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Effects of stress and aging on ribonucleoprotein assembly and function in the germ line

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Abstract

In a variety of cell types, ribonucleoprotein (RNP) complexes play critical roles in regulating RNA metabolism. The germ line contains RNPs found also in somatic cells, such as processing (P) bodies and stress granules, as well as several RNPs unique to the germ line, including germ granules, nuage, Balbiani bodies, P granules, U bodies, and sponge bodies. Recent advances have identified a conserved response of germ line RNPs to environmental stresses such as nutritional stress and heat shock. The RNPs increase significantly in size based on cytology; their morphology and subcellular localization changes, and their composition changes. These dynamic changes are reversible when stresses diminish, and similar changes occur in response to aging or extended meiotic arrest prior to fertilization of oocytes. Intriguing correlations exist between the dynamics of the RNPs and the microtubule cytoskeleton and its motor proteins, suggesting a possible mechanism for the assembly and dissociation of the large RNP granules. Similarly, coordinated changes of the nuclear membrane and endoplasmic reticulum may also help unravel the regulatory mechanisms of RNP dynamics. Based on their composition, the RNPs are thought to regulate mRNA decay and/or translation, and initial support for some of these roles is now at hand. Ultimately, the question of why RNP remodeling occurs to such a large extent during a variety of stresses and aging remains to be fully answered, but a current attractive hypothesis is that the plasticity promotes the maintenance of oocyte quality.

INTRODUCTION

Germ granules were identified as a unique component in germ cells during Hegner's observations of insect germ cells over 100 years ago.¹ The visible germ granules are one component of the germ plasm, a term coined by Weismann in 1892, for the substance that is passed down from parental germ cells to the germ cells of the next generation.² The precise role of germ plasm and germ granules in terms of contributing to the specification of germ cell identity or function was not immediately clear to early researchers. The eventual characterization of germ granules as well-conserved ribonucleoprotein (RNP) complexes contributed to hypotheses for functions in regulating mRNA metabolism, in particular post-transcriptional regulation specific to germ cells. The current view is that while germ granules are not required in all organisms to specify germ cell fate, all organisms rely on germ granules for germ cell function. Several excellent reviews summarize our current

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understanding of the structure of key protein components of germ granules,² germ granules in mammals,³ and the conserved components, dynamics, and function of germ granules in female and male germ cells.^{4,5}

Germ granules share several features with other conserved RNP complexes, notably processing (P) bodies. Several proteins localize to both types of RNPs, and both are dynamic structures in terms of their composition, size, and number. Recent investigations of the effects of environmental stress and aging on the germ line have highlighted the plasticity of germ line RNP complexes, including sponge bodies and P bodies in *Drosophila* and large RNP granules in *Caenorhabditis elegans*.^{6–9} This review focuses on describing the dynamic changes that occur in germ line RNPs and comparing and contrasting the changes that occur with different types of stress or aging. While the studies to date point toward novel cellular responses to stress and aging, they may also contribute to our understanding of the role of RNP complexes during normal development.

OVERVIEW OF GERM LINE RNP COMPLEXES

Within the germ lines of vertebrates and invertebrates numerous types of RNP complexes are described that vary in size, subcellular localization, complexity, and function. The best-described RNP complexes fall into two categories: germ granules and processing bodies. Germ granules have been most thoroughly described in female germ cells; however, they are also present in some male germ cells (reviewed in Ref 4), and in both types of primordial germ cells, the germ granules are considered a type of nuage.¹⁰ One of the shared features of many germ granules is the presence of the DEAD-box RNA helicase Vasa. Vasa and its homologs have been linked to a variety of processes in gametogenesis, germ cell specification, and stem cell biology (reviewed in Ref 11). Other characteristics of germ granules include a lack of a membrane, proximity to mitochondria, translationally repressed mRNAs, and RNA-binding proteins. Processing bodies (or P bodies) have been characterized in a myriad of eukaryotic species and cell types, from yeast to mammalian cells.¹² P bodies have roles in mRNA decapping and decay, and in mRNA storage and translational repression^{12–16} (Table 1). They are found in both somatic and germ cells, and some P body components are also observed in germ granules.^{20,21} The fact that some proteins localize to more than one type of RNP complex sometimes leads to an inconsistent use of terms in the literature, and causes confusion especially in understanding how various RNP complexes are related (Box 1). The changes in morphology and composition that occur during development and stress also contribute to the challenge of having a common understanding of the nomenclature and defining characteristics of different types of RNPs. Another confounding factor in synthesizing the literature is the distinction, or lack thereof, between biochemical studies of RNPs *in vitro* and cytologically defined RNPs *in vivo*. Considering the challenges of studying these dynamic structures, these complementary approaches are clearly beneficial²²; however, the intersection of these approaches in most cases is not yet clear.

