POST-TRANSCRIPTIONAL CONTROL — OPINION

RNA granules: post-transcriptional and epigenetic modulators of gene expression

Paul Anderson and Nancy Kedersha

Abstract | The composition of cytoplasmic messenger ribonucleoproteins (mRNPs) is determined by their nuclear and cytoplasmic histories and reflects past functions and future fates. The protein components of selected mRNP complexes promote their assembly into microscopically visible cytoplasmic RNA granules, including stress granules, processing bodies and germ cell (or polar) granules. We propose that RNA granules can be both a cause and a consequence of altered mRNA translation, decay or editing. In this capacity, RNA granules serve as key modulators of post-transcriptional and epigenetic gene expression.

The central dogma of molecular biology states that genetic information flows from nucleic acids to proteins[1]. In modern organisms, DNA makes RNA and RNA makes protein, but DNA might be a relative newcomer to the party. In ancient organisms, enzymatic RNAs and the proteins that they produced might have determined the nature of simple cells and specified the next generation[2]. In this early RNA world, selection for RNA-binding proteins capable of homotypic or heterotypic adhesion might have been a key event that reversibly concentrated enzymatic RNAs at functional foci. In modern organisms, analogous foci that contain proteins, mRNAs and non-coding RNAs are found in the cytoplasm of somatic and germ cells. Specific components of these RNA granules can alter DNA and RNA sequences, repress transcription and translation, and transmit genetic information in a form of cytoplasmic inheritance. RNA granule-associated factors that modulate gene expression include RNA-editing enzymes (for example, APOBECs[3–6] and ADAR1; P.A. and N.K., unpublished observations) that alter the coding regions of mRNAs to produce proteins that are not encoded in the genome; transposon-derived RNAs and proteins (for example, RNA from LINE1 retrotransposons and its protein product ORF1) that reverse the DNA–RNA–protein equation by encoding RNAs that are converted into DNA and inserted into the genome[7]; Argonaute-like Piwi proteins and Piwi-interacting RNAs (piRNAs) that repress transposon expression[8,9]; and maternal mRNAs that transmit information by cytoplasmic inheritance during embryogenesis[10,11]. We contend that RNA granule-mediated post-transcriptional and epigenetic alterations in protein expression are remnants of an ancient RNA world that have key roles in the survival and development of modern cells.

> "...RNA granule-mediated post-transcriptional and epigenetic alterations in protein expression are remnants of an ancient RNA world..."

The mRNA life cycle

As mRNA exits the nucleus, the cap-binding protein 20 (CBP20)–CBP80 complex initiates a pioneering round of translation that strips off exon-junction complexes (multiprotein complexes that are deposited at exon–exon junctions during splicing), and CBP20–CBP80 is replaced by the major cytoplasmic cap-binding protein eukaryotic translation initiation factor 4E (eIF4E), which recruits the translation initiation complex[12]. The remodelled messenger ribonucleoprotein (mRNP) is a substrate for competing enzymatic complexes that determine its functional fate. Recruitment of the translation initiation complex promotes protein synthesis and the assembly of polysomes (a string of translating ribosomes bound to circularized mRNA)[13], whereas recruitment of the CCR4–NOT1 complex promotes deadenylation and, eventually, mRNA decay[14]. In many cases, however, nascent transcripts are packaged into distinct classes of RNA granules that ensure regulated translation and decay[15], and function at different phases of the mRNA life cycle.

Nascent transcripts that emerge from the nucleus of somatic cells are packaged into transport granules (or neuronal granules in neurons) that prevent translation and decay as they are delivered to specific sites in the cytoplasm[16]. In germ cells, nascent maternal transcripts are packaged into germ cell (or polar) granules that associate with the nuclear envelope and maintain their contents in a protected, dormant state until needed for developmental progression[17]. In somatic cells, disassembly of translating polysomes can drive the assembly of either processing bodies (PBs)[18] or stress granules (SGs)[19]. Here, we summarize what we have learned about the structure and function of different types of RNA granules.

Properties of SGs

When polysome disassembly is initiated by stress-induced translational silencing, stalled initiation allows translating ribosomes to run off the transcript, thereby producing circular polyadenylated mRNPs that can either aggregate to form SGs[19] or assemble into PBs (reviewed in REF. 20 and REF. 21) (FIG. 1). The composition of these mRNPs is a major determinant of SG assembly, as homotypic or heterotypic interactions between specific proteins drive the aggregation step. The translational silencers TIA1 and TIAR (among others) contribute to stress-induced translational arrest, polysome disassembly and aggregation of client mRNPs[22–24]. Overexpression of these proteins induces the assembly of SGs in the absence of stress[25], and cells that lack TIA1 exhibit impaired SG assembly[22]. The TIA proteins are not components of polysomes[26], indicating that they are recruited to the mRNP in response to stress, possibly to promote the assembly of stalled translation initiation complexes. TIA1 and TIAR possess Gln or Asn (Q/N)-rich prion-related domains at their carboxyl termini that mediate self-aggregation[22]. Molecular chaperones (for example, heat shock protein 70 (HSP70)) determine whether these domains assume the aggregation-prone conformation that allows mRNPs to aggregate at SGs[22].

