Published in final edited form as:

Int Rev Cell Mol Biol. 2012 ; 295: . doi:10.1016/B978-0-12-394306-4.00013-7.

New Insights into the Regulation of RNP Granule Assembly in Oocytes

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Abstract

In a variety of cell types in plants, animals, and fungi, ribonucleoprotein (RNP) complexes play critical roles in regulating RNA metabolism. These RNP granules include processing bodies and stress granules that are found broadly across cell types, as well as RNP granules unique to the germline, such as P granules, polar granules, sponge bodies, and germinal granules. This review focuses on RNP granules localized in oocytes of the major model systems, *Caenorhabditis elegans*, *Drosophila*, *Xenopus*, mouse, and zebrafish. The signature families of proteins within oocyte RNPs include Vasa and other RNA-binding proteins, decapping activators and enzymes, Argonaute family proteins, and translation initiation complex proteins. This review describes the many recent insights into the dynamics and functions of RNP granules, including their roles in mRNA degradation, mRNA localization, translational regulation, and fertility. The roles of the cytoskeleton and cell organelles in regulating RNP granule assembly are also discussed.

Keywords

RNP granules; Oocytes; P granules; Polar granules; Sponge bodies; Germ granules; P bodies; Translational regulation

1. Introduction

The germline is distinct from the soma in several key areas; the germline must accurately transmit genetic information between generations, and it must establish and maintain pluripotency. While the mechanisms of these unique properties are not completely understood, one physical structure unique to germ cells is the germ granule, a component of the germ plasm. The history of studies focused on understanding the role of germ plasm in germ cells is long. The term germ plasm was coined by Weismann in 1892 (Arkov and Ramos, 2010), as the substance handed down from parent germ cells to the germ cells of the new individual. Hegner described visible granules in the germ plasm of insect germ cells for the first time in 1911 (Hegner, 1911).

We now understand that germ granules are well-conserved ribonucleo-protein (RNP) complexes in the germ plasm with an electron dense, membrane-free architecture. They have various names in different species, for example, sponge bodies and polar granules in *Drosophila*, P granules in *Caenorhabditis elegans*, germinal granules in the mitochondrial cloud in *Xenopus*, and chromatoid bodies (CBs) in mouse. For simplicity, these germline RNP granules will be referred to collectively as germ granules. In several invertebrate species, germ granules are found in germ cells continuously throughout development, while in mammals, germ granules have been mainly detected at later stages of germ cell differentiation. Thus, it appears that the functions of germ granules may vary across species and become specialized at different developmental times. Despite some differences, the molecular composition and morphology of germ granules have many shared features,

suggesting commonalities of function in germ cells in addition to potentially more specialized roles.

Within a particular species, germ granules are often quite dynamic. In *C. elegans*, for example, P granules localize to the germ cell precursors of the early embryo and remain localized to germ cells throughout the four larval stages and in the adult germline (Strome and Wood, 1982; Updike and Strome, 2009a). During development, their subcellular localization changes from perinuclear to a diffuse distribution throughout the cytoplasm. In addition, the composition of germ granules changes during development; several proteins are enriched in embryonic P granules that are not detected in the P granules of oocytes or the adult germline (Updike and Strome, 2009a; Table 7.1). In some cases, the data are incomplete, for example, oocytes in the adult germline have not been included in some studies of *C. elegans* RNP granule components. However, in many instances, the embryonic P granule proteins are either translationally repressed prior to fertilization or distributed diffusely throughout the cytoplasm of oocytes. Thus, P granules may function one way in the transcriptionally active meiotic cells of the adult germline and differently in the transcriptionally quiescent early embryo when additional proteins are recruited to P granules and their distribution is no longer perinuclear. Put another way, it seems reasonable to speculate that the function of germ granules in mitotic cells, such as those at the distal ends of the adult *C. elegans* germline and the early embryo, differs from their commonly hypothesized function of posttranscriptional regulation in meiotic cells. Oogenesis can occur over several days (flies) to several months or years (frogs) to decades (humans). Since many maternal messages are not used for an extended time after they are made, they must be regulated posttranscriptionally. Taken together, a focus on understanding the role of germ granules in meiotic cells seems critical to understanding oogenesis.

