P bodies: at the crossroads of post-transcriptional pathways

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Abstract | Post-transcriptional processes have a central role in the regulation of eukaryotic gene expression. Although it has been known for a long time that these processes are functionally linked, often by the use of common protein factors, it has only recently become apparent that many of these processes are also physically connected. Indeed, proteins that are involved in mRNA degradation, translational repression, mRNA surveillance and RNA-mediated gene silencing, together with their mRNA targets, colocalize within discrete cytoplasmic domains known as P bodies. The available evidence indicates that P bodies are sites where mRNAs that are not being translated accumulate, the information carried by associated proteins and regulatory RNAs is integrated, and their fate — either translation, silencing or decay — is decided.

Ribonucleoprotein (RNP). A complex of proteins and RNA. In many cases, the proteins bind directly to their cognate mRNA molecules (mRNPs). Proteins can also be recruited to the RNP particle through protein–protein interactions.

5′→3′ exoribonuclease An enzyme that has an important role in all aspects of RNA metabolism. It degrades RNA to 5′ mononucleotides in a 5′→3′ direction.

5′-cap structure A structure that consists of m^7GpppN (where m^7G represents 7-methylguanylate, p represents a phosphate group and N represents any base) that is located at the 5′ end of a eukaryotic mRNA.

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doi:10.1038/nrm2080

Eukaryotic gene expression involves several post-transcriptional events that share mRNA as a common substrate[1–3]. The primary transcript is processed by the removal of introns and the addition of a poly(A) tail, and the mature mRNA is exported from the nucleus to the cytoplasm for translation. Not all mRNAs are translated immediately; some are maintained in a translationally repressed state and might be transported to a specific cytoplasmic location where translation might be activated. In the cytoplasm, mRNAs are also subject to quality control and regulatory mechanisms that either promote mRNA degradation or repress mRNA translation. Translational repression and mRNA decay can be triggered by specific RNA-binding proteins and also by small, complementary regulatory RNAs in a process known as RNA-mediated gene silencing. Eventually, the mRNAs are degraded.

In eukaryotes, hundreds of proteins and small non-coding RNAs participate in post-transcriptional processes and their regulation. Many act on bulk mRNA, whereas others regulate specific transcripts. A subset of these factors, with functions in translational repression, mRNA silencing, mRNA surveillance (or quality control) and degradation, colocalize in discrete cytoplasmic domains referred to as mRNA-processing bodies (now known as P bodies), which reveals fundamental interrelationships among these processes.

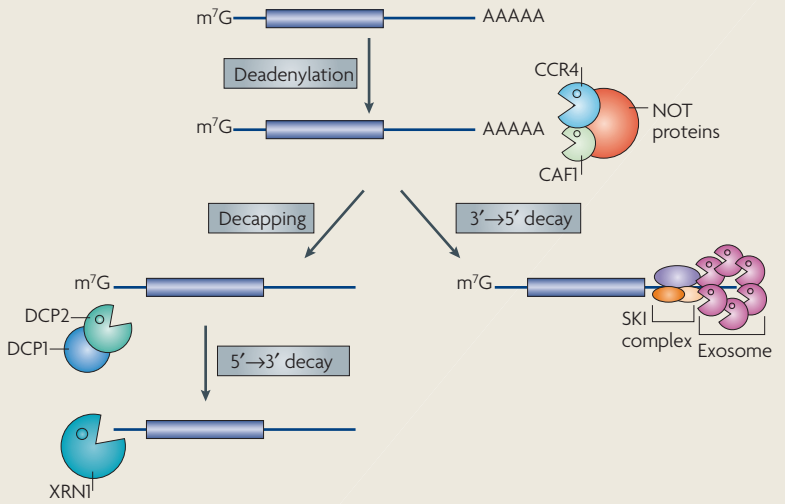
In this review, we focus on the role of P bodies in mRNA surveillance and mRNA decay, RNA-mediated silencing and translational control. We also discuss current models of P-body function and the link between P bodies and other messenger ribonucleoprotein (mRNP) granules that are present in stressed mammalian cells, in polarized cells such as neurons, and during oogenesis in diverse organisms.

Discovery and rediscovery of P bodies

In 1997, Bashkirov *et al.*[4] reported that XRN1, the main cytoplasmic 5′→3′ exoribonuclease in eukaryotic cells, was localized in small granular structures and was "highly enriched in discrete, prominent foci" within the cytoplasm of mammalian cells. This observation, the biological importance of which remained unappreciated for almost five years, paved the way for fundamental discoveries in the field of RNA metabolism and post-transcriptional gene regulation. Insights into the role of the XRN1 foci as sites where eukaryotic mRNA degradation can take place emerged from the discovery that the enzyme that cleaves the 5′-cap structure of mRNA (the decapping enzyme DCP2) and its cofactors colocalize with XRN1 in these foci in both mammalian and yeast cells[5–9]. The decapping enzyme functions upstream of XRN1 in the 5′→3′ mRNA-decay pathway (BOX 1); it hydrolyses the cap structure leaving an mRNA with a 5′ monophosphate, which is the preferred substrate for XRN1. The foci were called P bodies, DCP bodies or mRNA-decay foci.

XRN1 foci were rediscovered in 2002 by Eystathioy *et al.*[10] thanks to an autoimmune serum from a patient who suffered from motor and sensory neuropathy. This serum stained discrete cytoplasmic domains and recognized a novel human protein of unknown function,

Box 1 | **Degradation of bulk mRNA in eukaryotic cells**



Bulk mRNA decay is initiated by deadenylation (see the figure). There are several deadenylase complexes in eukaryotes: the PARN2–PARN3 complex is thought to initiate deadenylation (not shown), which is then continued by the CAF1–CCR4–NOT complex, the components of which localize to P bodies[24]. This complex consist of two deadenylases, CCR4 and CAF1, and the NOT proteins. Following deadenylation, mRNAs are degraded by exonucleolytic digestion at both ends. Degradation from the 3′ end of the transcript is catalysed by the exosome and the SKI complex[25]. The 5′→3′ mRNA-decay pathway requires the removal of the cap structure by the decapping enzyme DCP2 and exonucleolytic degradation by XRN1. Several proteins — DCP1, EDC3, LSm1–7, Pat1 (also known as CG5208) and Dhh1 (also known as RCK/p54, Me31B or CGH-1) — have been shown to stimulate decapping, but they might function by a different mechanism. In *Saccharomyces cerevisiae*, DCP1 interacts directly with DCP2, whereas in human cells, a larger complex is thought to exist and includes EDC3 (also known as LSm16) and Ge-1 (also known as Hedls or RCD-8) (not shown). Most proteins that are involved in the 5′→3′ mRNA-decay pathway localize to P bodies[4–9,13,14,16,17,19–23,26–35].

Decapping
The process of removing the 5′-cap structure from an mRNA, usually as a step prior to further 5′→3′-exonucleolytic digestion of the remainder of the mRNA. In eukaryotes, decapping is catalysed by the decapping enzyme DCP2 assisted by decapping co-activators such as DCP1, EDC3 and the LSm1–7 complex.

Deadenylase
An enzyme that catalyses the degradation of the 3′-poly(A) tail, which is present on most eukaryotic mRNAs. Although many deadenylases have been described, the principal cytoplasmic activity in yeast seems to reside in the CCR4–CAF1–NOT complex.

Exosome
A complex of at least 11 putative 3′→5′ exonucleases that functions in several different RNA-processing and RNA-degradation pathways in the nucleus and the cytoplasm.

GW182 (so called because of its molecular weight and the presence of glycine and tryptophan repeats)[10] (BOX 2). GW182-containing foci became known as GW bodies (GWBs)[10]. It did not take long to realize that GWBs are XRN1 or DCP foci[11]. Patients with the autoimmune disease primary biliary cirrhosis also have antibodies against P-body components, and this led to the identification of two additional P-body proteins, RAP55 (RNA-associated protein of 55 kDa, also known as LSm14) and Ge-1 (also known as Hedls (human enhancer of decapping large subunit) or RCD-8), which functions in mRNA decapping[12–17].

RAP55, Ge-1 and GW182 are markers of P bodies in multicellular organisms (FIG. 1) and are essential for P-body integrity, because their depletion leads to P-body loss[12–14,18]. There are no Ge-1 or GW182 orthologues in the budding yeast *Saccharomyces cerevisiae*, which provided the first indication of an increased complexity of P-body composition and function in higher eukaryotes. P bodies are easily detected in cells from multicellular organisms (FIG. 1). Their size ranges from 100 to 300 nm in mammalian cells[10,18], whereas in *S. cerevisiae*, the visualization of P bodies is often enhanced by overexpressing or depleting some of the P-body components or by exposing yeast cells to stresses such as glucose deprivation or hypotonic shock[19–21].