GTPase-activating protein SH3 domain-binding protein (G3BP; also known as G3BP1) is another self-aggregating protein that regulates SG assembly[27]. G3BP and its molecular partner ubiquitin-specific processing protease 10 (USP10)[28] are

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polysome-associated proteins (P.A. and N.K., unpublished observations) that move with untranslated mRNPs to SGs (FIG. 1). USP10 is a deubiquitylating enzyme that works with G3BP to promote the starvation-induced degradation of large ribosomal subunits in yeast[29]. In mammalian cells, knockdown of either G3BP or USP10 impairs SG assembly, suggesting that deubiquitylation of an mRNP component facilitates SG assembly[30]. This process is regulated by the phosphorylation of G3BP[27], which inhibits its interaction with USP10 and impairs SG assembly (P.A. and N.K., unpublished observations). Interestingly, G3BP has other SG-associated partners, including caprin 1 and histone deacetylase 6 (HDAC6), which are also involved in SG assembly[31,32]. Like TIA1 and TIAR, overexpression of G3BP[17] (but not of many other SG-associated proteins[19]) nucleates SG assembly in the absence of stress, but the extent to which it requires its specific partner proteins remains to be determined.

Additional components of the mRNP that are required for SG assembly are the multisubunit translation initiation factor eIF3 (see Glossary) and multiple ribosomal proteins. Knockdown of most eIF3 subunits strongly inhibits SG assembly without affecting PB assembly[30]. Similarly, knockdown of individual large and small ribosomal subunit proteins strongly inhibits SG assembly and, in some cases, PB assembly. Stress-induced modification of ribosomal proteins with *N*-acetylglucosamine also promotes the assembly of SGs[30]. This modification does not affect stress-induced translational arrest but is required for the aggregation of untranslated mRNPs at SGs[30]. Finally, cytoplasmic polyadenylation element-binding protein (CPEB)[33] and tristetraprolin (TTP; also known as ZFP36) can nucleate the assembly of SGs and PBs and also promote interactions between these distinct classes of RNA granules[34] (see below). It remains to be determined whether eIF3, ribosomal proteins or CPEB are required for stress-induced translational arrest, aggregation of mRNPs, or both.

Properties of PBs

A number of excellent reviews describe metazoan PB assembly in detail, including their similarities and differences with SGs[18,20,21]. It is clear that SGs and PBs share certain proteins, are assembled and disassembled in response to drugs that promote or inhibit polysome disassembly, and can contain the same species of mRNA. However, they differ in that SGs contain components of the translation initiation machinery, whereas PBs contain components of the mRNA decay machinery.

What determines whether a particular transcript assembles into a SG or a PB? In general, when polysome disassembly is initiated by CCR4–NOT1-mediated deadenylation, disruption of interactions between poly(A)-binding protein 1 (PABP1) interactions and eIF4G breaks the circle of the polysomal transcript, reduces the efficiency of translation initiation and allows translating ribosomes to run off the transcript to produce a linear, deadenylated mRNA (FIG. 1, left pathway). Factors that recruit the deadenylase complex (for example, TTP[35] and RNA-induced silencing complexes (RISCs), which comprise microRNAs (miRNAs), Argonaute proteins and possibly other proteins[36]) seem to promote this mode of polysome disassembly. Removal of PABP from these transcripts allows a decapping complex (which comprises decapping enzyme 1 (DCP1; DCP1A in humans), DCP2, EDC3, RCK (also known as DDX6) and HEDLS) and a decapping activator complex (which comprises PAT1 bound to LSM1–7) to bind[37]. Although this supercomplex can degrade its component mRNA, it can also aggregate with related mRNPs to produce a PB, which in metazoans requires the RISC machinery[20,21,38,39] and the decay machinery. The factors that determine whether an individual mRNA transcript is degraded or aggregates with other mRNAs to form a PB are poorly understood, but signalling pathways that phosphorylate, ubiquitylate or otherwise modify mRNP components have all been implicated[30]. Post-transcriptional gene regulation seems to be linked with post-translational protein modifications at many levels.

> “...the PB might, like the SG, contain translationally silenced mRNA.”

