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Proteomic composition of eukaryotic and bacterial RNA decay condensates suggests convergent evolution

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Abstract

Bacterial cells have a unique challenge to organize their cytoplasm without the use of membrane-bound organelles. Biomolecular condensates (henceforth BMCs) are a class of non-membrane bound organelles, which through the physical process of phase separation, can form liquid-like droplets with proteins/nucleic acids. BMCs have been broadly characterized in eukaryotic cells, and BMCs have been recently identified in bacteria, with the first and best studied example being Bacterial RNP-bodies. BR-bodies contain the RNA decay machinery and show functional parallels to eukaryotic p-bodies and stress granules. Due to the finding that mRNA decay machinery is compartmentalized in BR-bodies and in eukaryotic P-bodies/stress granules, we will explore the functional similarities in the proteins which are known to enrich in these structures based on recent proteomic studies. Interestingly, despite the use of different mRNA decay and post-transcriptional regulatory machinery, this analysis has revealed evolutionary convergence in the classes of enriched enzymes in these structures.

Keywords

biomolecular condensate; mRNA decay; RNase E; BR-bodies

INTRODUCTION

RNP BMCs are organized via phase separation

Historically, membrane-less compartments have been described as granules, foci, cellular bodies, cellular aggregates, and bodies. Recently, the general term “biomolecular condensates” (BMCs) has been proposed to describe their ability to spatially organize

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biomolecules[1]. BMC formation is most often driven by the physical process of phase separation, whereby the macromolecular mixture separates into two phases: a dense phase of concentrated molecules and a dilute phase (Fig1)[1,2]. Phase separation is facilitated by "driver" proteins/nucleic acids with multivalent weak interactions occurring in a homotypic fashion or in combination with other molecules in heterotypic fashion[3]. Phase separation is often promoted by proteins with large intrinsically disordered regions (IDRs), but IDRs are not present on all drivers of phase separation[4]. BMCs can have various material properties, ranging from liquid-like to gel-like or solid-like, and can achieve various levels of macromolecular complexity[5]. Drivers typically act as scaffolds with other protein/nucleic acid "client" binding sites (Fig1)[6,7]. Clients cannot phase-separate on their own, and recruitment occurs through direct interactions with the driver/scaffold or indirect associations with another client [1,6]. Some BMCs have been found to enrich hundreds of different clients[8–11], and some form complex shell-core like architectures[12]. Further, BMCs are dynamic structures and provide semi-permeable barriers that enable constituent molecules to rapidly exchange between BMCs and the surrounding dilute phase on a time scale of seconds to minutes[1]. By concentrating specific biomolecules, BMCs help to promote biochemical pathways and prevent unfavorable reactions inside the cell[1,7,13]. Additionally, BMCs sequester molecules from their enzymes to inhibit biochemical activity, differentially partition diverse substrates to increase substrate specificity, or can buffer fluctuations in cellular concentration of molecules[1,14–16]. In eukaryotes, BMCs have been found in most cellular compartments and extracellular milieu, and bacterial BMCs have been found in the cytoplasm, periplasm, and external structures like biofilms[17–19]. Despite the protein-centric view of BMCs, RNA or polyphosphate also appear to be able to form BMCs[20], suggesting that a diverse array of biomolecules may be capable for driving condensation. The largest class of BMCs are ribonucleoprotein (RNP) BMCs, which will be the focus of this review[1,21–23].

Most of what we know about RNP BMCs has been revealed in eukaryotic systems. RNP BMCs have been reported in the cytoplasm, nucleus, mitochondria, and chloroplasts of eukaryotic cells and are involved in a broad range of biological functions including gene expression, RNA metabolism, and signaling[1,23,24]. The nucleolus, cajal bodies, and paraspeckles are located inside the nucleus of eukaryotic cells and function in RNA metabolism and various aspects of gene expression[1]. Cytoplasmic BMCs such as P-bodies (PBs) and stress granules (SGs) are thought to regulate RNA degradation, mRNA translation, signaling pathways, and stress responses[1,25]. Mitochondrial degradosome-foci (D-foci) have been found to play a role in mitochondrial RNA degradation[26]. In bacteria, RNP BMCs have been observed in the cytoplasm and in the nucleoid[18,27]. RNA polymerase and Rho phase separate, suggesting that transcription can be compartmentalized in BMCs[28,29]. In addition, Hfq and RNaseE which are involved in mRNA regulation and decay have also been identified to form BMCs[17,30]. Finally, DEAD box RNA helicases (DDXs) from eukaryotes and bacteria form BMCs, suggesting their role in BMC organization is conserved[31]. These observations indicate that RNP BMC organization is broadly utilized in both eukaryotes and prokaryotes, in both RNA biosynthesis as well as RNA processing/decay. Further, condensates have been shown to improve the catalytic activity of ribozymes[32], suggesting condensates were present in the RNA world prior to modern cells.