Over the past few years, the number of proteins detected in P bodies has increased exponentially (TABLE 1). In addition to proteins that are involved in mRNA degradation, P-body components include proteins with roles in mRNA surveillance, RNA interference and translational repression. Ribosomal proteins and proteins that are involved in translation initiation[19,20,22] are notably absent, with the exception of the cap-binding protein eukaryotic translation-initiation factor-4E (eIF4E) and its binding partner, eIF4E-transporter (eIF4E-T), which are found in mammalian P bodies[22,23]. The presence of proteins that function in such a broad range of post-transcriptional processes indicates a central role for P bodies in the regulation of gene expression.

P bodies are sites of mRNA decay

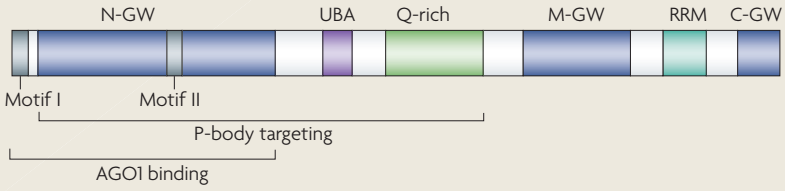
In eukaryotic cells, degradation of bulk mRNA occurs by two alternative pathways, both of which are preceded by removal of the poly(A) tail by deadenylases (BOX 1; reviewed in REF. 24). Following this first rate-limiting step, mRNAs can undergo 3′→5′ exonucleolytic decay, which is catalysed by the exosome, and the exosome activity is regulated by the SKI complex[1,24,25]. Alternatively, after deadenylation, the cap structure is removed by the decapping enzyme DCP2, rendering the mRNA susceptible to 5′→3′ digestion by XRN1.

mRNA decapping is an irreversible and therefore highly regulated process that requires the participation of several proteins. These proteins are collectively known as decapping co-activators, although the precise molecular function of most of these co-activators is unclear. Similar to DCP2 and XRN1, decapping co-activators are highly conserved in eukaryotes and include DCP1, EDC3 (enhancer of decapping-3, also known as LSm16), Ge-1, the heptameric LSm1–7 complex, the DExD/H-box RNA helicase Dhh1 (also known as RCK/p54, Me31B or CGH-1) and Pat1 (also known as CG5208), which is a protein of unknown function that interacts with LSm1–7 heptamers, XRN1 and Dhh1[13,14,24,26–31]. Strikingly, all proteins that function in the 5′→3′ mRNA-decay pathway have now been shown to localize to P bodies[4–15,18–23,26,32–35] (TABLE 1). By contrast, exosome and SKI-complex components are not detected in P bodies[8,20], which indicates a degree of compartmentalization of mRNA-degradation pathways within the cytoplasm.

The presence of mRNA-decay enzymes in P bodies has raised the question of whether P bodies represent the sites of mRNA degradation or whether they serve as storage sites for these enzymes. Several lines of evidence indicate that mRNA degradation takes place in P bodies. First, the inhibition of transcription with actinomycin D, or the exposure of cells to ribonuclease A, leads to P-body loss, which indicates that P-body assembly is dependent on RNA[9,19]. Second, when mRNA decay is blocked at an early stage — for example, by preventing deadenylation in cells that lack the deadenylase CCR4 — P bodies disappear[8,22]. By contrast, blocking mRNA decay at a later stage in cells that lack XRN1 or the decapping co-activator DCP1 results in an increase in

Box 2 | **Domain organization of GW182**

GW182 belongs to a family of proteins that are characterized by central ubiquitin-associated (UBA) and glutamine-rich (Q-rich) domains, a C-terminal RNA-recognition motif (RRM) and three blocks of glycine–tryptophan (GW) repeats at the N-terminal, middle and C-terminal domains of the protein[10,34] (see the figure). The N-terminal repeats (N-GW) are required for the interaction of GW182 with Argonaute protein-1 (AGO1), whereas P-body localization requires the UBA and the Q-rich domains, in addition to the N-GW repeats[34]. There are three GW182 paralogues in vertebrates (known as TNRC6A–C), but there is only a single orthologue in insects and there are no orthologues in fungi[34]. In *Caenorhabditis elegans*, the protein AIN-1 is similar to the N-terminal GW-repeats of GW182 proteins[66]. Although AIN-1 contains no UBA, Q-rich or RRM domains, its localization and function indicate that it is a functional analogue. Indeed, AIN-1, *Drosophila melanogaster* GW182, human TNRC6A (also known as GW182) and TNRC6B all localize to P bodies and have been shown to function in RNA silencing[33,34,62–66].



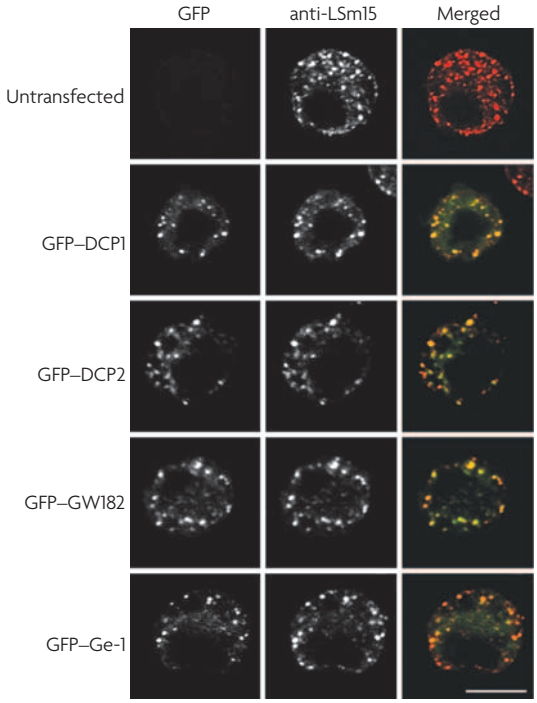


Figure 1 | **P bodies in *Drosophila melanogaster* Schneider cells.** The figure shows confocal fluorescent micrographs of *D. melanogaster* Schneider cells that are untransfected (top row) or transfected with vectors expressing green fluorescent protein (GFP) fusions of the decapping enzymes DCP1 and DCP2 and the P-body markers GW182 and Ge-1 (left panels). Cells were stained with affinity-purified anti-LSm15 (Tral) antibody (middle panels). For easy visualization, the GFP panels (green) and the anti-LSm15 panels (red) are shown in black and white. The right panels show the overlay of the red and green panels. The decapping enzymes, the decapping co-activator Ge-1, together with GW182, localize to endogenous P bodies stained with the anti-LSm15 antibody. Additional P-body components are listed in TABLE 1. Note that the expression of the GFP-fusion proteins does not alter the distribution of endogenous LSm15 (compare untransfected and transfected cells). The scale bar represents 5 μm.

P-body size and number[8,9,19,22]. The size and number of P bodies therefore depends on the amount of mRNA that is undergoing decapping. Last, mRNA-decay intermediates are detected in P bodies. Indeed, blocking the progression of XRN1 along an mRNA by the insertion of specific RNA sequences or by depleting XRN1 leads to the enlargement of P bodies owing to the accumulation of decay intermediates within these bodies[8,9,19]. Collectively, these observations definitively establish that mRNA decay can take place inside P bodies. More importantly, they indicate that the existence of P bodies relies on the presence of mRNPs that are committed to, or are undergoing, degradation.

Does mRNA decay also occur outside of P bodies in the diffuse cytoplasm? Most P-body components are distributed diffusely throughout the cytoplasm as well as being localized in P bodies[4–13] (FIG. 1). Quantitative information regarding the fractionation of these proteins between the cytoplasm and P bodies is still lacking, but given the volume of P bodies relative to that of the cytoplasm, the diffuse cytoplasmic fraction could be significantly larger, although the concentration of mRNA-decay factors might be much higher in P bodies. Also, P-body components dynamically exchange with the cytoplasmic pool[35], which indicates that decay enzymes and co-activators are not confined to P bodies. So, it is probable that mRNA decay also occurs, and might even be initiated, in the diffuse cytoplasm, and that P bodies arise by association of mRNA-decay intermediates induced by interactions between decay enzymes and cofactors (see below). The concentration of mRNA-decay intermediates and mRNA-decay enzymes in P bodies would be expected to increase the speed of degradation and, in turn, reduce any potential competition between these RNA fragments and normal mRNAs for limiting cellular proteins involved in mRNA regulation.