Diversity of Germ Line RNP Granules

Multiple classes of germ line RNP granules have been characterized in the *Drosophila* germ line based on developmental stage, localization, morphological criteria, and composition. The polar granules are perhaps best known; these RNP complexes are first detected in the posterior of late-stage oocytes and embryos.^{21,23,24} Polar granule assembly in oocytes requires Oskar, a protein with a conserved winged helix-turn-helix fold domain that is predicted to bind double-stranded RNA or may play a scaffolding role in RNP complexes.²⁵ Polar granules are enriched for Vasa, an ATP-dependent RNA helicase.^{26,27} In contrast, sponge bodies are detected earlier in development, in the nurse cells and oocyte of stage 6 egg chambers. They are RNP complexes that were first characterized by their endoplasmic reticulum (ER)-like cisternae or vesicles in a ribosome-free, electron-dense matrix.²⁴ By both immuno-electron microscopy and fluorescence microscopy, sponge bodies are enriched for Exuperantia, a protein required for localization of *bicoid* mRNA, and Me31B, a DEAD-box RNA helicase.^{21,24,28} The translational regulator Bruno is detected at high levels only in sponge bodies of nurse cells; whereas Orb, a regulator of polyadenylation, is concentrated only in sponge bodies of the oocyte.^{6,21,24} Based on their components, the function of sponge bodies appears to include a role in post-transcriptional regulation of gene expression and share similarities with P body function⁶ (Table 1). While both contain the translation regulator Me31B, the decapping protein Dcp1, the translation initiation factor eIF4E, and the eIF4E-binding protein Cup,^{20,29–31} the morphology of P bodies and sponge bodies differs, leading to speculation that they may represent differing stages in the hierarchy of RNP assembly.⁶ Adjacent to the germ line in the *Drosophila* somatic gonad is yet a different type of RNP granule. Cytoplasmic spheres called Yb bodies are detected in both ovaries and testes; they are enriched in the Tudor domain-containing protein Yb and function as a site for Piwi-associated RNA biogenesis.^{32,33}

Germ granules in *C. elegans* are named P granules.³⁴ Like polar granules in fruit flies, P granules contain Vasa homologs: GLH-1, -2, -3, and -4.^{35,36} While several components, including GLH-1 and -2, associate with P granules throughout the development, other components localize to P granules variably, for example, only during early embryonic development. In worms lacking *glh-1* and *glh-2* the germ line fails to develop completely at high temperatures, suggesting that these components are necessary for fertility.³⁵ Interestingly, in *pptr-1* mutants (that lack a regulatory subunit of the PP2A phosphatase) maternal P granules are not asymmetrically distributed to germ line blastomeres, and yet the germ cell fate is still specified normally. Thus, asymmetric segregation of P granules is not required to determine the germ cell fate, a surprising finding given the fertility defects in germ granule mutants such as *glh-1*. However, fertility of *pptr-1* decreases at high temperatures, suggesting a role in protecting the nascent germ line from stress.³⁷

The Balbiani body is prominent in zebrafish oocytes and is characterized by mitochondria, ER membranes, and Golgi, as well as germ plasm RNAs and germinal granules. During early oogenesis, the Balbiani body has a role in localizing maternal mRNAs and germinal granules to the vegetal cortex.^{38–42} An analogous structure in *Xenopus* is the mitochondrial cloud; it contains several localized maternal mRNAs that are translationally repressed during oogenesis, and is associated with the message transport organizer (METRO) pathway.^{43–47}

The Balbiani body is also detected in oocytes of mouse primordial follicles; these cytoplasmic structures Trailer hitch (Tral), a conserved protein with an Sm-like domain that localizes to several related RNPs including P bodies, *C. elegans* P granules, and *Drosophila* sponge bodies.^{48–51} Interestingly, in male mouse germ cells, Trailer hitch labels an RNP granule distinct from the Vasa-positive chromatoid body.⁴⁸ The chromatoid body is a well-described RNP granule in mammalian male germ cells that includes high levels of Dicer and several P body marker proteins.^{52–54}

The morphology and composition of germ line RNP granules varies across and within species as noted by examples above, and additional characteristics are compared in Table 1. One common feature of several RNP granules is distinctive substructure within the granules. In *Drosophila* sponge bodies, *oskar* mRNA and Stauf protein occupy subdomains within the RNP complex.⁶ In *Xenopus* Balbiani bodies, several mRNAs reproducibly localize to distinct subregions,⁴⁶ and in the large RNP granules in *C. elegans* meiotically arrested oocytes, PGL-1 protein and *pos-1* mRNA localize to overlapping domains within the large RNP complex.⁵⁵ The functional significance of the segregation of components into subdomains is not yet clear, although it could allow for differential regulation of RNA transport, degradation, or translation.

Dynamics of RNP Granules

Changes in Subcellular Localization—The subcellular localization of RNP granules is dynamic during development. In the larval and adult *C. elegans* gonad the P granules associate closely with clusters of nuclear pores on the nuclear envelope of the transcriptionally active immature germ cells (Table 1). The P granules become diffusely distributed throughout the cytoplasm of mature oocytes and early embryos, two developmental stages when transcription is not detected.^{34,55–57} Similarly, the chromatoid body associates with nuclear pore complexes during early spermiogenesis, but in secondary spermatocytes the chromatoid bodies become distributed throughout the cytoplasm and associate with mitochondria^{58,59} (Table 1). Dynamic changes also occur with the mitochondrial cloud and Balbiani body of *Xenopus* and zebrafish oocytes. In early-stage oocytes each localizes along the vegetal side of the nucleus, and it then moves toward the vegetal pole eventually resulting in germ granules being localized at the vegetal cortex.⁶⁰

Processes of Assembly and Dissociation—The process of building an RNP granule is dictated by the molecular interactions of its components. Some interactions are quite stable, such as those between Tudor and the symmetrically dimethylated arginines (sDMAs) of Piwi proteins,² whereas other interactions have lower specificity including those between RNA and protein. Several elegant live-imaging experiments show that the assembly of RNP granules is not as straightforward as the unidirectional movement of proteins from the cytoplasm into an RNP complex. FRAP (fluorescence recovery after photobleaching) experiments with GFP-tagged Stauf indicate a random exchange of the protein between the sponge body and the cytoplasm,⁶ and the highly dynamic nature of protein localization to *C. elegans* P granules is clear from photobleaching experiments using GFP::PGL-1.⁵⁷ As many of the same components of P bodies localize to germ line RNPs, additional insight may be gleaned from the kinetics of P body assembly and dissociation. In *Saccharomyces*

cerevisiae, P bodies assemble within 15min of glucose deprivation and dissociate within 10min of exposure to cycloheximide.⁶¹ Showing similarly rapid responses, *Drosophila* sponge bodies can disassemble within 5min of Dynein inhibition.⁶² However, without pharmacological treatments, the kinetics of RNP remodeling are somewhat slower in multicellular animals. When female flies are protein deprived for 72 h and then resupplied protein, GFP::Ypsilon schachtel (GFP::Yps) aggregates decrease within 2 h, a similar response time as the assembly of Yps aggregates in response to protein deprivation⁷ (Figure 1). Similarly, the large RNP granules in meiotically arrested *C. elegans* oocytes dissociate within 60 min of the resumption of meiosis.⁹ Future analyses of the *in vivo* dynamics of RNP complexes should continue to provide insight into the molecular interactions that result in macrolevel stability and microlevel dynamics of these granules.