General properties of RNA decay BMCs

The RNA decay machinery is compartmentalized in BMCs in eukaryotes, bacteria, and in mitochondria and chloroplasts (Table I), suggesting biomolecular condensation of RNA decay machinery is an ancient organizational strategy. Since the early 1990s, both SGs and PBs were found to contain mRNA decay machinery and have been topics of in-depth studies due to their importance in human disease[33,34]. SGs and PBs were subsequently observed from yeast to mammalian and plant cells[35,36], suggesting they are conserved across eukaryotes. PBs are found constitutively in unstressed cells and increase in size and number when subjected to stress conditions and recognized as the sites of mRNA degradation/storage in the cell[37]. Moreover, the major cytoplasmic 5' - 3' exonuclease Xrn1 accumulates in PBs and SGs. SGs have been observed to occur across various stresses, but in contrast to PBs are not constitutively present in unstressed cells. Due to the broad distribution of PBs and SGs, these structures have been proposed to be utilized across all eukaryotic cells. Organellar mRNA decay BMCs originated from bacterial endosymbiosis and have been found in mitochondria and in chloroplasts[26,38]. D-foci have been suggested to be constitutive and the site for the mitochondrial RNA degradation containing the mitochondrial-degradosome components SUV3 and PNPase[26], which are partially conserved with bacterial RNA-degradosomes[39]. Chloroplast stress granules (cpSGs) are mainly associated with mRNAs, poly(A)-binding proteins, and small ribosomal units and are induced upon stress[38,40].

Like eukaryotes, numerous bacterial RNP BMCs have now been identified. In 2018, the first bacterial RNP BMC, Bacterial Ribonucleoprotein-bodies (BR-bodies) were discovered containing the α-proteobacterial RNaseE RNA degradosomes[17]. Initial studies of *E. coli* RNaseE suggested it formed helical filament structures in the cytoplasm[41], however, TIRF microscopy revealed events of foci-fusion[42], suggesting they are RNP BMCs. Additional evidence suggests Cyanobacteria and *M. smegmatis* RNaseEs form RNP BMCs[43–45]. Further, Muthunayake et al. also demonstrated that when comparing bacteria from each major clade that possess RNase E, distinct patterns of charge blocks are present within the IDRs of RNase E which promotes phase separation[46]. While RNaseE is the most common mRNA decay enzyme, some bacteria use other RNases for controlling mRNA decay. In *B. subtilis,* RNaseY initiates mRNA decay and form RNaseY degradome BMCs[47] and *H. pylori* RNaseJ degradosomes form BMCs[48]. Overall, these observations suggest that BR-bodies containing the mRNA decay machinery are broadly distributed across bacteria. Other bacterial RNP BMCs outside mRNA decay also been identified, including RNAP/NusA[29,49], Rho[28], Hfq[20], and DDXs[31], however, "BR-bodies" will be used subsequently to describe "RNA-degradosome" condensates. While many of these RNP BMCs have been only observed in a single bacterial species, BR-bodies appear to be widely distributed across bacteria[46].