In yeast, an organism that lacks both the RISC machinery[21] and select components of the decay machinery[20], the aggregation of ‘decay mRNPs’ into PBs is also driven by Q/N-rich prion-related domains found in several proteins comprising the mRNP, in particular Edc3 and Lsm4 (REFS 40,41). When the aggregation domains of Edc3 and Lsm4 are deleted, microscopically visible PB assembly is prevented[40,41]. This indicates that the aggregation of mRNPs follows the recruitment of the decay complex. Thus, in the assembly of yeast PBs, form follows function. The rate of mRNA decay is only modestly decreased[41] or remains unchanged[40] in cells that lack the Lsm4 aggregation domain, indicating that the aggregation of deadenylated mRNPs into PBs is optional, rather than essential, for mRNA decay. Enhanced PB assembly occurs when individual decay enzymes are limiting, however, indicating that PBs are aggregates of deadenylated mRNPs awaiting degradation[18]. Because mRNPs can leave the PB to resume translation[42], the PB might, like the SG, contain translationally silenced mRNA.

In yeast, EGP bodies (sites at which eIF4E, eIF4G and PABP are concentrated)[43] have been proposed to be an intermediate

Glossary

CAR-1
(Cytokinesis, apoptosis, RNA-associated 1). An Sm-like domain-containing protein that has orthologues in mammals (RAP55), *Drosophila melanogaster* (TRAL) and *Caenorhabditis elegans* (CAR-1) RNA granules.

EDC3
(Enhancer of mRNA-decapping protein 3). A protein that is found in mammalian (EDC3) and yeast (Edc3) RNA granules.

eIF3
(Eukaryotic translation initiation factor 3). A multisubunit complex that serves as an adaptor between eIF2, eIF4G and the small ribosomal subunit, thus facilitating initiation and stabilizing the closed loop of polysomal mRNA. eIF3 is a key component of stress granules.

GW182
A large, multidomain GW repeat-containing metazoan protein that is associated with microRNAs (miRNAs) and is required for miRNA-induced gene silencing. Knockdown of GW182 inhibits the assembly of processing bodies.

PABP1
(Poly(A)-binding protein 1). A protein with orthologues in mammalian (PABP1) and *Caenorhabditis elegans* (PAB-1) stress granules and yeast EGP bodies (Pbp1).

PAT1
A translational repressor or enhancer of decapping orthologues that is found in mammalian (PAT1), yeast (Pat1) and *Caenorhabditis elegans* (PATR-1) RNA granules.

PGL-1
(P granule abnormality 1). A protein that is found in germ cell (or polar) granules that are adjacent to nuclear pores.

RCK
An RNA DEAD-box helicase that has orthologues in mammals (RCK), yeast (Dhh1), *Drosophila melanogaster* (ME31B) and *Caenorhabditis elegans* (CGH-1) RNA granules. These promote translational arrest, polysome disassembly and decapping.

TTP
(Tristetraprolin). A zinc-finger-containing protein that promotes the decay of AU-rich element (ARE)-containing mRNAs at processing bodies.