REMODELING OF RNPs IN RESPONSE TO STRESS, AGING, OR MEIOTIC ARREST

Given the dynamic changes of RNP complexes during normal development, it is perhaps no surprise that extensive remodeling of RNPs occurs in response to environmental stresses, meiotic arrest, starvation, or aging. Stress granules are one example of an RNP complex that is specifically induced in response to environmental stresses such as oxidative stress, heat shock, or osmotic shock. The components of stress granules overlap partially with those of P bodies; however, they have distinguishing characteristics including components of the translational initiation machinery such as eIF3 and ribosomal proteins⁶³ (Table 2). Stress granules have been observed in diverse species, from yeast to mammalian cells. Whether *bona fide* stress granules assemble in the germ cells of many species is not yet clear; however, environmental stresses do induce the remodeling of RNP complexes in several examples.

Environmental Stresses Induce the Assembly of Large RNP Granules

Heat Shock, Anoxia, and Osmotic Stress Induce RNP Granule Remodeling—

The distribution of mRNA in the adult *C. elegans* germ line normally appears diffuse throughout the cytoplasm with a slight enrichment at the site of P granules^{55,56} (Figure 2(a) and (b)). In response to heat shock, however, the distribution changes to a strong localization to discrete granules in three subcellular locations: at perinuclear P granules, in the syncytial cytoplasm of the gonad core, and in the oocyte cytoplasm (Figure 2(e) and (f)). Concomitant with the changes in mRNA distribution, the distribution of several RNA-binding proteins also changes. MEX-3 is a KH domain RNA-binding protein that is detected at the distal tip of the germ line and is uniformly distributed in the cytoplasm of the most proximal oocytes.⁶⁷ After heat shock, anoxia, or osmotic stress, its distribution changes to strong localization to large RNP granules in oocytes⁹ (Table 2). The changes occur gradually; discrete granules are first detected between 1 and 2h of heat shock, and they continue to increase in size until 5 h of heat shock at which point the worm's health rapidly deteriorates. The remodeling of MEX-3 is reversible; within 1h of removing heat stress, MEX-3 granules begin to dissociate, and they are no longer detected after 90 min of recovery.⁹

Additional components of the large, heat shock-induced RNP granules in oocytes include the stress granule markers TIA-1 (T-cell-restricted intracellular antigen) and PAB-1 [poly(A)-binding protein], and P body markers DCAP-2, CAR-1, and CGH-1⁹ (Table 2 and Figure 3). Most of these proteins are also enriched in the RNP granules in the distal gonad core. Given the functions of P bodies and stress granules, the hypothesis for RNP granule function is to modify mRNA metabolism during stress in order to preserve the oocytes for eventual fertilization. Interestingly, two of the major components of the small P granules normally found in oocytes, PGL-1 and GLH-1/Vasa, are detected neither in the large RNP granules of stressed oocytes nor in the gonad core. Instead, they appear diffusely cytoplasmic throughout the oocytes after heat shock.^{9,66} Thus, the assembly process of the large RNP complexes does not appear to use the existing P granules as a 'seed' from which to build the RNP granule.

Heat stress also induces RNP remodeling in the mouse testis. Stress granules assemble in the premeiotic male germ cells that contain DAZL, an RNA-binding protein that binds *Mvh* (mouse Vasa homolog) mRNA, TIA-1, eIF3, and phospho-eIF2 α ⁶⁴ (Table 2 and Figure 3). Interestingly, these granules do not colocalize with Tudor domain-containing 1, a marker of nuage or P body markers; thus, they differ from the heat shock-induced RNP granules in *C. elegans* oocytes. The stress granules appear to play a protective role against apoptosis in male germ cells that experience heat stress.⁶⁴

Nutrient Stress Reveals Plasticity of Sponge Bodies and U Bodies—The composition of sponge bodies changes as they transit from *Drosophila* nurse cells into the oocyte,⁶ and even more substantive changes in sponge body architecture occur in response to changing environmental conditions. When females are fed standard growth media supplemented with dried yeast, dispersed sponge bodies appear in *Drosophila* egg chambers as small puncta somewhat similar to P bodies (Figure 4(a)). In contrast, when no yeast supplement is provided, much larger sponge bodies are detected, which can appear as interconnected bodies; these are named reticulated sponge bodies⁶ (Figure 4(b)). Several homologs of the proteins enriched in the *C. elegans* RNP granules also localize to reticulated sponge bodies, including the RNA helicase Me31B (Table 2 and Figure 3). The levels of Me31B are much lower in the cytoplasm than in oocytes with dispersed sponge bodies, suggesting that the protein is aggregating or being recruited into the reticulated bodies⁶ (Figure 4(a) and (b)). The similar dynamics between CGH-1 and Me31B in oocytes (compare Figure 4(a) and (b) to Figure 2(g) and (h)) suggest that conserved RNA helicases in other species, such as *Xenopus* Xp54 and mouse RCK, may reveal similar plasticity upon environmental changes.