Proteomes of P-bodies, stress granules, and BR-bodies suggests convergent evolution

Despite the diverse RNA decay machinery in eukaryotes, their organelles, and bacteria, the proteomes of PBs, SGs, and BR-bodies appear to contain similar types of non-homologous enriched enzymes, suggesting evolutionary convergence. Hundreds of proteins in PBs and SGs have been identified and characterized through purification by particle sorting

or differential centrifugation followed by mass-spectrometry or by enzyme-catalyzed proximity labeling followed by mass spectrometry[8–11]. Over 100 proteins were also identified in *C. crescentus* BR-bodies isolated via differential centrifugation followed by mass-spectrometry[50]. Analysis of their proteomes revealed several categories of enriched proteins involved in many posttranscriptional processes including DDX, siRNA/miRNA/sRNA factors, major mRNA decay nucleases (RNaseE and Xrn1), accessory ribonucleases, translation repressors, and components of the deadenylation complex are present in PB, SG, and BR-body BMCs (Table II). Surprisingly, 5'-decapping enzymes are present in PBs and SGs, but bacterial 5'-decapping enzymes are not enriched in BR-bodies (Table II). The enrichment of decapping enzymes in PB and SG has been suggested to the presence of decapped translationally repressed mRNAs in these structures. While bacterial mRNAs do not contain a canonical m^7G cap, decapping enzymes RppH and NudC also are crucial for the stability of mRNAs where they can remove 5'-PPP or 5'-NAD(H) from RNA[51–53] and resulting 5'-P ends have enhanced cleavage by RNaseE[52], but these enzymes are not enriched in BR-bodies[50]. While it is known that RNaseE cleavage is accelerated with a 5'-P, the coordination of decapping with BR-bodies is not yet understood.

Heterogeneous client composition in BMCs

While most of the proteomics experiments are performed in bulk, this approximates each BMC as a homogenous mixture of the enriched proteins. However, an interesting finding is that, while a subset of BR-body proteins appears to uniformly be enriched in BR-bodies, certain BR-body enriched proteins are present in a subset of BR-bodies leading to heterogeneity in BR-body clients (Fig2). RhlB, Aconitase, and PNPase are enriched uniformly in BR-bodies and known to stoichiometrically assemble into the RNA degradosome[54]. Additionally, they are colocalized with RNaseE *in vivo* and strongly recruited into RNaseE droplets over other RNP BMCs *in vitro,* suggesting that the RNA degradosome likely composes the core clients within each BR-body. In contrast to the core BR-body clients, accessory proteins, RhlE, Hfq, and RNase D are contained in a subset of cellular BR-bodies (Fig2)[50]. This heterogeneous client composition in BR-bodies may promote distinct biochemical activities. In that way, perhaps BR-body RNA decay activity may be regulated upon the recruitment of these specialized factors.

In Eukaryotic PB and SG, evidence also exists which suggests specialization. The PB proteome revealed that PB proteins form a dense protein network which is three times denser than the SG protein network[9]. This network is mostly composed of RNA binding protein (up to two thirds of PB enriched proteins) including many DDXs (4 times more compared to SG enriched helicases) suggesting stronger PB importance[9]. Importantly, the protein composition of SGs varies according to stress, cell type, and disease state. For example, 20% of proteins were specific to arsenite-induced SGs vs heat-induced SGs[8,11]. Overall, these observations suggest that SGs assemble with different proteins in response to different stress conditions. The specific stress inducing SGs can also influence the material properties of SGs[55]. In yeast PB, core protein components were found to be highly concentrated and uniformly present in PB[56] and were able to reconstitute *in vitro* with similar dynamics and stoichiometries[57]. When scaffold molecules of PBs were deleted, the partitioning of many PB components was altered, whereas when client molecules

were deleted, only the immediate interacting components of the clients were affected suggesting that deleting scaffold molecules is likely have heterogeneous effects on the PB composition[56]. While PBs contain a more complex core interaction network than BR-bodies, the reconstitution of both structures *in vitro* should allow the functional dissection of how specialized clients might impact biochemical functions.

