in the SG core protein dataset which includes proteins that are most tightly associated with SGs [10,58]. It should be noted however that the majority of these shared proteins are also paraspeckle components. An example of a protein with dual SG-speckle localization is HspB1, which is a predominantly cytoplasmic protein sequestered into both SGs and speckles in cells subjected to heat shock [81,82].

5.3.3. *Stress granules and Cajal bodies/Gems*

Arsenite-induced SG assembly leads to reduced numbers of both Cajal bodies and Gems [21]. This profound effect is likely due to the translocation of snRNPs from Cajal bodies to the cytoplasm where they are recruited to SGs. Dissolving SGs with cycloheximide was sufficient to prevent Cajal body collapse in stressed cells [21]. SMN, the main component of Gems, is also recruited to SGs [83], which may decrease its nuclear pool and thereby dismantle Gems in stressed cells.

5.3.4. *Stress granules and PML bodies*

The SUMO-targeted ubiquitin ligase (STUbL) pathway is a part of the nuclear protein quality control machinery which enables the removal of target proteins through sequential SUMOylation and ubiquitylation [84]. This process is confined to the nucleus and PML bodies in particular [85]. Recently, the STUbL pathway has been found to control SG dynamics. In stressed cells, a RING-type ubiquitin ligase RNF4 that catalyses ubiquitylation of SUMOylated proteins binds and ubiquitylates multiple RBPs, which translocate to the cytoplasm and incorporate into SGs. RNF4 depletion or inhibition of the STUbL activity delays SG disassembly, and this effect can be recapitulated by disruption of PML bodies [86].

5.3.5. *P-bodies and nuclear bodies*

The majority of P-body components are predominantly cytoplasmic proteins, yet some of them shuttle between the two cellular compartments, including Pat1b, CPEB-1, eIF4E, and 4E-T [17,87]. In the nucleus, Pat1b concentrates in splicing speckles and in nuclear foci partially overlapping with PML bodies [17]. Recently, Pat1b has been also identified in Cajal bodies in a complex with the nuclear Lsm2-8

heptamer and tri-snRNP (U4/U6.U5), and its absence affected alternative splicing [88]. Similarly, SG/P-body proteins eIF4E and DDX6 are also able to translocate to the nucleus and localize to speckles/PML bodies and speckles, respectively [89–91]. A recent systems-level study revealed an intimate connection between P-bodies and the nucleolus [92]. Knockdown of certain genes led to enlarged nucleoli with concomitant P-body dissolution, while downregulation of others increased P-body numbers but negatively affected the nucleolus [92].

5.3.6. RNA transport granules and nuclear bodies

RNA transport granules demonstrated a striking enrichment in structural paraspeckle components. Out of 79 established RNA transport granule proteins, 38 (48.1%) appear in the paraspeckle proteome, with nearly all essential paraspeckle proteins included (SFPQ, NONO, FUS, hnRNP A1, hnRNP K, EWS) [16,57,73]. Furthermore, 28 RNA transport granule proteins are speckle components, and eight are nucleolar proteins, including nucleolin. Consistently, profiling of interacting partners of SYNCRIP, one of the major RNA transport granule proteins, in rat neurons identified several established components of speckles (SRSF2, SRSF6, SRSF7, hnRNP H), as well as nucleolin and another nucleolar marker protein, nucleophosmin [93].

6. Who regulates whom: molecular mechanisms maintaining communication between RNP granules

For RNP granules with physical contacts, e.g. paraspeckles and speckles, or SGs and P-bodies, efficient component exchange and hence granule crosstalk are enabled by their immediate interaction. Conceivably, communication between spatially separated RNP granules should be mediated by signal transduction; however the respective regulatory pathways are yet to be elucidated. A recent systematic analysis of genes whose knockdown alters the integrity and abundance of six RNP granules (nucleoli, Cajal bodies, speckles, PML bodies, P-bodies, and SGs) revealed that these granule types are controlled by distinct as well as shared signalling pathways [92]. For example, genes with splicing-related functions were found to modulate both SG assembly and nucleolar morphology. Signalling networks that underlie such RNP granule correlations supposedly bridge nuclear bodies and cytoplasmic RNP granules and integrate them into a network. Signalling pathways that have emerged as mediators of the RNP granule crosstalk are discussed below.