A second study has also described P body-like RNP complexes in *Drosophila* nurse cells and oocytes that are induced by a protein-poor diet for 24 h.⁷ These RNPs are described as having several, but not all, of the same components as reticulated sponge bodies, including the *oskar* mRNP. Components of the *oskar* mRNP in the large RNP complexes include *oskar* mRNA, Ypsilon schachtel (Yps), and Staufen^{6,7} (Figures 1 and 4). In contrast to the P body markers, two stress granule markers, eIF2 α and PABP, do not colocalize with the large reticulated bodies, suggesting that these RNP bodies are more similar to P bodies than stress granules (Table 2 and Figure 3).

Following upon the work describing the P body dynamics induced by nutritional stress, U bodies have also been shown to increase in size in *Drosophila* oocytes following nutritional deprivation.⁶⁸ U bodies are cytoplasmic structures that contain uridine-rich snRNPs, the SMN complex, and the snRNP assembly machinery.^{69,70} U bodies closely associate with P bodies in the cytoplasm of nurse cells and oocytes,⁷⁰ and mutations in U body components such as SMN and *gemin3* phenocopy mutations for some P body components during *Drosophila* oogenesis.^{71,72} When females are starved discrete U bodies are seen in egg chambers earlier in oogenesis compared to controls, and in stages 8–10 egg chambers U bodies are significantly larger than those from well-fed females.⁶⁸ U bodies also respond dynamically to heat stress, similar to RNP granule remodeling in *C. elegans* oocytes. In the nurse cells of early egg chambers SMN, a marker of U bodies and the determining factor for spinal muscular atrophy (SMA) in humans localizes to the nuage at perinuclear regions, and by stage 8 U bodies are increased in size throughout the egg chamber. The U body response to starvation is reversible, similar to the dynamic and reversible responses of P body-like sponge bodies and *C. elegans* RNP granules.⁶⁸ Interestingly, the composition of large, stress-induced U bodies does not appear to differ from that of smaller U bodies, unlike the increased complexity seen for *C. elegans* RNP granules. The reason for the increased size of U bodies in response to nutritional stress is not yet clear. The changes may be related to decreases in metabolic gene transcription and splicing activity during stress, with the increased size of U bodies allowing for increased and protected cytoplasmic storage of snRNPs.⁶⁸

Aging or Extended Arrest of Oocytes Induces RNP Granule Assembly

Dynamic changes in the composition and morphology of RNP granules occur in the germ line not only in response to stress but also in response to extended meiotic arrest or aging. In young *C. elegans* hermaphrodites, oocytes are ovulated and fertilized every 23 min; thus, no prolonged meiotic arrest occurs.⁷³ However, within 3.5 days of adulthood, the hermaphrodites run out of sperm, ovulation arrests, and the oocytes accumulate in the gonad arms. In the arrested oocytes of these middle-aged ‘females’, large RNP granules assemble that closely resemble those that form after heat shock^{55,65,74,75} (Figure 2(c) and (d)). Their composition includes RNA-binding proteins (including DCR-1, MEX-3, PUF-5, and MEX-5), at least three P body marker proteins, two stress granule marker proteins, and translationally repressed maternal mRNAs^{9,65,66} (Table 2 and Figure 3). One difference from stress-induced RNPs is the localization of P granule proteins, including GLH-1/Vasa, to the large RNP granules^{55,9,65,66} (Table 2). The GLH-1/Vasa protein not only localizes to the large RNP granules in arrested oocytes, it is also required for the localization of MEX-3 to the granules.⁶⁶ The RNP granules increase in size in oocytes the longer their delay in being fertilized, and their assembly is irreversible; within 1 h of being mated and sperm being replenished, the large RNP granules dissociate and the oocytes resume ovulation.⁹

The assembly of large RNP granules in arrested oocytes occurs in middle-aged hermaphrodites that have become depleted of sperm, and therefore is related to the normal aging of the worms. However, large RNP granules also assemble in the arrested oocytes of a subset of young worms. In mutants, such as *fog-2*, no sperm is made; thus, the worm is functionally a female.⁷⁶ Because of the lack of sperm, the oocytes are not ovulated and

instead accumulate arrested in meiotic prophase.⁷³ RNP granules assemble in *fog-2* oocytes within 4 h of adulthood, and increase in size and number the longer the oocytes are arrested (Schisa, unpublished results). Thus, it appears that the length of meiotic arrest is critical to stimulate RNP granule assembly, and not the age of the worm necessarily. The assembly of RNP granules in meiotically arrested oocytes is conserved in at least three male/female *Caenorhabditis* species.⁷⁴ How unmated females might benefit from a mechanism to protect oocytes during the time they are searching for a male is more obvious than the need for such a mechanism in a self-fertilizing hermaphrodite. One idea is that RNP granules may continue to assemble in *C. elegans* hermaphrodites owing to conservation from an ancestral gonochoristic state.^{74,77,78}

The response of RNP granules to oocyte age or delayed fertilization accompanying meiotic arrest has not been examined in many other species beyond nematodes; however, data from *Drosophila* may suggest a conserved response. In virgin female flies the large, reticulated sponge bodies assemble independent of nutrient stress.⁶ Thus, a parallel exists between unmated female *C. elegans* and virgin female *Drosophila*, in which similar responses occur in response to meiotic arrest; the assembly of RNP granules is dynamic and reversible in both. Additional studies in vertebrate species should uncover the extent of conservation of these cellular responses.

ROLE OF THE CYTOSKELETON IN REGULATING RNP ASSEMBLY

Many localized mRNPs rely on the cytoskeleton for transport through the cytoplasm or for localized anchoring. Examples include the requirement for Magellan, a microtubule actin cross-linking factor1, in localizing the zebrafish Balbiani body to the oocyte cortex.⁷⁹ In *Drosophila*, elegant live-imaging experiments show that gurken mRNPs are directly associated with microtubules as they transit from nurse cells to the oocyte, as well as a requirement for the microtubule motor protein dynein.^{62,80,81} Sponge bodies disassemble within 3–5min when Dynein is inhibited in stage 9 *Drosophila* oocytes, but not when microtubules are disassembled⁶² (Table 1).