Nonsense-mediated mRNA decay
(NMD). The process by which the cell destroys mRNAs, the translation of which has been prematurely terminated owing to the presence of a nonsense codon within the coding region.

Premature translation-termination codon
(PTC). An alternative name for a nonsense codon. An in-frame stop codon that terminates translation, which leads to C-terminal truncated proteins. PTCs elicit NMD.

P bodies in mRNA surveillance

In eukaryotic cells, mRNA surveillance or quality-control mechanisms have evolved to ensure that only fully processed and error-free mRNAs are translated[2]. Among these, the nonsense-mediated mRNA decay (NMD) pathway recognizes and degrades mRNAs that contain premature translation-termination codons (PTCs, also known as nonsense codons), thereby limiting the synthesis of potentially toxic protein fragments[36–38].

NMD is triggered by a premature translation-termination event, and this leads to the assembly of the so-called surveillance complex on the mRNA[36–38]. The surveillance complex comprises the UPF1–3 proteins, which are conserved from yeasts to humans, and four additional NMD effectors (SMG1 and SMG5–7), which are conserved in most multicellular organisms but have no clear orthologues in *S. cerevisiae*[36–38].

Table 1a | **Protein components of P bodies**

Name	Function	Organisms	Effects of depletion or overexpression on P-body integrity	References
XRN1, *Sc* Kem1	5′→3′ exonuclease	Human, mouse, *Sc*	Depletion: increase in P-body size and number (*Sc*)	4,6,8,9
GW182, *Ce* AIN-1	In the miRNA pathway	Human, *Dm*, *Ce*	Depletion: P-body loss (human)	10,11,18,34,62,63,66
DCP2, *Ce* DCAP-2	Decapping enzyme	Human, *Dm*, *Ce*, *Sc*	Depletion: increase in P-body size and number (human); overexpression: P-body loss (human)	5,8,9,14,22,34,66
DCP1, *Ce* DCAP-1	Decapping-enzyme subunit	Human, *Dm*, *Ce*, *Sc*	Depletion: increase in P-body size and number (*Sc*)	5,8,32,34,66
Hedls, Ge-1	Decapping co-activator	Human, *Dm*	Depletion: P-body loss (human); overexpression: increase in P-body size and number (human)	13,14,34
Dm CG5208, Pat1	Decapping co-activator	*Dm*, *Sc*	Depletion: slight reduction in P-body size (*Sc*); overexpression: increase in P-body size and number (*Sc*)	8,32,AE.& E.I., uo
EDC3 (LSm16)	Decapping co-activator	Human, *Dm*, *Sc*	Overexpression: P-body loss (human)	14,26, AE.& E.I., uo
LSm1–7	Decapping co-activator complex	Human, *Sc*	Depletion: P-body loss (human), but increase in P-body number (*Sc*)	6,8,22,27,33
RAP55 (LSm14)	Predicted decapping co-activator	Human	Depletion: P-body loss	12
RCK/p54, *Dm* Me31B, *Ce* CGH-1, *Sc* Dhh1	Decapping co-activator, translation regulator	Human, *Dm*, *Ce*, *Sc*	Depletion: P-body loss (human and *Sc*); overexpression: P-body loss, but increase in P-body size and number (human), P-body loss (*Sc*)	8,9,14,22,32,33, AE.& E.I., uo
eIF4E	Translation-initiation factor	Human, rat	Not determined	22,23
eIF4E-T	Translational repression	Human	Depletion: P-body loss	22,23
SMG7	NMD	Human	Overexpression: increase in P-body size	45
SMG5	NMD	Human (when co-expressed with SMG7)	Not determined	45
UPF1, *Sc* Nam7	NMD	Human (when co-expressed with SMG7), *Sc* (on depletion of DCP2, DCP1, XRN1, UPF2 or UPF3)	Depletion: no effect (*Sc*)	21,45
UPF2	NMD	*Sc* (on depletion of DCP2, DCP1 or XRN1)	Depletion: increase in P-body size	21
UPF3	NMD	*Sc* (on depletion of DCP2 DCP1 or XRN1)	Depletion: increase in P-body size	21
Argonaute proteins	In the siRNA and miRNA pathways	Human, *Dm*, *Ce*	Not determined	34,61,64
CCR4–CAF1–NOT complex	Deadenylation	Human, *Sc*	Depletion: loss of P-bodies (human and *Sc*)	8,9,22
CPEB	Translation regulator	Human	Not determined	70
FAST	Fas-activated serine/threonine phosphoprotein	Human	Not determined	35
TTP	ARE-mediated mRNA decay	Human	Not determined	14,35,96
Staufen	Double-stranded RNA-binding protein, mRNA localization	*Dm*	Not determined	71, AE.& E.I., uo

Table 1b | **Protein components of P bodies**

Name	Function	Organisms	Effects of depletion or overexpression on P-body integrity	References
Rbp1	RNA-binding protein, mediates decay of mitochondrial porin mRNA	*Sc* (under stress conditions)	Not determined	104
Rpb4	Subunit of RNA polymerase II	*Sc*	Depletion: increase in P-body size and number	105
Sbp1	Suppressor of decapping defects	*Sc*	Depletion: reduction in P-body formation under stress conditions; overexpression: increase in P-body size and number	106
Gemin5	Component of the SMN protein complex involved in assembly of U snRNPs	Human	Not determined	107
Dcs2	Stress-induced regulatory subunit of the scavenger decapping enzyme Dcs1	*Sc*	Not determined	108
APOBEC3G, APOBEC3F	Deoxycytidine deaminase with antiviral activity	Human	Not determined	109

Ce, *Caenorhabditis elegans*; CPEB, cytoplasmic polyadenylation element-binding protein; *Dm*, *Drosophila melanogaster*; EDC3, enhancer of decapping-3; eIF4E, eukaryotic translation-initiation factor-4E; eIF4E-T, eIF4E-transporter; miRNA, microRNA; NMD, nonsense-mediated mRNA decay; *Sc*, *Saccharomyces cerevisiae*; siRNA, small interfering RNA; SMN, survival of motor neurons; snRNP, small nuclear ribonucleoprotein; TTP, tristetraprolin; uo, unpublished observations.

UPF1 is an RNA helicase, the activity of which is regulated by cycles of phosphorylation and dephosphorylation, which requires the additional NMD factors (FIG. 2). Phosphorylation of UPF1 is catalysed by SMG1, a protein kinase that is related to phosphoinositide-3-kinase, and requires UPF2 and UPF3. This indicates that the formation of a trimeric UPF1–UPF2–UPF3 complex promotes UPF1 phosphorylation. Dephosphorylation of UPF1 is mediated by SMG5, SMG6 and SMG7, which are three related proteins that function as adaptors between phosphorylated UPF1 and protein phosphatase-2A (PP2A)[36–38] (FIG. 2).

Although it is currently unclear how NMD effectors assemble on mRNAs that terminate translation prematurely, it is well established that, once assembled, the surveillance complex recruits enzymes that are involved in general mRNA decay, thereby coupling premature-translation termination with accelerated mRNA degradation. In *S. cerevisiae*, the surveillance complex recruits decapping enzymes and XRN1, but can also accelerate deadenylation and 3′→5′ degradation by the exosome and the SKI complex[39,40] (BOX 3). A similar mRNA-decay pathway of NMD substrates is thought to occur in humans[41–43]. By contrast, in *Drosophila melanogaster*, the degradation of nonsense transcripts is initiated by endonucleolytic cleavage near the PTC (BOX 3). The resulting RNA fragments are degraded from the newly generated 3′ and 5′ ends by the exosome and XRN1, respectively[44].

So, the enzymes that are involved in general mRNA decay also function in NMD (BOX 3). These findings have raised the question of whether the decay of NMD substrates occurs in the cytoplasm or whether the entire surveillance complex escorts the nonsense mRNA to P bodies where it undergoes rapid decay. What could trigger the accumulation of NMD targets in P bodies? Insights into this question were provided by the observation that, in human cells, SMG7 localizes to P bodies and causes the accumulation of SMG5 and UPF1 in these bodies[45,46]. This, together with the finding that SMG7 binds to phosphorylated UPF1 and triggers its dephosphorylation (FIG. 2), indicates a mechanism that links the assembly of the surveillance complex to the degradation of NMD targets in P bodies. Accordingly, UPF1 is phosphorylated during the assembly of the surveillance complex on PTC-containing mRNAs. Phosphorylated UPF1 recruits SMG7 (most likely in a complex with SMG5 and PP2A (REFS 47,48)), which would, in turn, target the PTC-containing transcript for degradation in P bodies[45,46]. Alternatively, SMG7 might recruit P-body components to mRNPs that are marked by the surveillance complex; the localization to P bodies could then be a consequence of this recruitment. The association of SMG7, SMG5 and PP2A would also trigger the dephosphorylation of UPF1, which might be involved in releasing the NMD factors to mediate further rounds of NMD. It is unclear how SMG6 participates in this process.