CONCLUSIONS

All living organisms should have an ability to respond and adapt to ever-changing environmental conditions and survive under stress. BR-bodies globally stimulate bacterial mRNA decay and prevent the accumulation of toxic decay intermediates[17,58]. More like PBs, BR-bodies are constitutive and dynamic and both appear to promote mRNA decay, while SGs promote mRNA storage. Furthermore, Mateju et al., also demonstrated that localized mRNAs into SGs could undergo translation[59]. BR-bodies are dynamic structures under log growth conditions, they are in rapid exchange (Fig1C)[17]. RNA is essential for BR-body assembly, and they dissociate upon RNA degradation[17], suggesting in log-growth they predominantly facilitate RNA decay (Fig1C). While PBs are not required for mRNA decay[60], several studies have suggested that mRNA decay or storage can occur in PBs depending on growth conditions. The size of PBs were found to increase upon decapping enzyme deletion, suggesting that PBs are decay sites for decay[37]. Additionally, a rapid inducible decay of RNA (RIDR) showed that PBs promote faster RNA decay rates[61]. PBs have been found to switch from decay to mRNA storage upon acute Carbon-starvation[62]. In line with a storage function, Hubstenberger *et al.*, suggested that partitioning of mRNAs into PBs is not associated with temporary storage sites[9]. While the bulk of P-body data suggests they can switch from decay to storage upon different cellular conditions, it is still unknown whether the BR-bodies can switch from decay to storage under different conditions/stresses (Fig1C). Similar to SGs which perform storage, BR-bodies were found to be more strongly induced upon various stresses[17]. While BR-bodies have so far been studied extensively in log-growth conditions[17,50,58], it is unclear how their proteomes might change when strongly induced with different stresses. In addition, it is not known how the material properties of BR-bodies might vary in different stress conditions, and whether any changes in dynamics might affect RNA decay activity. As all current BR-body studies have focused on the functions in exponential-growth, it is important to test how activity and dynamics change upon stress.

Decapping enzymes are important components of 5’-end dependent mRNA degradation in both eukaryotes and bacteria. 5’-m^{7}G dictates the fate of mRNAs and decapping is required for cytoplasmic mRNA decay in nearly all eukaryotic mRNAs. While the decapping enzymes are found within PBs, it is poorly understood whether the decapping reaction occurs inside or outside PBs (Fig2B). Similar to eukaryotes, bacterial mRNA decay can initiate by removing the 5’-end modifications by decapping enzymes such as RppH and NudC followed by endonucleolytic cleavage by RNaseE[51–53]. RNaseE contains a 5’-P sensor domain that has affinity for 5’-P. In contrast to PBs, *C. crescentus* decapping enzymes, RppH and NudC are not enriched in BR-bodies, suggesting they function outside BR-bodies (Fig2C). However, it is unclear whether decapping enzymes will functionally impact BR-body phase separation and subsequent mRNA decay.

While eukaryotic and bacterial mRNA decay has been studied for many years, there is limited information about this process in the third domain of life, the archaea. Archaeal mRNA decay machinery resembles the eukaryotic RNA exosome machinery that is functionally similar to bacterial PNPase, however some clades of archaea lack exosome genes[63]. These archaea instead possess a bacterial RNaseR homolog[64], which is also a 3’ to 5’ exoribonuclease. In some archaea, 5’−3’ exoribonucleolytic activity is carried by archaeal RNaseJ[65]. Even though it is difficult to genetically manipulate archaea, recent cell biology advances offer improved genetic tools. Genetic tagging of protein coding genes with fluorescent proteins such as GFP, is now possible, opening the door to future studies to identify whether the RNA decay machinery of archaea way will also form a BMC. As a few eukaryotic and bacterial RNA decay related enzyme homologs have been identified in archaea, is now possible to determine if the archaeal mRNA decay machinery performs RNA decay in BMCs. Studies like these will tell us whether RNP BMCs are utilized across all three domains of life to organize the complex biochemical pathway of RNA decay.

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HIGHLIGHTS

- Similar enzyme functional classes are enriched in P-bodies/Stress granules/BR-bodies.
- RNA decay condensates show evolutionary convergence.
- Decapping enzymes are depleted in BR-bodies but enriched in P-bodies/ Stress granules.
- Heterogeneous RNA decay condensates suggest specialization.