A role for the cytoskeleton in regulating dynamic changes in RNP granules induced by stress or age is demonstrated in the germ lines of both *Drosophila* and *C. elegans*. The first line of evidence is the reorganization and cortical enrichment of microtubules that occurs concomitant with the assembly of large RNPs in *Drosophila* nurse cells and the *C. elegans* oocyte^{7,9,82} (Table 1). Second, live imaging of the growing ends of microtubules in nutritionally stressed egg chambers shows changes in microtubule dynamics.⁷ Third, disruption of microtubules by colchicine results in mislocalization of GFP::Yps to nurse cells and large cytoplasmic aggregates. Taken together, it appears that nutritional stress promotes the rearrangement of microtubules, which in turn leads to protein mislocalization in nurse cells.⁷ Also highlighting connections between microtubules and RNP granules are the results of a recent screen for regulators of RNP granule assembly in *C. elegans* oocytes in which a number of microtubule-associated proteins were identified (Schisa, unpublished results). More in-depth studies of these proteins and their associated complexes should illuminate more precisely the functional relationship between the cytoskeleton and RNP granules induced by stress or age.

ASSOCIATIONS OF RNP GRANULES WITH ER AND NUCLEAR MEMBRANES

RNP granules associate with the ER and/or nuclear membrane in a variety of organisms. Examples include the close association between the ER and germinal granules in the Balbiani body or mitochondrial cloud of *Xenopus*, *Drosophila*, zebrafish, and mouse oocytes.^{40,48} The ER is also thought to promote the association between U bodies and P bodies.⁷⁰ The original description of sponge bodies by transmission electron microscopy (TEM) included membranous, ER-like cisternae within an electron-dense material.²⁴ Moreover, the *Drosophila* Trailer hitch (Tral) protein, which resides in an RNP complex with Cup, Yps, and Me31B, localizes at a subset of ER exit sites in nurse cells.⁴⁹ Tral is also required for the secretion of the dorsal-ventral patterning factor Gurken (Grk) and the vitellogenin protein Yolkless (Yl). Thus, mRNA processing proteins within RNP complexes appear linked to the regulation of protein trafficking through the secretory pathway.

Recent studies examining the effects of stress and age are mixed in supporting a connection between RNP dynamics and the ER. In *C. elegans* oocytes the subcellular localization and morphology of the ER changes in response to heat shock or extended arrest of meiosis. Large sheets of ER are visible by TEM at the cell cortex, which are in close proximity to the large RNP granules.⁸ In contrast, no obvious changes in the organization of ER accompany the formation of reticulated sponge bodies in *Drosophila* egg chambers in response to nutritional stress.⁶ This difference could stem from the fact that the sponge bodies were viewed only at the light microscopy level, or sponge bodies may differ from *C. elegans* RNP granules in their regulation or function. Future ultrastructural studies may partially resolve this question.

Associations between a variety of germ line RNP granules and the nuclear membrane have been well documented (Table 1). Sponge bodies are enriched on the cytoplasmic face of the nuclear membrane in stages 7–10 of *Drosophila* oogenesis,²⁴ and *C. elegans* P granules associate with clusters of nuclear pore complexes where they interact with RNA export factors and newly synthesized mRNA.^{56,57} In mouse spermatocytes, the chromatoid body is connected to electron-dense material within nuclear pore complexes.⁸³ Together these studies suggest that the close proximity of RNP granules to nuclear pores in nuclear membranes may ensure that subsets of mRNA are properly targeted to ensure correct translational control. Recent studies of the DEAD-box protein UAP56 show that its nuclear localization is directly across the nuclear envelope from nuage granules containing Vasa.⁸⁴ More significantly, mutations that alter a conserved surface residue of UAP56 disrupt germ line piRNA production, transposon silencing, and perinuclear localization of Vasa, thus suggesting Vasa and UAP56 function in a piRNA-processing complex spanning the nuclear envelope.

Further connections between the nuclear membrane and RNP assembly are seen in response to stress or aging (Table 1). Increased numbers of nuclear membrane blebs are induced in stressed or old-aged *C. elegans* oocytes, as is the formation of annulate lamellae at the cortex.⁸ The timing of the increased blebbing is just prior to when the large RNP granules are detected at the cell cortex and along the nuclear membrane, and is consistent with a

model suggesting that the trafficking of the nuclear membrane material toward the cortex may facilitate the assembly of annulate lamellae and large RNP granules. The model is supported by RNAi of a subset of nuclear pore complex proteins that prevent the assembly of MEX-3 protein into RNP granules. Nuclear blebbing appears to be distinct from any other similar structures described in the literature, and it has not yet been described in any other species. It will be very interesting to determine if this is a conserved response to stress or aging in the germ line, and further tests should determine if nuclear blebbing promotes RNP granule assembly. An intriguing candidate regulator linking the stress-induced remodeling of the nuclear membrane, reorganization of the ER, and formation of RNP granules in the germ line is *lipin*, a phosphatidic acid phosphohydrolase in the glycerolipid biosynthetic pathway. Inactivation of the *lipin* homolog in worms results in ER disorganization in oocytes and early embryos and defects in nuclear envelope breakdown, and similar defects are seen in yeast.^{85,86} Future studies should identify any connections between the regulation of RNP granules in the germ line and *lipin*.