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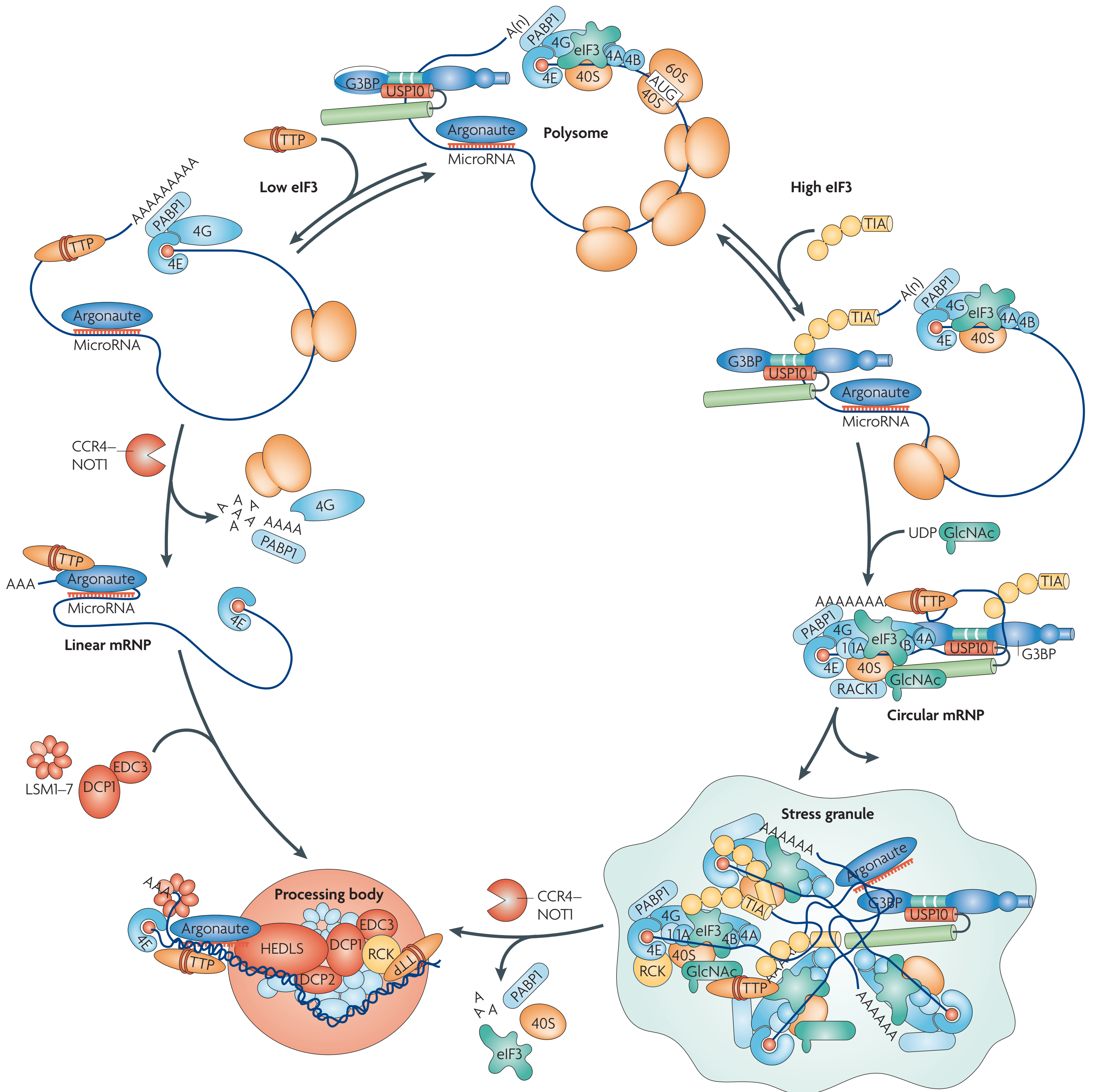

Figure 1 | **Dynamic relationship between metazoan RNA granules.** Polysomal RNA is circularized by interactions between poly(A)-binding protein 1 (PABP1) and eukaryotic translation initiation factor 4G (eIF4G), which are stabilized by eIF3. Polysome disassembly can be initiated by deadenylation (left pathway) or by translational silencing (right pathway). Linearized messenger ribonucleoproteins (mRNPs) seem to be destined for processing bodies (PBs), whereas circularized mRNPs are directed to stress granules (SGs). In the 'linear' pathway (left), the deadenylation complex CCR4–NOT1 is recruited by destabilizing factors, such as tristetraprolin (TTP), or RNA-induced silencing complexes (RISCs), which comprise Argonaute proteins and microRNAs. Breaking the circle by the loss of eIF3 and/or deadenylation-induced loss of PABP1 produces a linear transcript. This linear mRNA recruits a decapping complex (which consists of decapping protein 1 (DCP1; DCP1A in humans), DCP2, enhancer of mRNA-decapping protein 3 (EDC3), RCK (also known as DDX6) and HEDLS) and a decapping activator complex (PAT1 bound to LSM1–7; PAT1 is not shown). Q/N-rich domains in LSM4 and EDC3 promote the aggregation of these mRNAs into PBs. In the 'circular' pathway (right), transiently stalled initiation complexes recruit TIA1 and TIAR (together shown as TIA) as elongating ribosomes run off the transcript, converting the polysome into a circular, adenylated mRNP. Aggregation of bound TIA1 and TIAR or G3BP–USP10 (G3BP is GTPase-activating protein SH3 domain-binding protein and USP10 is ubiquitin-specific processing protease 10) and/or modification of ribosomes with O-linked *N*-acetylglucosamine (GlcNAc) promote the assembly of these mRNAs into SGs. It is possible that mRNPs in PBs or SGs can be remodelled to nucleate the assembly of other types of RNA granules. Alternatively, selected mRNPs might move from one type of granule to another, thus creating transient tethers between different granules.

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between PBs and polysomes[44], facilitating their eventual reinitiation. As mRNA can leave PBs to resume translation even in metazoan systems[45], it is possible that degradative mRNPs in PBs are remodelled through EGP bodies to produce translational mRNPs *en route* to initiation, a process that would allow EGPs to 'bud off' from PBs. However, the directionality of mRNA flux between PBs and EGP bodies has not been established. EGP bodies lack eIF3 and small ribosomal subunits, and they are therefore different from mammalian SGs. Moreover, mammalian SG and PB assembly are mutually independent[30].