The above model is unlikely to apply to *S. cerevisiae* or *D. melanogaster*, which both seem to lack an SMG7 orthologue[49]. Nevertheless, UPF1 and NMD targets localize to P bodies in yeast cells that lack UPF2, UPF3 or DCP1 (REF. 21). UPF2 and UPF3 also concentrate in P bodies in cells that lack DCP1, but this accumulation is dependent on UPF1 (REF. 21). Therefore, UPF1 elicits P-body formation and the accumulation of NMD

effectors and targets in these bodies when NMD is blocked at a stage after UPF1 binding, but before target degradation. This implies that under these conditions P-body formation is triggered by the accumulation and aggregation of mRNPs that are committed to NMD, because they are associated with UPF1, but can no longer be degraded. In agreement with a role for UPF1 in initiating P-body assembly, an interaction between UPF1 and decapping enzymes has been described in both yeast and human cells[14,50].

It is important to note that the degradation of NMD substrates is unlikely to require large P bodies in wild-type *S. cerevisiae* under normal growth conditions, because P bodies are hardly detectable in these cells[21]. Similarly, P-body integrity might not be required for NMD in *D. melanogaster*[51], as NMD is unaffected in *D. melanogaster* cells in which P bodies are disrupted by depletion of GW182. Given the different mechanisms by which nonsense mRNAs are degraded among species, it is still possible that P bodies have an active role in NMD in other organisms.

P bodies and gene silencing

Small interfering RNAs (siRNAs) and microRNAs (miRNAs) represent two novel classes of small RNA that regulate gene expression after transcription[52,53]. Although siRNAs and miRNAs differ in their mechanism of biogenesis, the regulatory functions of each class are effected by members of the conserved family of Argonaute proteins, with which they associate as part of RNA-induced silencing complexes (RISCs)[52,53].

Argonaute proteins promote decay and/or translational repression of mRNAs that are fully or partially complementary to the siRNAs and miRNAs involved[52,53] (BOX 4). siRNAs are fully complementary to their targets and guide the Argonaute proteins to cleave the mRNA in the region that is base-paired with the siRNA. Similarly, plant miRNAs usually promote endonucleolytic cleavage of cognate mRNAs.

Endonucleolytic cleavage of siRNA or plant miRNA targets is catalysed by the C-terminal PIWI domain of Argonaute proteins, which adopts a ribonuclease H (RNase H)-like fold[54]. Following this endonucleolytic cleavage, the resulting mRNA fragments are handed over to the general mRNA-decay machinery. In *D. melanogaster*, 5′ and 3′ mRNA fragments that are generated by Argonaute-mediated endonucleolytic cleavage are degraded by the exosome in conjunction with the SKI complex, and by XRN1, respectively[55] (BOX 4). *Arabidopsis thaliana* has several XRN1 paralogues, and the only cytoplasmic paralogue, XRN4, has been implicated in the exonucleolytic removal of 3′-mRNA fragments generated by endonucleolytic cleavage by Argonaute proteins[56].

In contrast to plants, most animal miRNAs are only partially complementary to their targets and silence gene expression by at least two distinct mechanisms: by repressing translation and/or by promoting mRNA decay[34,57] (BOX 4). In most cases, however, mRNA decay by animal miRNAs does not occur through endonucleolytic cleavage by the Argonaute proteins, but instead by directing mRNAs to the general mRNA-degradation machinery, which accelerates their decay[34,57–60]. Indeed, recent studies in zebrafish embryos, *D. melanogaster* and human cells have shown that miRNAs accelerate deadenylation of their targets[34,57–59].

Deadenylation and accelerated decay of miRNA targets requires the Argonaute proteins, the P-body component GW182, the CCR4–CAF1–NOT deadenylase complex, the decapping DCP1–DCP2 complex and XRN1 (REFS 34,57,60) (BOX 4). All of these proteins localize to P bodies. Moreover, Argonaute proteins interact with GW182, DCP1, DCP2 and RCK/p54, and might be present as complexes in P bodies[33,34,61–67]. In addition to the Argonaute proteins, miRNAs and miRNA targets are also detected in P bodies[61,67–69], which strongly indicates a role for these bodies in RNA silencing.

There is additional support for the involvement of P-body components in gene-silencing pathways. First, depletion of GW182 in human and *D. melanogaster* cells impairs silencing by miRNAs and to a lesser extent by siRNAs[33,34,57,62,63,65]. Second, the human orthologue of Dhh1, RCK/p54, is required for miRNA-mediated translational repression[33]. Third, the *Caenorhabditis elegans* protein AIN-1 (which is related to GW182) is required for gene regulation by at least a subset of miRNA targets[66]. Last, miRNA function is impaired in *D. melanogaster* and human cells that are depleted of the decapping DCP1–DCP2 complex[34,51,57,62].

Although these results clearly establish a link between RNA-silencing pathways and P bodies, they do not address the question of whether the spatial environment of P bodies is required for silencing. Recent studies have shown that P-body integrity (as judged by the detection of these structures by light microscopy), is not necessary for siRNA-mediated silencing. In human cells, the depletion of LSm1 or RCK/p54 leads to P-body loss and diffusion of Argonaute protein-2 (AGO2) throughout the cytoplasm, without affecting siRNA-guided mRNA cleavage[33]. Consistently, endonucleolytic cleavage by AGO2 is only slightly inhibited in human and *D. melanogaster* cells that are depleted of GW182[33,51,62,63]. Moreover, cycloheximide treatment, which also disrupts P bodies, does not affect siRNA function[33]. Similarly, miRNAs repress their targets in cells in which P bodies are disrupted by the depletion of LSm1 (REF. 33). So, although P-body components have a role in silencing pathways, siRNAs and miRNAs can function in the absence of detectable P bodies. Consequently, the accumulation of Argonaute proteins, siRNAs, miRNAs and their mRNA targets in P bodies could be the consequence, rather than the cause, of silencing (see below).

Role of P bodies in translational repression

miRNAs not only promote mRNA degradation but also repress translation of their targets, and in many cases can do so without affecting the amounts of mRNA, which indicates that translational repression occurs independently of mRNA decay in these cases[3]. How miRNAs regulate translation is not well understood, but P-body components have been implicated in this

Small interfering RNA
(siRNA). A non-coding RNA of ~22 nucleotides that is processed from a longer dsRNA during RNA interference. Such non-coding RNAs base pair with mRNA targets and confer target specificity on the silencing complexes in which they reside.

microRNA
(miRNA). A small RNA of ~22 nucleotides that is encoded by an endogenous gene. The miRNA regulates the expression of RNAs to which it is complementary in sequence.

Argonaute proteins
A family of proteins that are characterized by the presence of two homology domains, PAZ and PIWI. These proteins are essential for diverse RNA-silencing pathways.

RNA-induced silencing complex
(RISC). A complex that consists minimally of an Argonaute protein and the associated miRNA or siRNA. The RISC complex mediates miRNA- or siRNA-guided gene silencing.

PIWI domain
A conserved protein domain that is found in members of the Argonaute-protein family. It is structurally similar to ribonuclease-H domains and, in at least some cases, has endoribonuclease activity.