Several excellent reviews have recently summarized our current understanding of the structure of key protein components of germ granules (Arkov and Ramos, 2010), RNPs induced by stress, aka stress granules (Buchan et al., 2011; Moser and Fritzler, 2010), germ granules in mammals (Chuma et al., 2003, 2009), and the conserved components, dynamics, and function of germ granules in female and male germ cells (Voronina et al., 2011). This review focuses on a discussion of recent findings on RNP granules in oocytes and compares and contrasts the composition, dynamics, and function of oocyte RNP granules across several species (Tables 7.1 and 7.2).

2. Diversity of RNP Granules in the Germline

2.1. Germ granules

2.1.1. P granules in *C. elegans*—Germ granules in *C. elegans* were named P granules according to the embryonic cell lineage in which they are found (Strome and Wood, 1982). P granules are localized to the nuclear membrane of germ cells during most of development; however, in maturing oocytes, they detach and become distributed throughout the cytoplasm (Strome and Wood, 1982; Fig. 7.1). In newly fertilized embryos, P granules segregate asymmetrically to the posterior, and upon cell division they are inherited by the daughter cell that is the primordial germ cell (PGC). They remain distributed throughout the cytoplasm of the PGC until the eight-cell stage when they reassociate with the nuclear membrane. P granules continue to segregate asymmetrically at embryonic cell divisions such that a single PGC, that will give rise to the entire germline, contains all of the P granules. Of the first proteins identified as P granule components, two are homologs of the DEAD-box RNA helicase Vasa, GLH-1, GLH-2, and a third is the RGG-domain protein PGL-1 (Gruidl et al., 1996; Kawasaki et al., 1998). In worms lacking GLH-1, -2, or PGL-1, the germline fails to develop completely at high temperatures, suggesting a requirement of P granule components for fertility. The adult *C. elegans* gonad includes two U-shaped tubes

surrounding a central uterus. Examination of one arm of the gonad (Fig. 7.1; Pitt et al., 2000) illustrates mitotic nuclei at the distal end and meiotic nuclei throughout the majority of the gonad. The syncytial nuclei arrest in pachytene of meiosis in the medial region and enter diakinesis as the oocytes cellularize and undergo meiotic maturation in the proximal end. The P granules of pachytene nuclei are associated with clusters of nuclear pores along the nuclear envelope (Pitt et al., 2000). Approximately 26 proteins have been identified as components of P granules in oocytes (Table 7.1), though this number is difficult to pin-down as several studies have focused on P granules in embryos without describing protein localization prior to fertilization, and it is clear that the composition of P granules changes during development. Several maternal mRNAs also localize to P granules, including *nos-2* mRNA, which is also enriched in embryonic P granules (Table 7.1; Schisa et al., 2001; Subramaniam and Seydoux, 1999).

2.1.2. Sponge bodies, polar granules, and nuage in *Drosophila*—Multiple classes of RNPs have been characterized in *Drosophila* based on morphological criteria and subcellular localization. In *Drosophila* oogenesis, the eggs develop in a production line-like mode as egg chambers bud off from the germarium at the anterior end and mature as they progress toward the posterior of the ovariole (Fig. 7.2; Bastock and St Johnston, 2008). *Drosophila* oogenesis is often divided into 14 developmental stages, with the 14th stage being the polarized, mature egg (Spradling, 1993). The developing *Drosophila* oocyte is surrounded by 15 nurse cells in the egg chamber, and these cells are interconnected by small ring canals (Ashburner and Gubb, 1989). The function of the nurse cells is to generate mRNAs, proteins, and other materials essential for early development and deposit them into the growing oocyte (Bastock and St Johnston, 2008; Spradling, 1993). The term nuage is often used to describe the small RNP granules near the nuclei of nurse cells; sponge bodies are RNPs detected in both nurse cells and oocytes (Fig. 7.2C–H); and polar granules are larger RNP complexes detected in the posterior of late-stage oocytes and embryos (Fig. 7.2J, K; Liang et al., 1994; Nakamura et al., 2001; Wilsch-Brauninger et al., 1997). The RNPs in nurse cells and oocytes can be distinguished based on molecular markers. Both nuage and polar granules are positive for Vasa (Table 7.1; Hay et al., 1988a,b), whereas sponge bodies are positive for Exuperantia, a protein required for localization of the *bicoid* RNA, and the DEAD-box RNA helicase Me31B (Berleth et al., 1988; Nakamura et al., 2001; Wilsch-Brauninger et al., 1997). In nurse cells, sponge bodies appear to surround the nuage particles that have been implicated in the genesis of polar granules in the oocyte.