6.1. Molecular chaperones

Chaperones and their regulatory proteins are known factors in RNP granule remodelling during stress. A major heat-shock protein Hsp70 accumulates in nucleoli and maintains their organization and function in cells subjected to heat stress [94]. During the recovery phase, Hsp70 translocates to the cytoplasm to facilitate SG disassembly [95]. Inhibition of Hsp70 activity or its knockdown significantly delays dissolution of heat-induced SGs and re-initiation of translation [96]. Conversely, cultured cells overexpressing Hsp70 fail to assemble SGs upon proteasome inhibition [96,97]. One can speculate that the nucleolus modulates cycles of SG assembly/disassembly by controlling the availability of Hsp70 in the cytoplasm. HSF1, an upstream regulator of Hsp70 and other heat shock proteins, is a known nSB component [98]. Under basal conditions, HSF1 is repressed and associated with chaperones such as Hsp70, Hsc70 and Hsp90; in stressed cells, it is released from these chaperone complexes and becomes activated, acquiring transcriptional activity [98]. The build-up of HSF1 within nSBs coincides with Hsp70 accumulation in the nucleolus [99], and may also play a role in Hsp70-mediated turnover of SGs. Interestingly, HSF1 contributes to the expression of architectural lncRNAs for two nuclear bodies, paraspeckles and nSBs (NEAT1 and satIII, respectively) [100,101], providing yet another level of RNP granule regulation by the chaperone system.

6.2. Mammalian target of rapamycin (mTOR)

During stress, the mTOR pathway is central to controlling the translation of stress-related proteins [102]. Under basal conditions, raptor protein, a component of the mTOR complex 1 (mTORC1), is localized to the nucleoli where it may regulate ribosome biogenesis [103]. However, during SG-inducing stresses, raptor is targeted to SGs to prevent apoptosis driven by mTORC1 hyper-activation [104,105]. Therefore, mTORC1 activity may help maintain proteostasis during stress through functional coordination between SGs and nucleoli.

6.3. Dual-specificity kinases

Several dual-specificity kinases are present both in the nucleus and cytoplasm and are recruited to RNP granules in both compartments. Such proteins are seemingly key to coupling phase separation and signal transduction and hence inter-granule communication. The most well-studied example is DYRK3, which is capable of phase transition and associates with several RNP granules. DYRK3 is an established SG component which also accumulates within splicing speckles in the nucleus [105,106]. When overexpressed, DYRK3 phase-separates and nucleates *de novo*, P-body like structures in the cytoplasm, but also leads to splicing speckle collapse [92,106]. It is plausible that these two events are linked, and sequestration of the bulk of speckle components to these cytoplasmic DYRK3-rich granules is the primary cause of speckle disassembly. Chemical inhibition of DYRK3 promotes its accumulation in speckles and prevents its exit from SGs and their dissolution [92,106], suggesting that kinase activity of the protein is required for its modulatory effect on RNP granules. Interestingly, DYRK3 was shown to mediate mTORC1 sequestration into SGs and hence mTORC1 signalling during stress [105]. Another member of the class, DYRK1A, is also a speckle component and controls speckle integrity via its kinase activity [107] however, possible coupling with cytoplasmic RNP granule assembly/disassembly for this kinase is yet to be established.

Two members of another class of dual-specificity kinases, HIPK1 and HIPK2, are intimately connected to PML bodies. Their overexpression causes depletion of PML body components such as Sp100, but not PML itself [92,108,109]. Given that PML bodies regulate SG dissolution and prevent SG persistence [86], the regulatory activity of HIPKs might be one of the molecular mechanisms that maintain the communication between PML bodies and SGs.