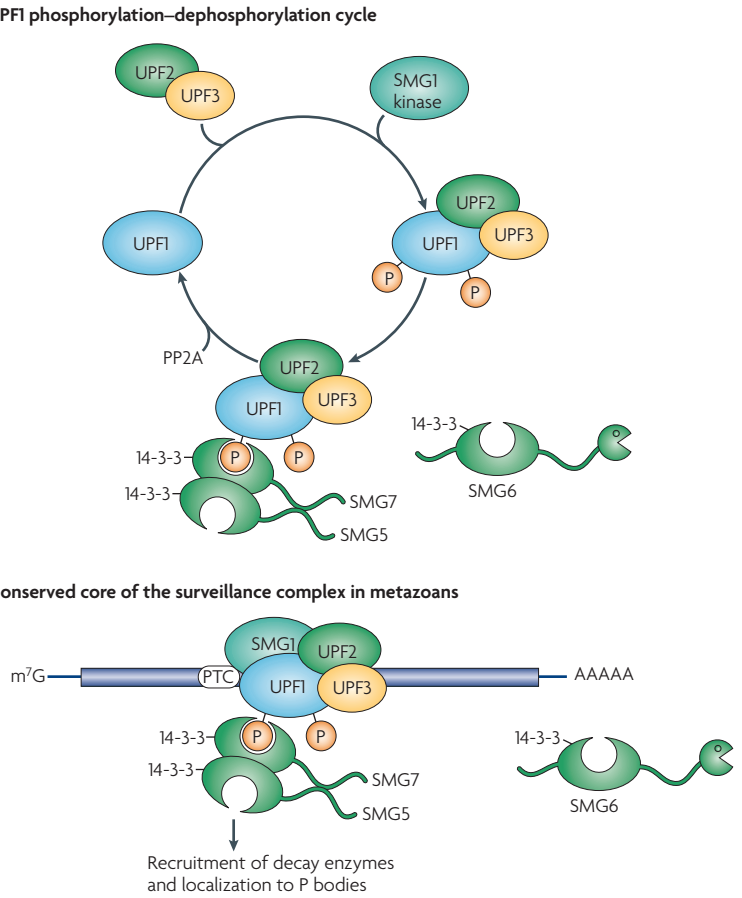


Figure 2 | **Molecular link between NMD and P bodies. a** | UPF1 is a conserved RNA helicase that is essential for nonsense-mediated mRNA decay (NMD). UPF1 has N- and C-terminal extensions with multiple serine residues that are targets for phosphorylation. Phosphorylation of UPF1 is catalysed by SMG1, a phosphoinositide-3-kinase-related protein kinase, and requires UPF2 and UPF3. Dephosphorylation of UPF1 is mediated by SMG5, SMG6 and SMG7, which are three similar, but not redundant, proteins. These proteins are characterized by the presence of a 14-3-3-like domain that binds phosphorylated UPF1, and they trigger UPF1 dephosphorylation by recruiting protein phosphatase-2A (PP2A). **b** | The precise mechanism by which NMD effectors assemble onto mRNAs that terminate translation prematurely is not completely understood and differs among species[36,37,38]. It is generally accepted that UPF1 is recruited by terminating ribosomes and then interacts with UPF2 and UPF3. Formation of the UPF1–UPF2–UPF3 complex on the mRNA triggers UPF1 phosphorylation by SMG1. Phosphorylation of UPF1 leads to the recruitment of SMG7 (most likely in association with SMG5) through specific interactions with the 14-3-3-like domains[45–48]. SMG7 then targets the bound mRNA for decay. Decay might occur in the cytoplasm or in P bodies. Indeed, SMG7 localizes to P bodies and promotes the accumulation of UPF1 and SMG5 in P-bodies[45,46]. Additionally, SMG7 and SMG5 recruit PP2A[47,48], resulting in UPF1 dephosphorylation and dissociation from the 14-3-3-like binding sites (not shown). This probably has a role in the recycling of NMD effectors for a new round of NMD. The role of SMG6 in NMD remains unclear. This protein does not localize to P bodies, but has a C-terminal domain with nuclease activity[103]. m^{7}G, 7-methylguanosine; PTC, premature translation-termination codon.

process. In particular, depletion of GW182 in human or *D. melanogaster* cells relieves translational repression by miRNAs[33,34,57,62]. This has been observed not only for targets that are regulated at the mRNA level, but also for those regulated mainly at the translational level[34,57,62]. Similarly, as discussed above, RCK/p54 has recently been shown to be required for miRNA function[33].

Polysome
Two or more ribosomes that are bound to different sites on the same mRNA.

The role of P-body components in translational repression and degradation by miRNAs shows that these processes are linked. The contribution of each of these processes to miRNA-mediated gene silencing seems to differ for each miRNA–target pair, and it is probably influenced by additional proteins that are associated with the mRNA (which might confer different accessibility to nucleases)[57]. In agreement with this, binding of the specific RNA-binding protein human antigen R (HuR) to the 3′-untranslated region (3′ UTR) of an *miR-122* target can reverse miRNA-mediated silencing under conditions of stress[68].

P-body components not only have a role in translational repression by miRNAs, but are also implicated in translational repression observed under diverse physiological conditions. For example, in *S. cerevisiae*, glucose deprivation leads to a rapid loss of polysomes, an increase in P-body size and number, and the accumulation of repressed mRNAs in P bodies[19,20,32], and these events require Dhh1 and Pat1. In mammals, proteins with established roles in translational repression that localize to P bodies include RCK/p54, the cytoplasmic polyadenylation element-binding protein (CPEB) and eIF4E-T[22,23,33,35,70].

The interplay between translational repression and P-body formation is underlined by the observation that stabilizing mRNAs into polysomes by treatment with cycloheximide (which inhibits translation elongation) leads to P-body loss[8,9,19,20,22,71], whereas drugs or mutations that inhibit translation initiation enhance P-body assembly[19,70,71]. Therefore, mRNAs must exit translation to enter into P bodies, lending additional support to the hypothesis that P bodies are formed by the accumulation of non-translating mRNPs and hence rely on them for their existence.

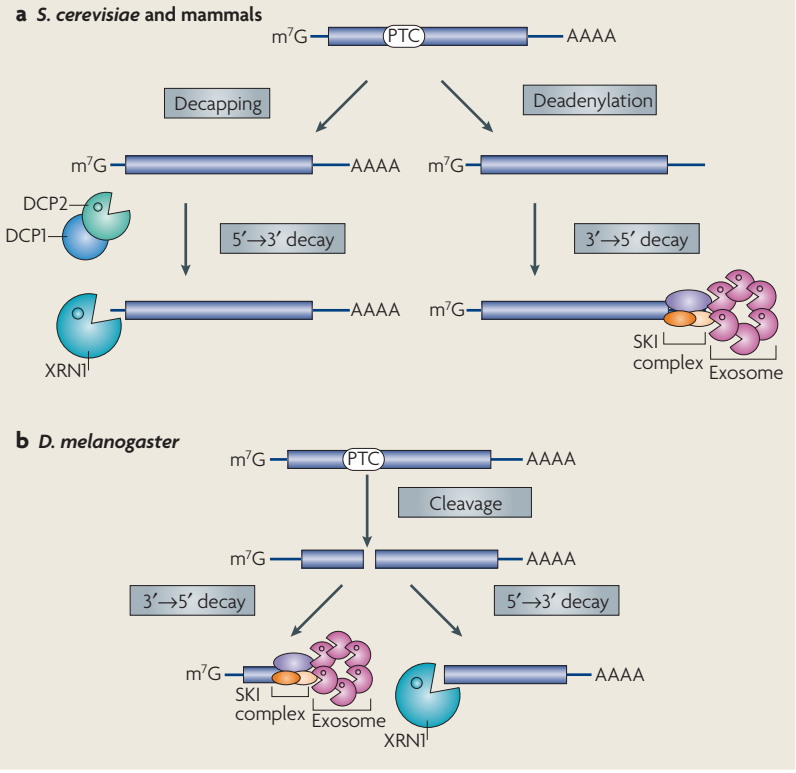
Interplay between P bodies and mRNP granules

In addition to P bodies, large RNP particles that contain dormant mRNAs have been observed in higher eukaryotic cells under stress, during oogenesis and in neuronal cells[71,72] (TABLE 2). Strikingly, these particles share with P bodies certain components and one common function — that of storing non-translating mRNAs. Although a detailed discussion of this topic is beyond the scope of this review, in the following paragraphs we summarize commonalities and differences between these particles and P bodies (reviewed in REFS 71,72; see TABLE 2).