FUNCTIONS OF RNPS IN THE GERM LINE DURING STRESS AND AGING

Effects of RNP Dynamics on mRNA Stability

A general role for germ line RNPs in the regulation of mRNA stability has been supported in a variety of studies. The localization of several decapping and decay factors to P bodies suggests that mRNAs localized to P bodies undergo active degradation. However, Boag et al. identified approximately 300 CGH-1-interacting mRNAs that are maintained during *C. elegans* oogenesis. Moreover, in the absence of CGH-1, several maternal mRNAs become destabilized, supporting a role for CGH-1 RNPs in protecting maternal mRNAs from degradation.⁸⁷ One attractive hypothesis is that the localization of multiple mRNAs to a single granule may provide global protection for the mRNAs from the degradation machinery.⁸⁸ In studies of RNPs in the *Drosophila* and *Xenopus* germ lines, the importance and challenge of separating the function of RNA localization from RNA stability has been made clear, and RNA localization can also contribute to translational repression. Further studies are needed to resolve these complex and interwoven functions in regulating gene expression in the germ line.

In the large RNPs that are induced by stress and age, roles in the regulation of mRNA stability have also been postulated. In the case of the *C. elegans* germ line maternal mRNAs are not transcribed in oocytes, and many are needed for embryonic development. When extended delays in fertilization of the oocytes occur, an increased need for inhibition of decapping or degradation activities may arise.^{9,65} In the case of loss of CAR-1, the levels of the maternal mRNA *glp-1* are not affected. In contrast, after RNAi of *cgh-1*, *glp-1* mRNA levels are decreased in late-stage oocytes, pointing to a possible role for CGH-1 in maintenance of mRNAs during extended arrest.⁶⁵ These studies are somewhat complex to interpret because of the abundance of factors that localize to RNP granules. The analysis of individual mRNA levels after knock down of single protein components provides a starting point, but additional studies are warranted to more comprehensively examine the effects on mRNA levels when RNP granule assembly is perturbed.

Effects of RNP Granule Dynamics on the Regulation of mRNA Translation

Strong correlations exist between the localization of mRNAs to RNP granules and their translational repression. In arrested *C. elegans* oocytes, several maternal mRNAs that are not actively translated, including *pos-1*, *skn-1*, *par-3*, *nos-2*, and *glp-1*, strongly localize to large RNP granules, whereas mRNAs that are translated, including *rme-2*, do not.^{55,65} In addition, the same 3'-UTR elements promote translational repression and localization to RNP granules. Finally, with the loss of *car-1*, CGH-1 localization to RNP granules is disrupted. No derepression is seen in the distal germ line; however, in the proximal oocytes ectopic GLP-1 protein is detected.⁶⁵ Thus, one consideration in trying to delineate these functions is that the roles of RNPs may be stage-specific within the germ line. Other studies indicate that mRNA repression does not strictly require sequestration into RNP granules, and examples from yeast and fly studies show that disruption of P bodies does not interfere with repression or mRNA decay.^{89,90,65} Taken as a whole, studies to date are consistent with the idea of RNP granules acting as a reinforcing center for mRNA metabolism control.

In the case of nutritionally stressed *Drosophila* egg chambers, the Yps-positive granules have been hypothesized to function as a storage site for translationally repressed *oskar* mRNA.⁷ The Staufen protein is not enriched in dispersed sponge bodies but becomes highly concentrated in subcompartments of reticulated sponge bodies, as does *oskar* mRNA which it regulates post-transcriptionally.⁶ It is not yet known if *oskar* mRNA is ectopically translated if it, or Staufen, does not localize to reticulated sponge bodies in nutritionally stressed environments. The complexities of dynamic RNP granule composition, coupled with the interplay between RNA decay and translational regulation, require additional studies to fully explore different models of RNP granule function.

The localization of mRNAs and RNA-binding proteins to RNP granules in response to environmental stress can impact RNA metabolism in a variety of ways. In addition to regulating RNA stability and translation, the formation of enlarged U bodies in *Drosophila* egg chambers suggests that splicing activity is also controlled. In response to starvation, transcription and splicing of metabolic genes is downregulated.^{91,92} The increased size of U bodies in response to starvation is suggested to represent an increased cytoplasmic storage of snRNPs in a protected compartment.⁶⁸ U bodies also increase in size in response to heat shock; however, in early egg chambers the U bodies localize specifically to the perinuclear nuage. It is not yet known if changes in levels of transcription or translational repression occur in parallel with the changed distribution of SMN protein in U bodies.

Impacts of RNP Dynamics on the Physiology of Stressed or Aged Germ Lines

One overarching question regarding the assembly of large RNP granules in the germ line is whether there is any global benefit to the germ cells or organism. Studies to date indicate no deleterious effect of RNP granule assembly on germ cells in terms of oocyte or germ line quality. In egg chambers in which reticulated sponge bodies form, abdominal patterning and viability of the subsequent embryos appear normal,⁶ and in the offspring resulting from arrested *C. elegans* oocytes containing large RNP granules, >90% are viable.⁹ Moreover, a positive effect can be inferred from the results of *fog-2; kgb-1* females in which RNP granules fail to assemble normally; after mating into the females, embryonic viability

decreases significantly, suggesting decreased oocyte quality.⁸ As more regulators of RNP granules are identified, the ability to interfere with assembly or dissociation and query the effects on germ cell function, oocyte quality, and fertility in the next generation will increase. The next chapters in these studies should yield more direct answers to the many remaining questions.

CONCLUSION

The exciting discovery that mRNA decapping and 5' → 3' degradation in yeast occurs at discrete and cytologically visible cytoplasmic sites (P bodies) has contributed significantly to the rapid pace of discovery over the past decade.¹³ P bodies have now been described in an array of species and cell types. The variety and large number of P body-related RNP complexes discovered in recent years may reflect improvements in the resolution of microscopy and live-imaging techniques, in combination with the leveraging of genomic and proteomic approaches. Many RNP complexes reside uniquely in the germ line, and one of the unresolved questions for germ cell biologists is why germ cells assemble unique RNP complexes such as germ granules that are hypothesized to control similar processes as those in somatic cells, such as regulation of gene expression and/or mRNA turnover.