CLK1 is a multifaceted kinase localized to nuclear speckles under basal conditions, where it phosphorylates the key splicing factors - SRSF proteins - to control their activity [110]. In addition, CLK1 is recruited to thermal stress-induced nSBs, and this event is crucial for re-phosphorylation of the SRSF9 protein and enhanced intron retention in a spectrum of transcripts during the recovery phase [48]. Therefore, cellular response to thermal stress is characterized by coordinated CLK1 redistribution between speckles and nSBs. CDKs are also important in the nucleolus maintenance [111]. CDK1 shuttles between the nucleus and cytoplasm, but less is known about the role of CDK1 in cytoplasmic granules. Given the role of the nucleolus in SG turnover [94] and inverse correlations between nucleoli and P-bodies [92] as well as SGs and speckles [20], it would be important to investigate possible CLK1 roles in the crosstalk between nuclear and cytoplasmic RNP granules.

Importantly, the above kinases possess IDRs required for their efficient partitioning into RNP granules. For example, the N-terminal domain of CLK1 can be considered an IDR because of the low sequence complexity, and it is essential for CLK1 recruitment to nSBs [48]. IDRs therefore may govern the function of dual-specificity kinases in the RNP granule crosstalk by enabling their association with these structures.

7. RNP granule network disruption as a driver of cellular pathology in human disease

RNP granule dysfunction is typical for many neurodegenerative

disorders, cancer and viral infections. Mutations in multiple RNP granule components lead to neurodegenerative diseases, most prominently ALS [36], and cause some types of cancer [112]. Viruses can subvert cellular RNA metabolism for their own needs, and their interactions with SGs are well-established [113]. Below we will focus on ALS as a prototypical disease originating from disrupted RNP granule homeostasis.

Mutations in over 30 genes cause ALS, and over a half of them code for RNP granule components or modulators of RNP granule assembly [36]. Many of these proteins are found in more than one RNP granule type, as summarized in Table 2. On top of their negative effect on physiological RNP granules, some ALS mutations cause formation of *de novo* RNP granules, whose primary component is either an abnormal RNA or mutant protein, i.e. expanded repeats in the *C9ORF72* gene or mutant FUS protein, respectively, as described in Sections 4.1 and 4.2. Effects of these *de novo* RNP granules on physiological RNP granules are also described below and in Table 2.

7.1. Initiation and spread of pathology within the RNP granule network in ALS

Although the majority of ALS-linked mutations are expected to have a pleiotropic effect on the RNP granule network, some types of granules become affected more significantly and earlier than others. Since a large proportion of ALS-associated proteins are SG proteins and because SGs are large structures that sequester multiple components and modulators of other RNP granules, the impact of compromised SG metabolism on the rest of the network would be dramatic. At least twelve ALS-linked mutants have a confirmed negative effect on SGs, with the majority being RBPs (Table 2). In contrast, effects on RNP granules other than SGs have been investigated and reported only for a limited number of ALS proteins (Table 2).

7.1.1. TDP-43 (TARDBP)

Mutations and/or abnormal localization of TDP-43 protein affect several RNP granule types, namely paraspeckles, Gems, nSBs, RNA transport granules, and SGs, presumably leading to a profound dysregulation of the RNP granule network. TDP-43 loss of function (LoF) is typical for ~95% of ALS cases, where the protein is cleared from the nucleus and aggregated in the cytoplasm [42]. Since TDP-43 is a negative paraspeckle regulator [64], its LoF presumably contributes to paraspeckle hyper-assembly in ALS motor neurons; however, the effect of ALS mutations in TDP-43 on paraspeckles is yet to be investigated. Both TDP-43 LoF and presence of mutant protein negatively affect Gems [114,115]. TDP-43 is a confirmed nSB component however, common ALS-linked TDP-43 mutants did not visibly interfere with its recruitment to these RNP granules [62], and it is currently unclear whether TDP-43 LoF changes nSB integrity and/or properties. Cells expressing mutant or cytoplasmically accumulated TDP-43 form larger and less dynamic neuronal RNA transport granules [116]. Since these granules sequester

Table 2
ALS-linked RNP granule components and the effect of abnormal/mutant variants on major RNP granules.