Stress granules. In mammalian cells, different types of stress, including ultraviolet (UV) irradiation, heat shock and oxidative stress inhibit translation of bulk mRNA, which aggregates in cytoplasmic structures known as stress granules (SGs)[35,70–74]. SGs are dynamic and reversible; they assemble in response to environmental stress and disperse after recovery. A striking feature that distinguishes SGs from P bodies is that SGs contain translation-initiation factors and 40S ribosomal subunits[35,70–74]. Furthermore, some proteins are found exclusively in SGs, such as eIF3 and eIF4G, the cytoplasmic poly(A)-binding protein-1 (PABPC1) and the Ras-GTPase-activating SH3-domain-binding protein

Box 3 | Degradation of aberrant mRNAs with nonsense codons

mRNAs with premature translation-termination codons (PTCs, also known as nonsense codons) are degraded by a conserved quality-control mechanism, known as the nonsense-mediated mRNA decay (NMD) pathway[36–38]. Current models indicate that prematurely terminating ribosomes signal the presence of a nonsense codon, and this leads to the recruitment of NMD factors that assemble onto faulty mRNAs to form a surveillance complex (see FIG. 2). The surveillance complex then recruits the general decay enzymes. In *Saccharomyces cerevisiae* and mammals (see the figure, part **a**), PTC-containing mRNAs are degraded by decapping (which is catalysed by the decapping enzyme DCP2 and the co-activator DCP1) and 5′→3′-exonucleolytic degradation by XRN1 (without undergoing deadenylation), or by deadenylation and 3′→5′ degradation by the exosome and the SKI complex[39–43]. In *Drosophila melanogaster* (see the figure, part **b**), degradation is initiated by endonucleolytic cleavage, the resulting RNA fragments are degraded from the newly generated 5′ ends by XRN1 and from the 3′ ends by the exosome and the SKI complex[44]. This implies a link between NMD factors and P-body components. In agreement with this, human SMG7, which is an NMD factor, localizes to P bodies and recruits the surveillance-complex protein UPF1 and another NMD factor, SMG5 (see FIG. 2)[45,46]. Furthermore, UPF1 triggers P-body formation and the accumulation of the other surveillance-complex components UPF2 and UPF3 and NMD substrates in these bodies in *S. cerevisiae* cells that lack DCP1 (REF. 21).



(G3BP) (TABLE 2). Conversely, DCP1, DCP2 and GW182 are found exclusively in P bodies, indicating that SGs and P bodies are functionally distinct[35,70,71].

In spite of these differences, a dynamic link between SGs and P bodies has been observed[35,70,71], and several protein components and mRNA species are shared between these structures. The proteins RCK/p54, CPEB, XRN1, eIF4E, Fas-activated serine/threonine phosphoprotein (FAST) and tristetraprolin (TTP) are present in P bodies, but relocalize to SGs in stressed cells[35,70,71]. The T-cell intracellular antigen-1 (TIA-1) and TIA-1 related (TIAR) proteins are found predominantly in SGs, but a small fraction is also detected in P bodies[35,70,71]. More importantly, a close association and fusion events between P bodies and SGs have been observed in living cells[35,70]. Finally, the engulfment of P bodies by large SGs has been observed in cells that overexpress CPEB, which provides evidence for the exchange of components between these bodies[70].

A remarkable common feature of mammalian P bodies and SGs is that they are both dispersed by drugs that stabilize polysomes (for example, cycloheximide), and their numbers and size are increased by drugs that release ribosomes from mRNA (for example, puromycin)[8,9,19,20,22,35,70,71], which supports the notion that mRNAs must exit the translation cycle to enter SGs or P bodies. Because mRNAs in SGs, but not in P bodies, are associated with translation-initiation factors, it is reasonable to assume that mRNAs that have been released from polysomes in stressed cells are first directed to SGs[35]. After cell recovery from stress, these mRNAs might once again be actively translated or be subject to further remodelling steps in which translation factors and PABPC1 are stripped off the mRNA and proteins that specify mRNA degradation and P-body localization are recruited.

It is currently unclear whether there are SGs in *S. cerevisiae* cells, but the observation that P-body formation in *S. cerevisiae* is enhanced under several stress conditions[19] indicates that yeast P bodies might represent ancestral structures with roles in both the presence and absence of stress. During evolution, P bodies in multicellular organisms might have acquired additional components, such as components of RNA-silencing pathways that are absent in *S. cerevisiae*, and the constitutive and stress functions of these bodies might have segregated into distinct structures.

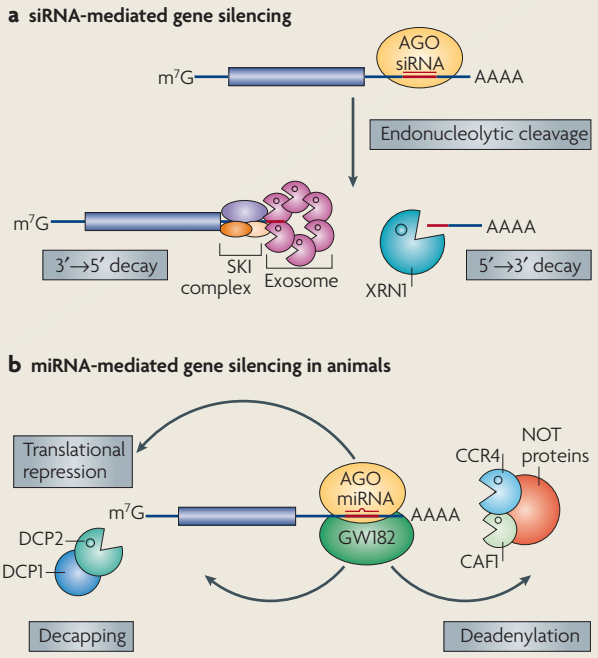
Germ-cell mRNP granules. During oogenesis, many maternal mRNAs are translationally repressed and transported to different locations in the oocyte. Some are directed to the posterior pole where they form polar granules or germinal granules, which are important determinants of the germ line in several organisms[71,72]. In the cytoplasm of nurse cells in insects, where maternal mRNA synthesis takes place, and during transport, some maternal mRNAs are found in large mRNP granules of different appearance and composition, such as sponge bodies and nuage (TABLE 2). Proteins that are associated with maternal mRNP granules that are also present in P bodies include eIF4E, Me31B (CGH-1), the double-stranded RNA-binding protein Staufen, and the *D. melanogaster* and *C. elegans* proteins Tral and CAR-1, respectively, which are highly related to RAP55 (REFS 12,75–81) (TABLE 2). Staufen was initially identified as an essential component of maternal mRNPs in *D. melanogaster* oocytes[72,81]. More recently, Staufen has been shown to be present in stress and neuronal granules in mammals[82] and in P bodies in somatic *D. melanogaster* and human cells (REF. 71, A.E. and E.I., unpublished observations).

A recent study revealed that the decapping co-activator DCP1 (but not the DCP2 enzyme) is also a component of posterior localized mRNP granules in *D. melanogaster*[83]. The absence of DCP2 in these granules indicates that DCP1 might have other roles in addition to its stimulatory

Nurse cell
An auxiliary cell that supplies the oocyte with synthesized mRNAs and proteins during insect oogenesis.

Box 4 | **RNA-mediated gene silencing**

Small interfering RNAs (siRNAs) and microRNAs (miRNAs) differ in their biogenesis and origin. siRNAs are generated by the processing of long double-stranded RNA molecules of diverse origin by Dicer, whereas miRNAs are processed from genome-encoded highly structured transcripts. Plant miRNAs and siRNAs trigger endonucleolytic cleavage of complementary targets (see the figure, part **a**). Cleavage is catalysed by Argonaute (AGO) proteins[52,53], and the resulting mRNA fragments are degraded by XRN1, the exosome and the SKI complex[55,56]. By contrast, animal miRNAs are not fully complementary to their targets. They promote accelerated deadenylation by the CAF1–CCR4–NOT deadenylase complex and decapping by the DCP1 and DCP2 enzymes of at least a subset of target mRNAs[34,57–59] (see the figure, part **b**). This requires AGO proteins and the P-body component GW182 (REFS 34,57). In agreement with this, AGO proteins, miRNAs and miRNA targets have been localized to P bodies[34,57,61–69]. Also, miRNAs repress translation, with or without affecting the amounts of mRNA. The mechanism by which miRNAs repress translation of their targets remains controversial[3].



role in decapping. Alternatively, the decapping function of DCP1 might only be required at early embryogenesis when maternal mRNAs are degraded. Note that in *C. elegans* it is DCP2 that localizes to polar granules[84]. So, it seems that maternal mRNAs are transported with a partially pre-assembled but inactive degradation machinery, and this might facilitate degradation of these mRNAs at the zygotic transition[83].

Interestingly, the chromatoid body, which is a perinuclear granule that is localized in the cytoplasm of mammalian male germ cells, shares components with somatic P bodies and *D. melanogaster* nuage, including Argonaute proteins, miRNAs and DCP1, which indicates a role for both types of body in post-transcriptional regulation[85].