Germ cell or P granules

In lower metazoans, RNA granules have important roles in gametogenesis and embryonic development. In *Caenorhabditis elegans*, several distinct classes of RNA granules have been implicated in the regulation of maternal mRNA expression in germ cells[46–49] (see below). In *Drosophila melanogaster*, germ cell granules also contain maternal mRNAs that are held in a translationally silenced state until they are needed for germ cell development[17]. These germ cell granules contain PB components[50] that are known to regulate protein translation and miRNA function. In *D. melanogaster* gonads, nurse cells possess PBs that contain DCP1 and/or DCP2, the 5′→3′ exonuclease XRN1 and the RNA helicase ME31B[51]. In oocytes, DCP1 is a component of a different class of granule that lacks DCP2 and XRN1 (REF. 51) and is insensitive to the translation elongation inhibitor cycloheximide, which distinguishes these granules from mammalian PBs. In germ cells, RNA granules can produce heritable changes in gene expression that are independent of changes in DNA sequence or chromatin remodelling. This is thought to result from the cytoplasmic delivery of granule-associated RNAs (for example, maternal mRNAs, piRNAs and miRNAs) that regulate gene expression during embryogenesis.

The distinct classes of RNA granules described in *C. elegans* oocytes have various roles in mRNA metabolism. Classical PGL-1-containing germ cell granules that associate with the nuclear membrane probably contribute to the storage and translational repression of maternal mRNAs. More recently, a second class of maternal mRNA storage granule has been observed in the gonadal syncytium[46–48]. Like germ cell granules, these maternal storage granules contain the PB markers CGH-1 (conserved germline helicase 1) and CAR-1, but they lack PGL-1 and the PB-associated decapping enhancers DCAP-2 (mRNA-decapping enzyme 2) and PATR-1 (REF. 49). Surprisingly, they also contain components of mammalian SGs (for example, PAB-1 and ataxin-related 2 (ATX-2)), suggesting that they might both stabilize and repress the translation of maternal mRNAs[49], which is consistent with the finding that CGH-1 selectively stabilizes maternal transcripts[49]. Compositionally similar 'PB-related' granules appear in the gonadal syncytium of aged hermaphrodites that no longer produce sperm[47,48]. In addition to CGH-1 and CAR-1, these granules contain SG-associated proteins that suppress the translation of maternal transcripts[47,48]. Interestingly, *rme-2* maternal transcripts, which are repressed in the distal gonad but translated in the proximal gonad, are restricted to granules that are found in the distal syncytium[48]. Taken together, these results describe a granule that encompasses properties of both SGs and PBs. The extent to which these RNA granules deliver maternal mRNAs to the embryo and contribute to maternal inheritance remains to be determined.

In mammalian spermatids, the chromatoid body is a perinuclear RNA granule, the composition of which is similar to PBs. Chromatoid bodies contain Dicer enzyme, GW182, Argonaute proteins, MIWI (a mammalian homologue of *D. melanogaster* PIWI) and miRNAs, indicating a link to small non-coding RNA function[10,52,53]. Chromatoid bodies also contain DCP1A and XRN1, which implies that they are involved in mRNA decay. Although little is known about the fate of chromatoid bodies during cell division, the segregation of these RNA granules with the germ lineage could epigenetically modulate gene expression during embryogenesis. This hypothesis awaits experimental validation.

Dynamic regulation of RNA granules

The mRNA inside PBs[20,54], SGs[54] and possibly neuronal granules[55] (the verdict is not yet in on transport granules and germ cell granules) is in dynamic equilibrium with the surrounding pool of mRNA that is being translated. Thus, PB and SG assembly is determined by the rate at which mRNPs enter (R_{ENT}) and exit (R_{EX}) the granule. When $R_{ENT}>R_{EX}$, PBs and SGs are assembled. When $R_{ENT}\leq R_{EX}$, new PBs and SGs are not assembled and existing PBs and SGs are disassembled. This RNA granule–polysome connection is revealed by drugs that inhibit translation elongation (for example, cycloheximide and emetine) and thus trap mRNA in polysomes. These agents dissolve PBs and SGs by depleting their core constituents[42,56]. Factors that increase R_{ENT} include deadenylases[57], aggregation factors[40,41] and stress-induced translational silencing[25]. Factors that increase R_{EX} include decapping enzymes[58], exonucleases[58] and translation initiation activators[22]. These factors ensure that PBs are assembled when decay mRNPs accumulate at a rate that exceeds mRNA degradation, and SGs are assembled when translationally stalled mRNPs accumulate at a rate that exceeds mRNA translation reinitiation or degradation.