Protein	Primary molecular pathway	RNP granule component	RNP granules affected by protein dysfunction	Main ref.
RNA and protein products of repeat expansions in *C9ORF72* gene	–	- De novo RNP foci in the nucleus (repeat-containing RNA) - De novo DPR protein aggregates (MLOs other than RNP granules)	- Nucleolus (DPR proteins/RNA foci; disruption) - SGs and P-bodies (DPR proteins; disruption) - Paraspeckles (DPR proteins; accumulation) - Speckles (DPR proteins/RNA foci; disruption) - Cajal bodies (DPR proteins; disruption)	[135,137–139]
FUS	RNA metabolism	- Paraspeckles (core protein) - SGs - RNA transport granules - De novo cytoplasmic FUS assemblies (mutants)	- SGs (mutant protein has higher affinity; disruption by de novo FUS aggregates) - Paraspeckles (increased formation but dysfunctional); - Gems and Cajal bodies (disruption)	[21,26,41,114,119]
TDP-43	RNA metabolism	- Paraspeckles (negative regulator) - SGs - RNA transport granules - Nuclear stress bodies	- SGs (reports of effects inconsistent) - Paraspeckles (TDP-43 loss - increased formation) - Gems (disruption) - RNA transport granules (enlarged, impaired dynamics and functionality)	[41,62,64,114,116,154]
VCP/p97	Protein degradation	- SGs	- SGs (impaired clearance)	[121,122]
Ubiquilin-2	Protein degradation	- SGs	- SGs (may affect dynamics)	[155]
hnRNP A1	RNA metabolism	- Paraspeckles (structural component) - SGs - RNA transport granules	- SGs (altered dynamics) - Paraspeckles?	[156]
hnRNP A2/B1	RNA metabolism	- SGs - RNA transport granules	- SGs (altered dynamics)	[156]
Matrin-3	RNA metabolism	- Paraspeckles (negative regulator)	- Paraspeckles (MATR3 loss –increased formation)	[157]
Ataxin-2	RNA metabolism	- SGs - RNA transport granules	- SGs (structural component) - P-bodies (elevated levels exert negative effect)	[158]
TAF15	RNA metabolism	- Paraspeckles (structural component) - SGs	- SGs and paraspeckles?	[159]
EWS1	RNA metabolism	- Paraspeckles (structural component) - SGs	- SGs and paraspeckles?	[160]
TIA1	RNA metabolism	- SGs (structural component)	- SGs (altered dynamics)	[161]
Angiogenin	RNA metabolism	- SGs	- SGs (decreased assembly)	[162]
Profilin-1	Cytoskeleton organization	- SGs	- SGs (altered dynamics)	[163]
SFPQ	RNA metabolism	- Paraspeckles (structural component) - RNA transport granules	- Paraspeckles?	[164]
CREST	Chromatin remodelling	- Paraspeckles - SGs	- No effect on SGs or paraspeckles	[165]

multiple components of nuclear bodies [16,73,93], the latter can become disrupted, especially with long-term persistence of larger and less mobile RNA transport granules. Reports on how TDP-43 LoF or mutations affect SGs are inconsistent, with the only consensus being that TDP-43 dysfunction is damaging for SG homeostasis [117]. Interestingly, TDP-43-containing RNA transport granules found in neuronal processes are also positive for SG markers and co-occur with P-bodies [116]. Therefore, RNA transport granules may represent "precursors" of SGs in neurons that, if they contain mutant TDP-43, can affect SG formation. Disrupted sequence of RNP granule assembly during stress in cells exhibiting TDP-43 LoF may be of primary pathological significance. At least in cultured cells, the assembly nSB and paraspeckle occurs early and late during stress, respectively [15,48]. However, ALS neurons would enter a stress state with a pre-activated paraspeckle response, which may disrupt stress-specific reshuffling of the RNP granule network and result in inadequate stress signalling. Further research is warranted to determine the consequences of such "primed" state for neurons.

TDP-43 has been recently reported to form a distinct type of nuclear bodies ("TDP-43 NBs") in arsenite-stressed cells, a fraction of which contain the core paraspeckle lncRNA NEAT1 [118]. Since TDP-43 is found both in nSBs and paraspeckles [57,62], and arsenite stress induces both nuclear bodies [15,63], TDP-43 NBs can represent a heterogeneous group comprised of both granule types. In addition, Gems contain TDP-43 [114] and may also account for a fraction of the TDP-43 NBs. The same study reported that an ALS-linked mutation in one of the TDP-43 RRMs (D169G) decreases the protein's phase separation capacity and its partitioning into nuclear bodies. At the same time, it promotes its cytoplasmic localization and SG incorporation [118]. This result points to unequal and even differential effects of certain mutant variants on nuclear and cytoplasmic granules, and indeed, a very complex effect on the RNP granule network as a whole. It would be important to test the differential impact of common mutations in the TDP-43 IDR domain [42] on its partitioning into nuclear and cytoplasmic RNP granules.