Neuronal granules. In neurons and in most other polarized cells, mRNAs are transported to specific cytoplasmic locations, for example, into axons or dendrites. During transport, these mRNAs are repressed and assembled into large particles that, in contrast to SGs and P bodies, are thought to contain both large and small ribosomal subunits[71,72,86]. Given the functional similarity of these particles with those observed during oogenesis (that is, mRNA repression and transport), it is not surprising that they share common components, including CPEB and Staufen[81,82,87–90].

It would be of interest to determine whether additional P-body components are associated with neuronal mRNPs and, in particular, whether mRNA-degradation enzymes are transported along with their future substrates. Indeed, localized mRNA decay, together with localized mRNA translation, could represent important mechanisms by which asymmetrical spatial and temporal distribution of cellular components is achieved.

Decay of mRNAs with AU-rich elements

The most notable example of integration of post-transcriptional regulation is perhaps provided by mRNAs that contain adenosine and uracil (AU)-rich elements (AREs). AREs are *cis*-acting RNA elements that are usually located in the 3′ UTR of short-lived mRNAs, which encode transiently expressed proteins such as cyclins, cytokines, growth factors and proto-oncogenes. These mRNAs have been shown to undergo rapid decay by the so-called ARE-mediated mRNA decay (AMD) pathway[91]. The effects of AREs on mRNA half-lives are mediated by several ARE-binding proteins. Some promote rapid decay, such as the ARE-binding protein TTP and butyrate response factor-1 (BRF1); others stabilize the transcript, such as HuR; and at least one AU-binding protein (AUF1 or heterogeneous nuclear (hn)RNP D) can stabilize or destabilize the transcript depending on the isoform[91].

Several studies indicate that ARE-containing mRNAs are degraded primarily in the 3′→5′ direction by the exosome[92–94], but recent studies have shown that 5′→3′ mRNA decay also has an important role in degrading ARE-containing mRNAs in mammalian cells[14,95,96], which is similar to observations in *S. cerevisiae*[97,98]. Consistently, depletion of XRN1 or LSm1 stabilizes ARE-containing mRNAs[23,96].

In agreement with a role for the 5′→3′ mRNA-decay pathway in AMD, TTP localizes to P bodies (and SGs) and interacts with P-body components in mammalian cells[14,95,99]. Also, depletion of the P-body component eIF4E-T, which is thought to be involved in translational repression, inhibits AMD[23,96]. By contrast, the knockdown of GW182, which causes P-body disruption, does not affect AMD[96], which indicates that

Dicer
An RNase III-type nuclease that is required for the processing of double-stranded-RNA precursors into siRNAs.

ARE-mediated mRNA decay
(AMD). A process of rapid degradation of mRNAs that harbour A- and U-rich sequence elements (AREs) that are generally located in the 3′-untranslated region of the mRNA.

Table 2 | **Common and unique components of mRNP granules**

Name	Organism	Brief description	Protein components common to P bodies	Unique protein components	References
Stress granules	Mammals	Cytoplasmic granules that are induced in response to environmental stress	CPEB, eIF4E, FAST, RAP55, RCK/p54, Staufen, TIA-1, TIAR, TTP, XRN1	eIF2, eIF3, eIF4G, FMRP, G3BP, HuR, PABPC1, Smaug, SMN, TRAF2	12,35,70–74, 100,110, 111
P granules	*Ce*, *Dm* (polar granules), *Xl* (germ-cell granules), other amphibians (dense bodies)	Granules present in germ cells or germ-cell precursors that contain maternal mRNAs and proteins required for germ-cell specification or differentiation	CAR-1, DCP1, DCP2, eIF4E, Me31B, Staufen, Tral	eIF5A, GLD2 poly(A) polymerase, Oskar, Tudor, Vasa	12,75–84, 112–114
Sponge bodies	*Dm*, *Xl* (mitochondrial cloud)	Cytoplasmic subcellular structures in nurse cells and oocytes, which might function as an intracellular compartment for assembly and transport of maternal products involved in RNA localization	Me31B	Exu, YPS	76,77,115
Nuage (fibrous bodies)	*Dm*	Electron-dense granules that are localized to the cytoplasmic face of the nuclear envelope in nurse cells	Not known	Aubergine, Bruno, Maelstrom, Spindle-E, Tudor, Vasa	116–121
Chromatoid body	Mammals	Electron-dense granules that are localized to the cytoplasmic face of the nuclear envelope in postmeiotic spermatids	Argonaute proteins, DCP1a, miRNAs	Dicer, VASA	85
Neuronal granules	Mammals	Dense granules present in neurons that transport mRNAs into dendrites for subsequent site-specific use at synapses	CPEB, eIF4E, Staufen	eIF2, HuR	82,86,122, 123

Ce, *Caenorhabditis elegans*; CPEB, cytoplasmic polyadenylation element-binding protein; *Dm*, *Drosophila melanogaster*; eIF, eukaryotic translation-initiation factor; FAST, Fas-activated serine/threonine phosphoprotein; FMRP, fragile-X mental retardation protein; G3BP, Ras-GTPase-activating SH3-domain-binding protein; HuR, human antigen R; miRNA, microRNA; mRNP, messenger ribonucleoprotein; PABPC1, poly(A)-binding protein-1; RAP55, RNA-associated protein of 55 kDa; SMN, survival of motor neurons; TIAR, T-cell intracellular antigen-1 (TIA-1) related; TRAF2, tumour necrosis factor receptor-associated factor-2; TTP, tristetraprolin; *Xl*, *Xenopus laevis*.

decapping and the 5′→3′-decay enzymes that are present in the diffuse cytoplasm are sufficient for AMD, which therefore does not require the presence of visible P bodies.

miRNAs, and consequently the Argonaute proteins, have also been implicated in ARE-mediated mRNA decay[100]. Jing *et al.*[100] showed that RISC complexes are directed to ARE-containing mRNAs by imperfect base pairing between *miR-16* and AREs, and this indirectly stabilizes the binding of TTP. Although it remains to be seen whether miRNAs have a role in the decay of all ARE-containing mRNAs, this finding highlights how different post-transcriptional cellular processes are integrated and cooperate to regulate mRNA stability.

P bodies are dynamic and reversible structures

Many P-body components interact to form multimeric protein complexes. In *S. cerevisiae*, DCP1 interacts with DCP2 (REF. 24), whereas in human cells a complex that consists of DCP1, DCP2, Ge-1, RCK/p54 and EDC3 has been found[14]. Pat1 interacts with Dhh1, XRN1 and the LSm1–7 complex[28,29]. GW182, RCK/p54, DCPs and Argonaute proteins associate in an RNA-independent manner[33,34,62–66], and TTP and UPF1 co-immunoprecipitate with DCP1, DCP2, Ge-1 and EDC3 (REF. 14). These interactions imply that the accumulation of proteins and associated mRNAs in P bodies might not result from an active targeting process that involves specific carriers, but could reflect their inherent affinity for each other.

Nevertheless, P bodies are unlikely to represent non-specific precipitates of RNP complexes because their number and size changes dynamically in response to different cellular conditions, and proteins and mRNAs can enter and be released from P bodies in a reversible manner[19,20,32,35,68,70]. That P bodies are highly dynamic structures is shown by the observation that their number and size changes throughout the cell cycle. P bodies are larger and more abundant in late S and G2 phases, but are absent in mitotic cells[10,18]. Similarly, the number of P bodies is high in proliferating cells and low in quiescent cells[18]. In *S. cerevisiae*, P-body assembly is triggered by a range of cellular stresses including glucose deprivation, osmotic stress and UV irradiation[19,20].