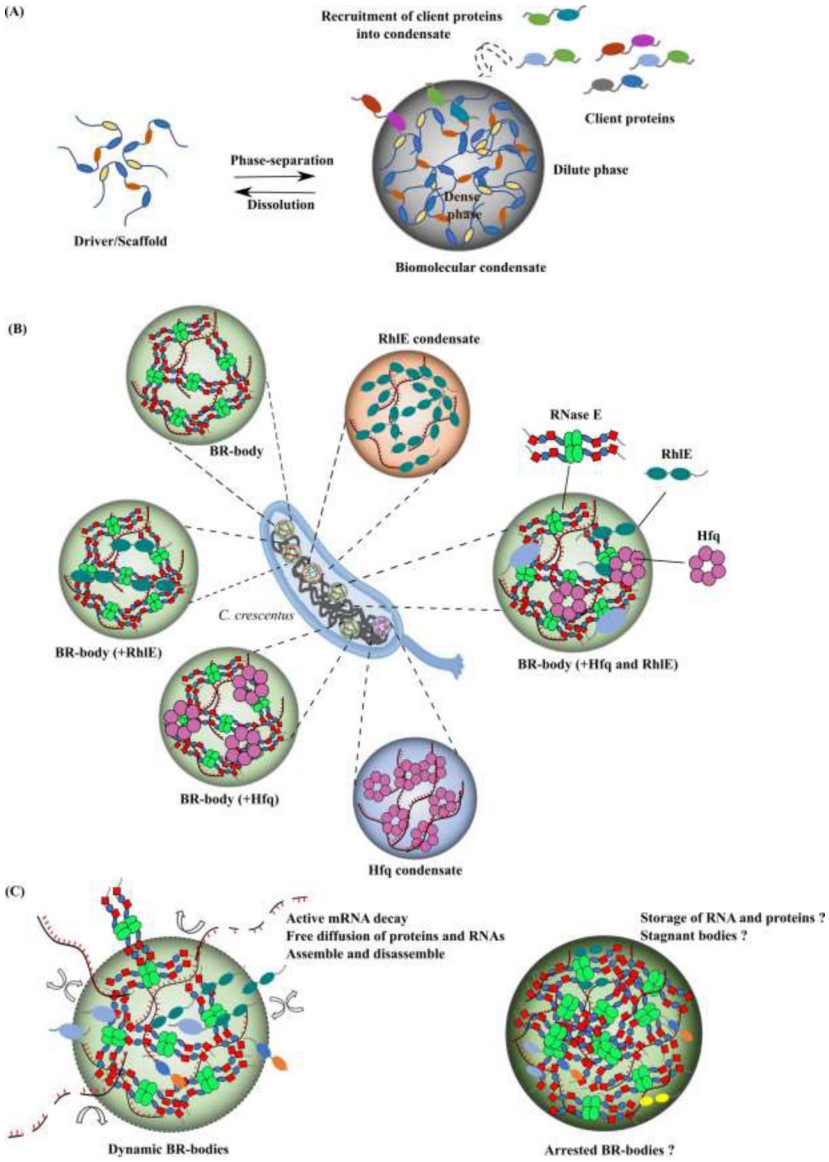

Figure 1.

(A) Schematic of biomolecular condensate phase separation. Biomolecules that undergo phase separation are called drivers/scaffolds. Molecules that interact with scaffolds and are recruited into condensates are called clients. (B) BR-bodies compose heterogeneous client composition within a single cell. The schematic shows BR-body accessory proteins, RhlE, and Hfq are contained in a subset of cellular BR-bodies within a cell and assemble into RhlE and Hfq only condensates. Each cell contains a heterogeneous mixture of condensates, suggesting RNP condensates are specialized to facilitate mRNA processing/decay. (C) Properties of BR-bodies under different cellular environments. (Left) BR-bodies are dynamic structures under exponential growth conditions and they rapidly exchange RNAs and proteins with the surroundings and organize active mRNA decay in bacteria. (Right) It is unknown whether BR-bodies act as storage condensates under different conditions.