7.1.2. FUS

Similar to TDP-43, the presence of FUS mutants leads to paraspeckle accumulation [119] as well as disruption of Cajal bodies and Gems [21] in unstressed cells, but also impaired SG dynamics [43,117,120]. In stressed cells, SG dysfunction can lie upstream of the effect on Cajal bodies/Gems and paraspeckles, since mutant FUS was found to form larger SGs [43,117,120]. Sequestration of increased amounts of negative paraspeckle regulators into these enlarged SGs would be predicted to enhance paraspeckle accumulation and deplete Cajal bodies and Gems.

7.1.3. Other ALS-linked proteins

Experimental evidence on the impact of ALS-associated mutant versions on RNP granules beyond FUS and TDP-43 proteins is still very scarce. Mutations in paraspeckle/SG proteins hnRNP A1, TAF15, EWS and SFPQ were identified in a small proportion of ALS cases [25], however it is still not known whether and how paraspeckles and SGs are affected by these pathogenic variants. Mutant proteins other than RBPs can also affect SG homeostasis in ALS, as exemplified by VCP/p97. This protein is required for efficient SG clearance post-stress, while ALS mutants are deficient in this pathway [121]. Interestingly, SUMOylation of VCP facilitates its localization to SGs, whereas SUMOylation of its mutants is altered [122]. Given the role of PML bodies in protein SUMOylation and SG turnover [86], it has to be investigated whether the deficiency of mutant VCP in SG clearance originates, at least in part, from its inefficient modification within PML bodies.

RNP granule components for which ALS mutations have not been reported can be affected in other ways, e.g. by abnormal protein depletion or aggregation and resultant LoF. For instance, level of hnRNP K, a nucleating paraspeckle protein [57], is reduced in ALS caused by TDP-43 mutations [123]. Depletion of this protein may compromise protective paraspeckle assembly in this disease subtype. Recently, we found that UBAP2L, a core SG protein [49,50] and a negative paraspeckle regulator [57], is downregulated or aggregated in spinal motor neurons in familial and sporadic ALS [15]. This phenotype is predicted to lead to loss of SG function with concomitant paraspeckle hyper-assembly.

Overall, the pleiotropic effect of ALS-causative variants on the RNP granule network dictates the need for research into the relative impacts of these proteins on different RNP granule types and on the temporal patterns of their assembly during stress. Detailed understanding of the primary cause of network disruption in different ALS subtypes can inform therapeutic developments targeting the most relevant RNP granule type in each subtype.

7.2. Impact of ALS-linked de novo RNP granules on the RNP granule network

7.2.1. De novo FUS granules and their assemblies

In our early studies, using the candidate-based approach, we found that FUS aggregates sequester RBPs – components of P-bodies (Dcp1a), SGs (TIAR, G3BP1) and RNA transport granules (DDX5) – thereby depleting their functional pool and causing disruption of respective physiological RNP granules [26]. Our recent proteomic analysis of affinity-purified FUS assemblies induced by arsenite identified 487 proteins, and this dataset was highly enriched in the components of RNP complexes (Cellular Component GO1990904: Ribonucleoprotein complex) [124]. Nearly half of these proteins are also known SG proteins, and 7.5% – P-body proteins, including decapping complex components (Dcp1a, Dcp1b and Edc4), translational repressor Pumilio 1, and nonsense-mediated decay factors Upf1 and NCBP1. Furthermore, cytoplasmic FUS assemblies sequester major RNA transport granule proteins SYNCRIP, Staufen1, FMRP, hnRNP D, and RBM3. These assemblies were also found to contain multiple components of nuclear bodies, including structural paraspeckle proteins DAZAP1, EWS1 and PSPC1, as well as the Gem-specific protein SMN. FUS assemblies are very large cytoplasmic structures; even those made of endogenous protein are comparable in size with SGs [43]. Therefore they are expected to trap the above proteins in quantities sufficient to exert a negative functional effect on other RNP granules in stressed cells.