An important consequence of the dynamic movement of mRNA and proteins is that SGs and PBs act like capacitors, collecting untranslated mRNAs that exceed the capacity of the translation and/or decay machinery. Under stress conditions, the size and number of SGs and PBs increase, implying that they 'store' mRNA (at least transiently) under stress conditions. Although the dynamic nature of germ cell granules is not well defined, they are also thought to store maternal mRNAs. In stressed cells, sequestration of mRNA in granules might contribute to translational repression and either promote or inhibit mRNA decay. Because some RNA granules are capable of motor-dependent movement along microtubules[59], sequestered mRNAs might be delivered to specific sites in the cell for later activation. These dynamic properties allow RNA granules to exert post-transcriptional control over gene expression (see below).

Modulation of gene expression

Protein-induced aggregation of RNA negatively regulates protein expression. Specific examples in both germ cells and somatic cells support a role for RNA granules in the post-transcriptional and epigenetic control of gene expression.

Germ cells. All RNA granules contain translationally repressed transcripts. During cell division, cytoplasmic RNA granules can be symmetrically or asymmetrically sorted into daughter cells to affect a form of cytoplasmic inheritance. This is most striking in P blastomeres of *C. elegans* embryos, which contain both germ cell granules and PBs[46–49]. Germ cell granules contain maternal transcripts that are required for specification of the germ line[60,61]. During mitosis, germ cell granules shrink and disappear from the part of the cell that will become the soma and fuse or enlarge in the part of the cell that

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Box 1 | RNA granules and non-coding RNAs

Translationally inactive mRNA is the common constituent of all RNA granules. Consequently, knockdown of RNA polymerase II prevents the arsenite-induced production of both stress granules (SGs) and processing bodies (PBs)[30]. Intriguingly, knockdown of RNA polymerase III subunits also prevents the assembly of both SGs and PBs[30], suggesting that non-coding RNAs, such as tRNAs, might also contribute to RNA granule assembly. SGs, PBs and germ cell (or polar) granules all contain Argonaute proteins and associated microRNAs (miRNAs)[19], which suggests that RNA-induced silencing complexes (RISCs) can promote the assembly of distinct messenger ribonucleoproteins (mRNPs) that drive the assembly of different classes of RNA granules. This might reflect the different mechanisms by which RISCs silence protein expression. On the one hand, RISCs can recruit the decay machinery to promote deadenylation, decapping and mRNA decay[36]. This would result in the assembly of 'decay mRNPs' that aggregate at PBs (FIG. 1, left pathway). If interactions between Argonaute and tristetraprolin (TTP) are involved in this process[76], the availability of TTP might drive RISC-mediated PB assembly. On the other hand, RISCs can inhibit translation initiation to assemble a translationally silent mRNP that aggregates at SGs (FIG. 1, right pathway). The presence of Argonaute proteins in germ cell granules suggests that miRNAs might contribute to the silencing of maternal mRNPs that aggregate at perinuclear granules. Because germ cell granules also contain Argonaute-like Piwi proteins, it is likely that Piwi-interacting RNAs (piRNAs) are also concentrated in these particles. It remains to be determined whether they also regulate maternal mRNA translation or any other aspect of germ cell function.

will remain germline[46]. The differentiation of somatic and germline daughter cells is determined in part by the absence or presence of germ cell granules. These are also required to specify the germ line in the next generation, which represents a clear example of epigenetic inheritance.

In germ cells, a variant of RISC is composed of an Argonaute-like Piwi protein bound to piRNAs, a germ cell-restricted class of small RNAs that are transcribed and processed independently of miRNAs. In *C. elegans*, Piwi-associated piRNAs are prominent components of germ cell granules[62,63] that are important for gene expression. When germline blastomeres divide, their germ cell granules are asymmetrically sorted and remain restricted to the germ lineage[46]. The finding that Piwi mutants have defective germ cell maturation implicates piRNAs in the process of germline specification[62,63]. As such, maternal inheritance of several different classes of RNA might be required for normal gametogenesis and embryonic development (BOX 1).

Piwi-associated piRNAs also contribute to maternal inheritance, as shown in studies of hybrid dysgenesis in *D. melanogaster*[8]. In this phenomenon, the offspring from crosses between wild-caught males and laboratory strain females exhibit defective gametogenesis and sterility. By contrast, crosses between genetically identical wild-caught females and laboratory strain males are fertile. This difference results from maternal inheritance of piRNAs that suppress the expression of transposons found in the genomes of wild-caught, but not laboratory strain, organisms. Piwi proteins and associated piRNAs can also exert epigenetic control over heterochromatin/euchromatin ratios at a subtelomeric region in *D. melanogaster*[9]. This involves histone modifications that promote both heterochromatin and euchromatin in different parts of the chromosome. Since these piRNAs are likely to reside in germ cell granules, the observed asymmetric segregation of RNA granules could potentiate these forms of epigenetic inheritance.