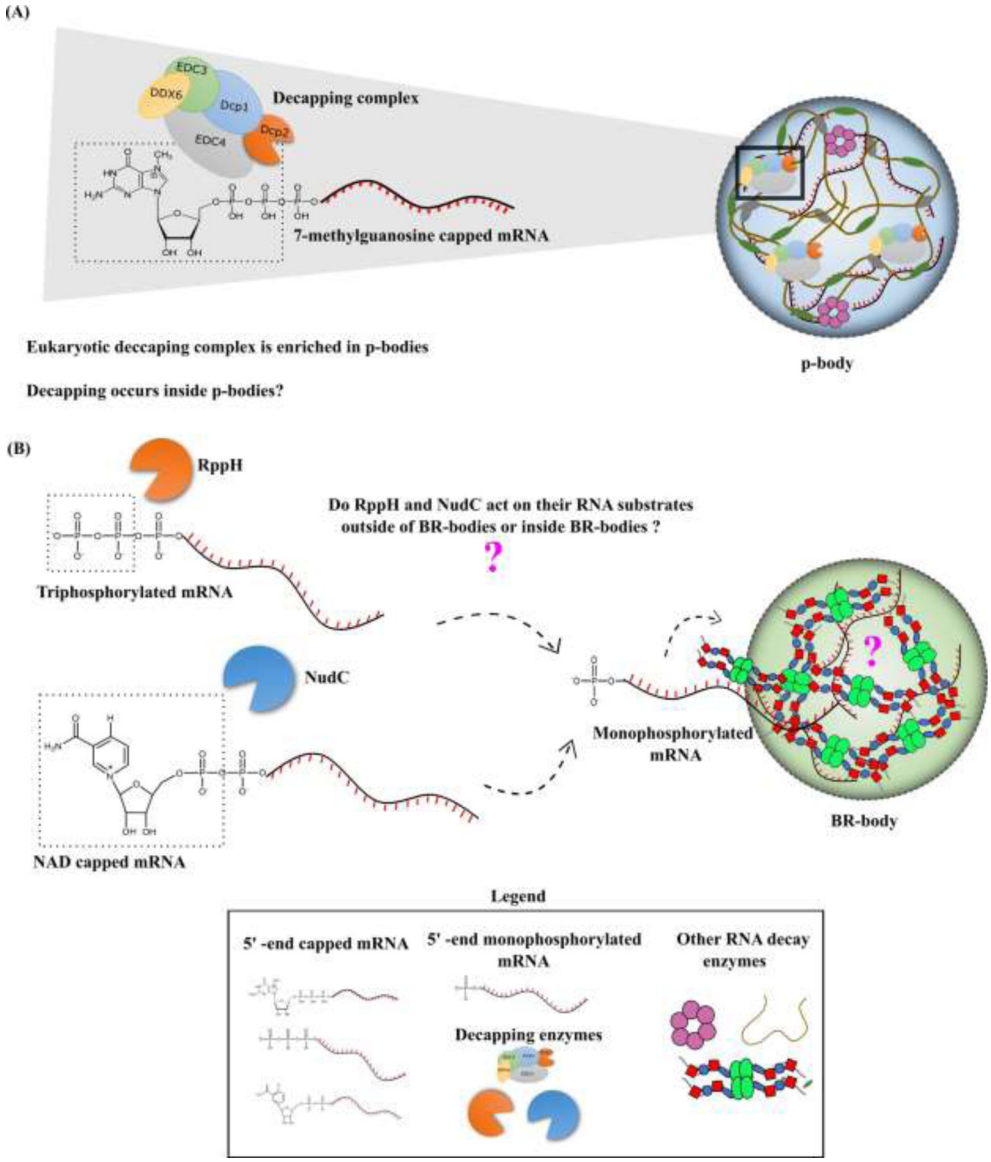

Figure 2.

Key differences in mRNA decay condensates in eukaryotes and bacteria. (A) The eukaryotic decapping complex that remove the 5’ -end 7-methyl guanylate (m7G) cap attached to the 5’-end of mRNA is localized in p-bodies s. (B) Bacterial decapping enzymes RppH and NudC that remove 5’ -end triphosphate (PPP) or nicotinamide adenine dinucleotide (NAD+) modified nucleotides leaving a 5’-end monophosphate (5’P) at the 5’-end are depleted from BR-bodies.

Table I.

Known RNA decay condensates across species (Blue – Animals, Orange – Yeast, Green- - Plants, Yellow – Bacteria).