7.2.2. Abnormal C9ORF72 gene products

Abnormal products of the *C9ORF72* gene are predicted to affect the RNP granule network at several levels. Sense and antisense repeat-containing RNAs transcribed from the locus form nuclear foci that are positive for a number of RBPs – RNP granule components. These RNA foci are enriched in SRSF1 and SC35, the marker proteins of speckles, with splicing perturbed in foci-containing cells [125]. Multiple splicing factors were also identified as G4C2 expanded RNA interactors in another study [126]. The major nucleolar protein nucleolin was found to preferentially bind to the G-quadruplex formed between G4C2 repeat DNA and RNA, where repeat RNA expressing cells showed signs of nucleolar stress [127]. Pur-α, one of the core components of neuronal transport granules [16] and modulator of SG formation [128], has been detected in G4C2 repeat RNA foci in a number of studies [129]; although experimental evidence for altered integrity of SGs or RNA transport granules in these cells is yet to be obtained. Several other well-established SG proteins, including YBX1, ELAVL1, PABP1, hnRNP A1, TDP-43 and FMRP, were detected in *C9ORF72* foci [130,131]. Paraspeckle biogenesis and/or maintenance factors SFPQ, NONO, RBM14 and FUS can be hijacked by G4C2 RNA to build "paraspeckle-like structures"; presumably, paraspeckle integrity is affected by entrapment of these proteins in the foci [132].

The presence of RNA foci on its own is likely not sufficient to cause the disease, and protein products of repeat-containing transcripts – dipeptide repeat (DPR) proteins – become an additional base point for spreading the pathology to other nodes of the RNP granule network. Interestingly, SRSF1, a major component of speckles, plays a crucial role

in the transport of pathological *C9ORF72* transcripts from the nucleus and hence DPR production [133]. DPRs build up both in the nucleus and cytoplasm of neurons [134] and undergo phase separation [135], leading to widespread disruption of RNP granules. In particular, DPRs may force the assembly of less dynamic SGs in cultured cells [135]. Furthermore, a mouse model exhibiting DPR protein pathology is characterized by aberrant deposition of SG components G3BP1, Ataxin-2, eIF3η and TIA1 within cytoplasmic DPR aggregates, and impaired SG dynamics [136]. Some DPR species are able to enter the nucleus and accumulate in the nucleolus, thereby compromising nucleolar dynamics and function [137]. Abnormalities in P-bodies, splicing speckles, Cajal bodies and paraspeckles were also evident in cells expressing DPR proteins [138,139]. In particular, the effect on Cajal bodies and splicing speckles may be mediated by the binding of DPRs to U2 snRNP and loss of this snRNP from the nucleus [140]. In addition to the entrapment of RNP granule proteins, DPR may undermine RNP granule structure and function by interacting with their architectural and other RNAs [139].

8. Conclusions and outlook

RNP granules are found in all eukaryotic organisms, from yeast to human; the processes they control are conserved and lie at the heart of cellular metabolism. Their studies have provided a discovery-rich haul and will continue doing so in years to come. While an integrated view of the RNP granule network is still lacking in the literature, the published experimental evidence makes a strong case for the existence of such cell-wide network regulated by numerous molecular mechanisms. A concerted effort is needed to systematically analyse this network and build its map, detailing the modes of inter-granule communication.

The following aspects of the RNP granule network regulation will likely become the focal points for research in the field in the upcoming years:

- *Molecular basis of RNP granule identity and autonomy.* Inclusion of specific protein and RNA components and exclusion of the others define the RNP granule type. However how exactly a granule with a specific composition (and hence function) is assembled, i.e. how the stoichiometry and hierarchy of its components is controlled, is yet to be understood. Most strikingly, despite extensive component exchange, the borders between physically interacting RNP granules do not become blurred, and granule types preserve their identity. In vitro, droplets readily fuse when they come into contact, whereas RNP granules in living cells do not. Factors maintaining the boundaries of RNP granules and hence their autonomy await further investigation.
- *Temporal patterns of RNP granule assembly and how they are affected in disease states.* It is still not clear how the cell controls the lifecycles of RNP granules. For example, the peak of paraspeckle assembly does not coincide with that of SG assembly [15]. Similarly, a clear negative correlation between the assembly of nucleolus and P-bodies has been reported [92]. Systematic analysis of functional cellular states that trigger, maintain, or dismantle different types of RNP granules should shed light on the rules governing temporal patterns in RNP granule assembly. This knowledge will ultimately translate into a better understanding of the disease states characterized by dysfunction of the RNP granule network.
- *Heterogeneity of RNP granules*. Further studies are needed to establish whether some RNP granules represent a heterogeneous collection of RNP granules currently known under an umbrella name. For instance, nSBs belong to two subtypes, nSB-M and nSB-S, containing either hnRNP M or SAFB protein [141]. Similarly, several morphological subclasses of paraspeckles have been described, e.g. globular and elongated ones, the latter being less dynamic [142]. Neuronal transport granules are a highly heterogeneous class of structures. A consensus needs to be reached on the classification, terminology and experimental approaches to the studies of RNP granule subclasses. New insights into RNP granule sub-categories should help understand the fine structure of the RNP granule network.
- *Role for post-translational protein modifications (PTMs) in the RNP granule crosstalk*. Multiple structural and functional aspects of the RNP granule biology and pathology are regulated by PTMs of their constituents and most prominently, RBPs and other IDR-containing proteins. Rapid and reversible changes elicited by PTMs that do not require changes in the primary protein structure were found to not only regulate protein's subcellular localisation but also differentially impact on its ability to associate with RNP granule types. A prominent example is the regulation of SG recruitment and nuclear export of FUS protein by methylation of arginine residues in its RGG domains; this PTM may also contribute to pathological FUS aggregation in neurodegenerative diseases [143]. Paraspeckles critically depend on FUS nuclear abundance [11], and SG size and dynamics are modulated by cytoplasmic FUS [120]. PTMs of proteins with dual nucleo-cytoplasmic distribution presumably play a significant role in the crosstalk between spatially separated RNP granules, including in disease states. They also represent a yet untapped resource for therapeutic targeting of RNP granule mis-communication in neurodegeneration and beyond.
- *Connections between the network of RNP granules and other MLOs.* In this review, we have focused on the RNP granule network comprising MLOs rich in RNA and protein. However, MLOs other than RNP granules also communicate both with RNP granules and with each other. For example, cilia, the protrusions found on the surface of cells and associated with centrosomes, are formed through LLPS and can be considered MLOs. Cilia have been found to actively exchange protein components with splicing speckles as well as SGs and P-bodies [144]. Likewise, multiple components of the nuclear pore complex (NPC) – macromolecular assembly that forms a channel across the nuclear envelope – can be sequestered into SGs [145]. Further exploration of structural and functional crosstalk between different classes of MLOs in healthy and diseased cells is warranted.

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Abbreviations

ALS	amyotrophic lateral sclerosis
ARE	AU-rich element
CHART	capture hybridization analysis of RNA targets
CHIRP	chromatin isolation by RNA purification
CLIP	cross-linking and immunoprecipitation
DPR	dipeptide repeat
FRAP	fluorescence recovery after photobleaching
FUS	Fused in Sarcoma
Gems	Gemini of Cajal bodies
IDR	intrinsically disordered region
LLPS	liquid-liquid phase separation
LoF	loss of function
lncRNA	long non-coding RNA
MALAT1	Metastasis Associated Lung Adenocarcinoma Transcript 1
MLO	membraneless organelle
mTOR	mammalian target of rapamycin
NEAT1	Nuclear Paraspeckle Assembly Transcript 1
NLS	nuclear localization signal
NPC	nuclear pore complex
nSB	nuclear stress body
P-body	Processing body
PML	promyelocytic leukaemia
PTM	post-translational modification
RBP	RNA-binding protein
RNP	ribonucleoprotein
SG	stress granule

SMN	survival motor neuron
snoRNPs	small nucleolar RNPs
snRNPs	small nuclear RNPs
STUbL	SUMO-targeted ubiquitin ligase

Declaration of competing interest

The authors have nothing to disclose.

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