One lesson learned regarding germ line RNPs is that they must be regarded as extremely dynamic complexes. During normal development, photobleaching experiments demonstrate a rapid flux of proteins in and out of discrete granules, and the subcellular localization of germ line RNP complexes is also dynamic. The plasticity of germ line RNP complexes has been underscored by their reversible changes in composition, size, and morphology in response to stress, aging, or meiotic arrest. The foundational characterization of stress-induced RNP granules has been largely accomplished. Dozens of components of sponge bodies, P body-like bodies, and large RNP granules have been identified, and roles for the cytoskeleton in regulating the assembly of sponge bodies and large RNP granules in *C. elegans* oocytes have begun to be elucidated. A major goal remains to determine the function of these dynamic RNP complexes and how those functions aid the germ line in managing environmental stresses such as nutritional stress and heat shock. What we learn from studies of RNP complexes in the germ line may also have implications for better understanding RNP complexes in neurons that are linked to disease states such as amyotrophic lateral sclerosis (ALS) and SMA. For example, mutations associated with ALS result in a deficiency of RNP granule assembly in neuronal tissue.⁹³ Thus, increasing our understanding of the regulation and function of RNP complexes may have direct implications for human health. Further detailed investigations will be needed to fully explain if and how the assembly of large RNP complexes in the germ line regulates RNA metabolism, and whether RNP granules might directly regulate translation or are instead a consequence of repressed mRNAs.

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BOX 1**WHAT'S IN A NAME?**

When it comes to the nomenclature of RNP complexes in the germ line, a variety of names are used. Below is a brief description of the large number of cytoplasmic RNP complexes, with references included in the main text of the review.

RNPs detected during normal development:

Germ granule: refers collectively to the electron-dense, RNP granules in vertebrate and invertebrate germ lines, often given a specific name in a species

P granule: *C. elegans* germ granules

Nuage: refers to germ cell-specific organelles including small RNP granules near the nuclei of *Drosophila* nurse cells and the chromatoid body of mouse spermatids

Polar granule: *Drosophila* germ granules found in the posterior of late-stage oocytes and embryos, usually larger than small granules of nuage

Sponge body (or dispersed sponge body): small RNP puncta dispersed in *Drosophila* egg chambers that differ from nuage and polar granules in not containing Vasa RNA helicase

Mitochondrial cloud: electron-dense region in *Xenopus* oocyte containing germinal granules, localized mRNAs, and numerous mitochondria

Balbani body: structure in zebrafish (and other) oocytes analogous to the mitochondrial cloud

Subcortical aggregate: RNP in mouse oocytes containing maternal mRNAs and P body proteins

Chromatoid body: nuage component of mammalian spermatogenic cells; contains similar proteins as germ granules in female germ cells

P body: conserved RNP complex in somatic and germ cells with roles in mRNA decay and storage

U body: concentrations of uridine-rich small nuclear RNPs and their assembly machinery, the survival motor neuron (SMN) complex, in the cytoplasm of *Drosophila* somatic cells, nurse cells and the oocyte, often associated with P bodies

Yb body: a cytoplasmic body in the *Drosophila* somatic gonad with a role in piRNA biogenesis

RNPs induced by stress, age, or meiotic arrest:

Stress granule: RNP complex that assembles in somatic and germ cells in response to environmental stresses; composition partially overlaps with P bodies but has a different size and morphology

Reticulated sponge body: interconnected RNPs found in virgin females and induced by mild nutritional stress in *Drosophila*

P body-like RNP: RNP aggregates induced by nutritional stress in *Drosophila*, may be related to reticulated sponge body

RNP granules (or RNP foci): large RNP granules in the *C. elegans* gonad core and oocyte that assemble in response to meiotic arrest or environmental stresses

gR body: alternate name for large RNP granules in *C. elegans* germ line (above)

U body (increased size): in response to starvation and heat stress, U bodies in the *Drosophila* egg chamber increase in size

FIGURE 1.

A working model of the *Drosophila* egg chamber response to nutrient stress. In the presence of protein-rich food, GFP::Yps particles are transported during stage 6 from the nurse cells to the oocyte along microtubule bundles (orange lines); they also move to the oocyte by diffusion (pink lines). In the presence of protein-poor food, microtubules become cortically enriched, and transport is disrupted. GFP::Yps assembles into larger aggregates and accumulates in the perinuclear region of nurse cells. (Reprinted with permission from Ref 7. Copyright 2011 Elsevier)

FIGURE 2.

RNA granules assemble in the *Caenorhabditis elegans* gonad core and oocytes in response to meiotic arrest or heat shock. (a–f) Fluorescent micrographs stained with SYTO 14 to visualize RNA. (a and b) RNA is distributed evenly throughout the core and oocyte cytoplasm of young wild-type worms. Concave arrows indicate strongly staining nucleoli. (c and d) RNA is concentrated into discrete granules (arrows) in the core and oocytes of meiotically arrested oocytes in *fog-2(q71)* worms. (e and f) RNA is concentrated into granules in the core and oocytes of wild-type worms after 3 h of heat shock. (g) CGH-1 is diffusely cytoplasmic and in small puncta in wild-type oocytes. (h) CGH-1 accumulates in large granules in arrested oocytes. (Reprinted with permission from Ref 9. Copyright 2008 Elsevier)

FIGURE 3.

Euler diagram of germ line ribonucleoproteins (RNPs) induced by stress or aging and their shared components. Note that only selected components are shown. The focus is on shared components among the three RNP complexes induced by stress or aging in the germ line and components shared with P bodies and stress granules (but not those shared between P bodies and stress granules). Note that of the *Caenorhabditis elegans* proteins, only DCR-1 and MEX-3 (asterisks) localize to RNP granules induced by stress; all proteins listed localize to RNP granules induced by meiotic arrest/aging.