	RNA decay machinery condensates	Organism
Eukaryotes	P-bodies	*Homo sapiens*[66]
	P-bodies	*Mus musculu*[34]
	P-bodies	*Drosophila melanogaster*[67]
	P-bodies	*Caenorhabditis elegans*[68]
	P-bodies	*Saccharomyces cerevisiae*[37]
	P-bodies	*Schizosaccharomyces pombe*[69]
	P-bodies	*Arabidopsis thaliana*[35]
	Mitochondrial Degradosome Foci(D-foci)	*Homo sapiens*[26]
	Stress granules	*Homo sapiens*[33]
	Stress granules	*Mus musculu*[70]
	Stress granules	*Drosophila melanogaster*[71]
	Stress granules	*Brachionus manjavacas*[72]
	Stress granules	*Trypanosoma brucei*[73]
	Stress granules	*Saccharomyces cerevisiae*[74]
	Stress granules	*Schizosaccharomyces pombe*[75]
	Stress granules	*Aspergilus oryzae*[76]
	Stress granules	*Arabidopsis thaliana*[35]
	Chloroplast stress granules	*Arabidopsis thaliana*[40]
	Chloroplast stress granules	*Chlamydomonas reinhardtii*[38]
Bacteria	BR-bodies	*Caulobacter crescentus*[17]
	BR-bodies	*Agrobacterium tumefaiens*[17]
	BR-bodies	*Sinorhizobium meliloti*[17]
	BR-bodies	*Escherichia coli*[29]
	BR-bodies	*Helicobacter pylori*[48]
	BR-bodies	*Mycobacterium smegmatis*[77]
	BR-bodies	*Mycobacterium tuberculosis*[77]
	BR-bodies	*Synechocystis sp. Strain PCC 6803*[43]
	BR-bodies	*Anabaena PCC 7120*[44]
	BR-bodies	*Bacillus subtilis*[47]

Table II.

Protein clients of RNA decay condensates. Stress granule, P-body, and BR-body proteome table were generated from recent proteomics based studies[8–11,50]. Yeast homologs are indicated in parentheses. RNA substrates of RNA decay condensates[9,58,78].

	Stress granules[8,10,11,78]	**P bodies[8–10]**	**BR-bodies[50,58]**
Rate limiting enzyme	Xrn1	Xrn1	RNase E
DEAD Box RNA helicases (DDXs)	DDX6 (Dhh1), DDX1, DDX3X (Ded1), DDX5, DDX17, DDX19A, DDX47, DDX50, DHX9, EIF4A (Tif1)	DDX6 (Dhh1), DDX1, DDX5, DDX3X, DDX50, DDX41, DDX47, DDX54, DDX18	RhlB, RhlE, DEAD
LSM proteins	LSM14A/B (Scd6), LMS12	LSM14A/B (Scd6), LSM1–7	Hfq
siRNA/miRNA silencing pathway	AGO2, GW182, TNRC6B, EIF3A (Rpg1), MOV10, PUM1	AGO1/2/3, MOV10, ZCCHC3, GW182, TNRC6A/B, PUM1	Hfq
Ribonuclease activity	XRN1, G3BP, SND1, DDX1, CCR4-NOT	XRN1, XRN2, CCR4-NOT	RNase E, PNPase, RNase D
Translation repressors	TIA-1/TIAR (Pub1/Ngr1), Caprin-1, FMRP/FXR1/2, Ataxin-2	CPEB, EIF4E-T	Hfq
Deadenylases/complex	CCR4-NOT	CCR4-NOT	PNPase
Decapping enzyme/ enzyme complexes	DCP1A, EDC4	DCP2, DCP1A, DCP1B, EDC4, EDC3, LSM1–7, DCP1, PAT1, PATL1	***Depleted from BR-bodies (RppH,NudC)***
mRNA	Poorly translated mRNAs	Poorly translated mRNAs	Poorly translated mRNAs
Length of RNA	Long mRNA; containg longer coding and untranslated regions, long noncoding RNAs (lincRNA)	mRNAs with variable poly(A) tail lengths, long noncoding RNAs(lincRNAs),	Long mRNAs
ncRNAs	lincRNAs, small RNAs, antisense RNAs, small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), pseudogene RNAs	antisense RNAs, pseudogene RNAs, small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), lincRNAs, micro RNAs(miRNA), small interfering RNAs (siRNAs)	small regulatory RNAs (sRNAs), antisense RNAs