Although many details regarding P-body assembly (and disassembly) remain to be discovered, it is possible to envisage several mechanisms for these processes. P-body assembly could be facilitated by protein–protein interactions among P-body components bound to non-translating mRNPs. The absolute requirement of RNA for P-body formation could be explained if additional interaction surfaces were exposed or the affinities between P-body components increased on RNA binding. Furthermore, many P-body components are large,

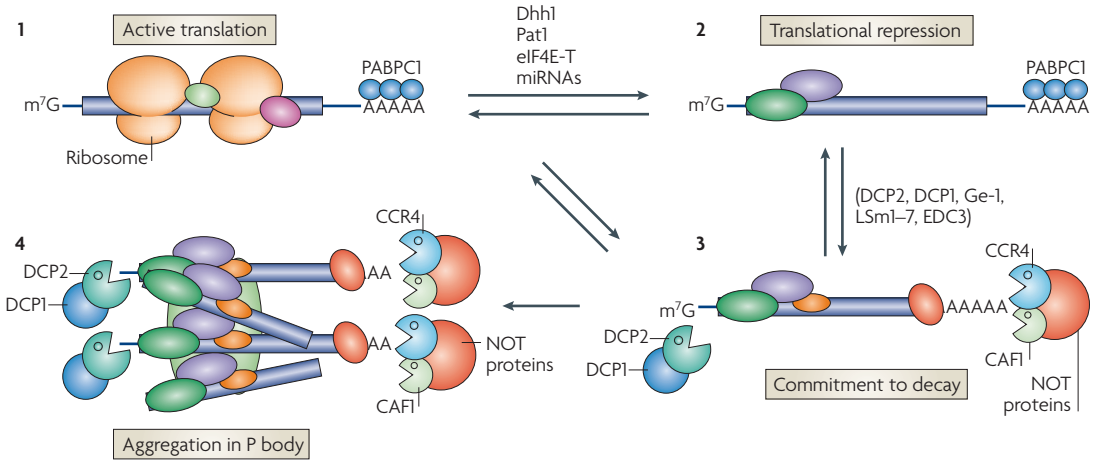


Figure 3 | **Model for P-body assembly.** Messenger ribonucleoproteins (mRNPs) can be in a translationally active state that is associated with polysomes (**1**) or in a translationally inactive state (**2–4**). The transition of mRNPs from a translationally active to an inactive state requires the dissociation of translation factors and the binding of translational repressors (for example, the eIF4E transporter (eIF4E-T)) and proteins that are generally referred to as decapping co-activators (for example, the RNA helicase Dhh1 (also known as RCK/p54, Me31B or CGH-1) and Pat1 (also known as CG5208)). In the figure, the transition between a translationally active and translationally inactive state is illustrated by different proteins bound to the mRNA (the light-green and pink ovals in **1** represent proteins associated with actively translated mRNAs, whereas the green and purple ovals in **2** represent translational repressors and cofactors). Translational repression can also be mediated by the binding of microRNAs (miRNAs) to complementary sequences that are usually present in the 3′-untranslated regions (3′ UTRs) of mRNAs (not shown). The transition between a translationally active and an inactive state is reversible, and repressed mRNPs can eventually re-enter a pool in which active translation occurs. Alternatively, additional proteins that commit the mRNP to degradation might bind (**3**). These include the decapping enzyme DCP2; decapping co-activators such as DCP1 , Ge-1 (also known as Hedls or RCD-8), enhancer of decapping-3 (EDC3; also known as LSm16) and the LSm1–7 complex; and the CCR4–CAF1–NOT deadenylase complex. Repressed mRNPs and mRNPs that are marked for decay (represented by the orange and red ovals) might have an inherent affinity for each other and aggregate to form P bodies (**4**). It is proposed that P-body formation reinforces translational repression and/or the commitment to decay, although these processes are probably initiated prior to the recruitment of mRNPs into P bodies. m^7G, 7-methylguanosine; PABPC1, poly(A)-binding protein-1.

multidomain proteins that could bind more than one RNA molecule or RNP simultaneously, bringing several components into close proximity and thereby nucleating the formation of P bodies.

GW182 and Ge-1 are good candidates for a scaffolding role in P-body assembly, because their depletion leads to the dissolution of P bodies, whereas their overexpression leads to the formation of large P bodies[10,11,13,18]. Consistently, the expression levels of GW182 parallels the presence of P bodies in mammalian cells: GW182 is highly expressed in late S and G2 phases and in proliferating cells, and is expressed in low amounts in mitotic or quiescent cells[10,11,18]. GW182 is a phosphorylated protein[10], which indicates that its ability to nucleate P-body formation might be regulated. Nevertheless, there is no obvious GW182 orthologue in *S. cerevisiae*[34], indicating that several mechanisms probably contribute to the assembly of P bodies.

In addition to GW182 and Ge-1, the depletion of LSm1, RCK/p54, eIF4E-T and proteins that are involved in miRNA processing, such as Drosha and its binding partner DGCR8, results in the loss of mammalian P bodies[22,23,33,69]. How can the absence of these different proteins affect P-body integrity? A possible answer to this question is that P-body assembly might require a crucial concentration of non-translating mRNPs (which are the building blocks of P bodies) in the cytoplasmic compartment. In the absence of LSm1, RCK/p54 or eIF4E-T, or in the absence of a functional miRNA pathway, the concentration of repressed mRNPs in the cytoplasm is reduced below the threshold that is required for P-body assembly. Indeed, RCK/p54 (Dhh1) functions as a general translational repressor in *S. cerevisiae*, *Xenopus laevis* oocytes and human cells[32,33,101,102]. A similar function has been attributed to eIF4E-T[22,23]. The LSm1–7 complex has a role in the transition of mRNAs from active translation to degradation in *S. cerevisiae*[29–31]. The role of this complex in higher eukaryotes is not completely understood, but it probably differs from that of its yeast counterpart because depletion of LSm1 in human cells disperses P bodies[22,33], whereas in *S. cerevisiae*, it increases the number and size of P bodies[8,27].

The observation that blocking the miRNA pathway leads to P-body disassembly in human cells is surprising, because it indicates that miRNA targets represent a significant fraction of repressed mRNPs in these cells. It would be of interest to determine whether P-body formation can be induced independently of the miRNA pathway, for example, by treating Drosha-depleted cells with puromycin (to generate repressed mRNPs independently of the miRNA pathway).

Reinforcing commitment to repression or decay

Based on the observation that P-body assembly requires non-translating mRNAs and that ribosomes or translation factors are not present in P bodies, it has been proposed that a crucial step in post-transcriptional control is the transition of mRNPs from a translationally active state associated with polysomes to a translationally inactive state[32] (FIG. 3; reviewed in REF. 3). Exit from the translation cycle probably involves a structural remodelling of the mRNP in which translation factors dissociate and translational repressors and decapping co-activators are recruited[22,28,31,32]. These rearrangements in mRNP composition could be facilitated by RNA helicases, such as RCK/p54 (Dhh1), or by the recruitment of proteins such as Pat1, LSm1–7 or eIF4E-T[22,23,32,33,101,102].

Repression is in many cases reversible and mRNPs can exit from the repressed state and re-enter a pool in which active translation occurs[20,32,68]. Alternatively, mRNPs can undergo further rearrangements that promote binding of additional proteins that commit them to degradation (FIG. 3). Repressed mRNPs and mRNPs marked for decay might have an inherent affinity for each other and aggregate to form P bodies. Sequestration of these mRNPs in P bodies probably reinforces the repressed state by shielding the mRNP from the translation machinery. Therefore, rather than having a role in the establishment of mRNA repression, gene silencing or the initiation of mRNA decay, the function of P bodies might be to reinforce and maintain the repressed state of mRNA or to irreversibly commit an mRNA for degradation.

Concluding remarks

Although P-body components have key roles in post-transcriptional gene regulation, an important question that remains unanswered is whether the environment of large, microscopically visible P bodies is required for regulation to occur or whether these processes take place as efficiently in the diffuse cytoplasm or in non-detectable sub-complexes. Recent studies have indicated that P-body integrity might not be required for RNA silencing and AMD in mammalian cells[33,96]. Therefore, an important goal is to determine whether the spatial confinement of mRNA-decay intermediates and repressed mRNPs in P bodies is required for cellular functioning or rather reflects the consequence of cellular activity.

It seems clear that many details remain to be discovered regarding the interplay between P bodies and other mRNP granules and the mechanisms by which assembly and disassembly of these structures is regulated. A fascinating question is how common protein and RNP components are sorted and specific markers segregated among these granules. To address these questions, a quantitative assessment of the fractionation and flux of components among these structures in living cells is required. The composition of P bodies is more diverse in higher eukaryotes than in yeast, which raises the question of whether yeast P bodies represent ancestral structures. A better understanding of how P bodies evolved and additional mRNP granules emerged in multicellular organisms is also likely to provide important insights into the roles and relationships of these different mRNP granules.

A significant future challenge will be to unravel the remodelling steps that underlie the transition of an mRNP from the exit of translation to degradation. The complete repertoire of proteins that are involved in post-transcriptional regulation should be established, and their molecular functions and interactions determined, so that in the not too distant future it might be possible to predict the fate of an mRNP by knowing its complement of associated proteins.

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Acknowledgments
We are grateful to D.J. Thomas for comments on the manuscript. This study was supported by the Max Planck Society and the Human Frontier Science Program Organization (HFSPO). A.E. and I.B-A. are recipients of fellowships from the Portuguese Foundation for Science and Technology and the European Molecular Biology Organization (EMBO), respectively.

Competing interests statement
The authors declare no competing financial interests.

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