> "...RNA granules can exert post-transcriptional control over gene expression."

Somatic cells. Environmental stress induces a profound reprogramming of protein expression, which is mediated, in part, by the inclusion and exclusion of specific mRNAs in SGs[25]. Whereas 'housekeeping' transcripts that are subject to stress-induced translational silencing are routed into SGs, mRNAs that encode molecular chaperones and repair enzymes and that are preferentially translated during stress are excluded from SGs[64]. For example, mRNAs that encode stress-inducible HSP70 and HSP90 are selectively excluded from SGs[65,66]. Although a molecular explanation for this phenomenon is lacking, we speculate that mRNAs transcribed during stress recruit distinct RNA-binding factors that confer preferred translation status, or lack protein marks that confer selective repression. Regardless of the mechanism, these examples show how RNA granules can exert post-transcriptional control over gene expression.

In somatic cells, PBs[67–69] and SGs[70] contain miRNAs and Argonaute proteins, components of the RISC that regulate the translation and decay of many mRNAs. It is likely that RISC-mediated translational silencing induces RNA granule assembly, as cells lacking Dicer, an enzyme that is required for miRNA production, lack miRNAs and exhibit reduced numbers of PBs[71]. When RNA granules are sorted into daughter cells during cell division, their repertoire of miRNAs might further influence gene expression in the next generation.

In addition to mRNAs and small non-coding RNAs, SGs contain LINE1 retrotransposon-derived RNA that encodes an RNA-binding protein (ORF1) and a reverse transcriptase or endonuclease (ORF2)[72]. Both proteins are required for retrotransposition, a process that alters the genome by inserting a reverse-transcribed duplicate element into another part of the genome. Remarkably, LINE1-encoded ORF1 nucleates SG assembly[72], although whether its recruitment to SGs is part of the host anti-transposon response or has different functions is not clear. However, RNA-editing enzymes with antiviral activity (for example, APOBEC1 (P.A. and N.K., unpublished observations), APOBEC3G[3,4] and adenosine deaminase I[73]) are also present in both PBs and SGs. Moreover, an inosine-dependent antiviral RNA nuclease, TSN, is also associated with SGs[73]. Proteins that alter RNA and DNA sequences that reside in SGs might allow for epigenetic regulation of gene expression in somatic cells as well as germ cells.

Interactions between RNA granules

Different classes of RNA granules can transiently interact with one another (FIG. 2), implying that mRNAs, miRNAs, piRNAs and associated proteins might move from one granule to another. The distinctive enzymatic milieu inside different types of granules can influence mRNA translation and decay to modulate protein expression. In mammalian cells, the mRNA-destabilizing CCCH-finger proteins TTP, BRF1 and BRF2 promote interactions between SGs and PBs[54], which likely allow decay-bound mRNAs to be degraded in PBs. Another RNA-binding protein, ZBP1, stabilizes selected transcripts by preventing their transfer from SGs to PBs[66]. Observations using live microscopy reveal that mammalian PBs move towards SGs, transiently dock and then move on[54].

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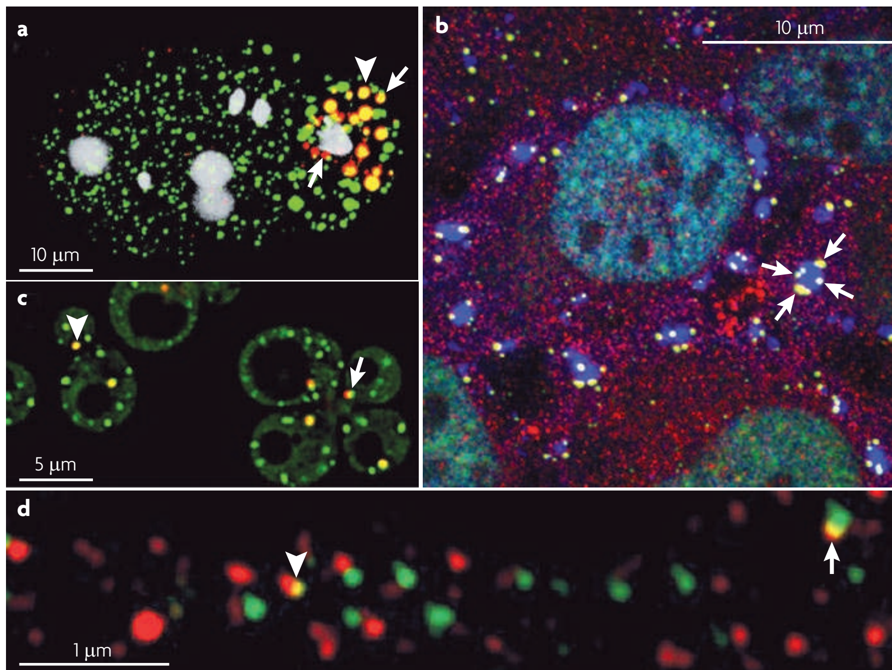