FIGURE 4.

Plasticity of sponge bodies. (a and b) Distribution of Me31B::GFP in egg chambers with dispersed and reticulated sponge bodies. The oocyte is on the right, and some of the nurse cells are on the left. Note the reduction in diffuse cytoplasmic staining in (b) relative to (a). The nurse cell to oocyte boundary is marked by a dashed line in (b). (c and d) *osk* mRNA is dispersed in the cytoplasm when dispersed sponge bodies are present, and localizes to reticulated sponge bodies when these structures form (d). (Reprinted with permission from Ref 6. Copyright 2009 Wiley-Liss, Inc)

TABLE 1

Overview of Ribonucleoprotein (RNP) Granules in the Germ Line

Type of RNP Granule	Species	Role of Microtubules (MT)	Association with Endoplasmic Reticulum (ER) or Nuclear Membranes	Role in Post-Transcriptional Regulation ¹
Normal development				
P bodies	Yeast/all eukaryotes	MT-regulated; dependent on MT motor proteins	ER	RNA decapping and decay, translation
Polar granules	<i>Drosophila</i>	MT-regulated	ER	RNA localization and translation
Sponge bodies (dispersed)	<i>Drosophila</i>	MT-associated; dependent on MT motor proteins	Nuclear envelope and ER	RNA localization and translation
U bodies	<i>Drosophila</i>	ND	ER	Splicing
P granules	<i>Caenorhabditis elegans</i>	MT-regulated (in embryo)	Nuclear pores	Translation, stability, and turnover
Balbiani bodies	Zebrafish	Yes	Nuclear envelope and ER	RNA localization and translation
Mitochondrial cloud/germinal granules	<i>Xenopus</i>	None ²	Nuclear envelope and ER	RNA localization and translation
Chromatoid bodies	Mouse	MT-regulated	Nuclear pores	Translation and stability
Induced by stress, age, or meiotic arrest				
Sponge bodies (reticulated)/P body-like RNPs	<i>Drosophila</i>	MT rearrangement; MT-regulated	No ER changes	Translation
U bodies (enlarged)	<i>Drosophila</i>	ND	ND	Splicing
RNP granules/grP bodies	<i>C. elegans</i>	MT rearrangement; MT-regulated	ER changes and nuclear pore and AL association	RNA stability and translation
Stress granules in testis	Mouse	ND ³	ND	Translation

ND, not determined; AL, annulate lamellae.

¹In several cases, the roles in regulation are proposed or correlative but not yet demonstrated.²Acetylated microtubules were not disrupted in these *Xenopus* experiments, and acetylated microtubules are organized in the Balbiani body.¹⁷³Microtubules are required for the assembly of stress granules in cell culture.^{18,19}

TABLE 2
Protein Components of Stress or Age-Induced Ribonucleoprotein (RNP) Complexes in the Germ Line

	Function	Normal Distribution	<i>Caenorhabditis elegans</i>				References
			Heat Shock	Age/Mitotic Arrest	Mouse Heat Stress	<i>Drosophila</i> Nutrition Stress	
TIA-1	RNA-binding protein	Nuclear enriched	+	+	+	ND	9,64
PAB-1/PABP	Poly(A)-binding protein	Cytoplasmic	+	+	ND	–	6,9
CGH-1/Me31B	Regulate translation	P granules, P bodies, sponge bodies, and cytoplasmic	+	+	–	+	6,7,9,64,65
CAR-1/Tra1	Regulate translation	P granules, P bodies, sponge bodies, and cytoplasmic	+	+	–	+	6,9,64,65
DCAP-2/Dcp1	Decapping of mRNAs	P granules, P bodies, sponge bodies, and cytoplasmic	+	+	–	+	6,7,9,64,65
PGL-1	RNA-binding protein	P granules	–	+	NA	NA	9,55,65
GLH-1/Mvhl/Vasa	DEAD-box RNA helicase	Germ granules	–	+	–	–	66
MEX-3	Regulate translation	Cytoplasmic	+	+	ND	ND	55
PUF-5	Regulate translation	Cytoplasmic and P granules	ND	+	ND	ND	65
MEX-5	CCCH RNA-binding protein	Cytoplasmic	ND	+	NA	NA	65
DCR-1	Produces small RNAs	Cytoplasmic, nuclear, and P granules	ND	+	ND	ND	66
DAZL	Regulate translation	Cytoplasmic	ND	ND	+	ND	64
Phospho-eIF2α	Regulate translation	Cytoplasmic	ND	ND	+	–	7,64
eIF3	Translation	Cytoplasmic			+		64
Exuperantia	RNA localization	Sponge bodies, P bodies, and cytoplasmic	NA	NA	NA	+	6,7
Bruno	Regulate translation	Sponge bodies and cytoplasmic	ND	ND	ND	+1	6
Orb	Regulate translation	Sponge bodies and cytoplasmic	ND	ND	ND	+2	6
eIF4E	Translation	Cytoplasmic and P bodies	ND	ND	ND	+	7
Pacman	5' $\rightarrow$ 3' exonuclease	P bodies	ND	ND	ND	+	7
Ypsilon schachtel	RNA binding	Cytoplasmic	ND	ND	ND	+	7
Staufen	Regulate translation	Cytoplasmic	ND	ND	ND	+2	6,7

NA, not applicable; ND, not determined.

Bold indicates that protein is a marker of stress granules.

Bruno is detected predominantly in sponge bodies of nurse cells but not in oocytes.
Orb and Staufen are not localized to large granules in nurse cells, but are present in oocyte granules.

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