Figure 2 | **Interactions between RNA granules. a** | *Caenorhabditis elegans* embryos stained with anti-CAR-1 (green) and anti-PGL-1 (red) reveal processing bodies (PBs) and germ cell (or polar) granules, respectively. In the germ cell lineage, these granules interact (arrow) and sometimes merge (arrowhead). Nuclei are grey. **b** | Arsenite-treated DU145 cells stained with anti-TIA1 (blue), anti-DCP1A (decapping enzyme 1A; red) and anti-GE1 (also known as HEDLS; green) reveal blue stress granules (SGs) and yellow PBs, respectively. Many SGs are rimmed by a corona of PBs (arrows), although some PBs are free in the cytosol. **c** | *Saccharomyces cerevisiae* (stably transfected with PUB1–GFP and EDC3–mCh) cultured in the absence of glucose display EGP bodies (sites at which eukaryotic translation initiation factor 4E (eIF4E), eIF4G and poly(A)-binding protein (PABP) are concentrated; green) and PBs (red). Although these granules are distinct, they often interact with (arrows) or merge with (arrowheads) one another. **d** | Primary murine hippocampal neuronal dendrites stained with anti-DCP1 (green) and anti-staufen 2 (red) reveal PBs and neuronal granules, respectively. These granules interact (arrows) and sometimes fuse (arrowheads) with one another. Image in part **a** courtesy of P. Boag and K. Blackwell, Joslin Diabetes Center, Boston, USA. Image in part **c** courtesy of R. Buchan and R. Parker, University of Arizona, Tucson, USA. Image in part **d** is reproduced, with permission, from REF. 77 © (2008) Society for Neuroscience.

These movements do not suggest that one type of RNA granule ‘buds off’ from another. Rather, they indicate that directed granule movement occurs in combination with specific intergranule interactions. PBs contain myosin motor proteins[74] that might be involved in their directed movements along microtubules[59], and the movements of PBs and SGs can be disrupted by the microtubule inhibitor nocodazole[59,75]. Despite these intriguing results, however, direct evidence for the transfer of mRNA between SGs and PBs is lacking. Similar interactions between PBs and germ cell granules have been observed in *C. elegans* oocytes[47,48]. Given the importance of CCCH-finger proteins (for example, MEX-5, MEX-6 and POS-1) in the expression of maternal mRNAs, their role in the transfer of mRNA between different classes of granules warrants investigation[46].

Conclusions and perspectives

Compositionally and functionally distinct RNA granules are found in the cytoplasm of somatic and germ cells in both higher and lower eukaryotes. Granule assembly occurs when compositionally similar mRNPs accumulate in response to metabolic events in the cell. The components of most RNA granules are in dynamic equilibrium with the translational pool allowing rapid shifts between translation, storage and decay.

RNA granules modulate gene expression in somatic cells by regulating translation, mRNA decay and the availability of miRNAs, and modulate gene expression in germ cells by regulating translation of maternal mRNAs and transmission of piRNAs. Although the assembly and composition of different types of RNA granules is distinct, all RNA granules contain transcripts that are translationally silent. This common feature might echo an ancient RNA world, in which the aggregation of primitive proteins with RNAs was an essential step in the evolution of organelles and organisms. Modern RNA-binding proteins that regulate and aggregate specific mRNPs into SGs, PBs and other types of RNA granules might be living fossils of an early prebiotic organization. The question of whether granules are a cause or an effect of RNA processes (for example, stress- or miRNA-mediated translational silencing) might be analogous to that of whether the chicken preceded the egg, or whether viruses are alive. Viruses and the SGs they sometimes trigger might both be remnants of an RNA world in which form and function were, and are, inextricably linked.

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DATABASES
UniProtKB: http://www.uniprot.org
ADAR1 | APOBEC1 | APOBEC3G | CBP20 | CBP80 | CGH-1 | DCAP-2 | DCP1A | DCP2 | Edc3 | eIF4E | G3BP | HSP70 | Lsm4 | PGL-1 | TIA1 | TIAR | TTP | USP10

FURTHER INFORMATION
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