Sponge bodies were first described by Wilsch-Brauninger et al. (1997) as a subcellular structure in nurse cells and oocytes consisting of an electron dense mass containing ER-like membranes, an association with mitochondria, an absence of surrounding membrane, and an enrichment of the Exuperantia protein (Fig. 7.2B). Since the sponge body contains high levels of RNA, it was initially proposed that the structure might function in the assembly and transport of mRNAs and proteins needed for RNA localization. The composition of sponge bodies differs somewhat between nurse cells and the oocyte; while some proteins such as Me31B and Exu are detected in both cell types, Bruno is concentrated in nurse cells and Orb is at high levels in the oocyte (Table 7.1, Fig. 7.2E, H; Nakamura et al., 2001; Snee and Macdonald, 2009a,b; Wilsch-Brauninger et al., 1997). Since sponge bodies have been observed moving between nurse cells and from nurse cells to the oocyte via ring canals, the composition differences suggest dynamic changes are coupled with transport (Snee and Macdonald, 2009a, b; Theurkauf and Hazelrigg, 1998). As additional components of sponge bodies have been identified (Tables 7.1 and 7.2), two themes have emerged in regards to their function. The first is support for a role in posttranscriptional regulation of gene expression and the second is a function similar to that of processing bodies (P bodies) (Snee and Macdonald, 2009a,b). P bodies are well-conserved RNPs in somatic and germ cells with roles in RNA degradation and RNA storage; they are discussed in more detail in Section 2.2.

The extent of the similarity of components, including Me31B, the decapping protein Dcp1 (Lin et al., 2006), the eIF4E binding protein Cup (Ferraiuolo et al., 2005; Wilhelm et al., 2003), and the translation initiation protein eIF4e (Nakamura et al., 2004), has led some to speculate that sponge bodies are *Drosophila* P bodies (Lin et al., 2006) and others to use the terms interchangeably (Decker and Parker, 2006). On the other hand, the morphology of P bodies and sponge bodies are not identical; therefore, the structures may instead represent differing hierarchies of a related RNP (Snee and Macdonald, 2009b). The precise relationship among sponge bodies, P bodies, and polar granules is one unresolved question for the field that will likely benefit from additional functional studies.

Polar granules are first detected in the late-stage oocyte. The polar granule materials are transported from the nurse cells into the posterior of the oocyte. It is now clear that Oskar (Osk) is the critical component for assembly of polar granule components in the posterior of the oocyte, but it does not act alone as Vasa is also required (Lehmann and Nusslein-Volhard, 1991; Schupbach and Wieschaus, 1991). One recent model proposes that Osk recruits Vasa and Valois independently, and Valois mediates recruitment of Tudor by interacting with Osk and Tud (Anne, 2010). Additional components of the *oskar* mRNP complex include dDcp1 and Me31B (Baat et al., 2004; Nakamura et al., 2001), suggesting that polar granules, like sponge bodies, may also be closely related to P bodies (Thomson et al., 2008). Live-imaging studies with GFP-Aubergine, a protein in the Piwi subfamily of Argonautes, indicate that although polar granules contain some of the same proteins present in nuage particles in nurse cells, polar granules assemble *de novo* in the fly oocyte. The polar granules also appear to incorporate new components more slowly than nuage, suggesting that they may be less dynamic RNPs (Snee and Macdonald, 2004). While Oskar is required for the assembly of polar granules at the posterior of the oocyte, it is not necessary for the formation of nuage (Lasko and Ashburner, 1990).

2.1.3. Mitochondrial cloud and germinal granules in *Xenopus*—The mitochondrial cloud (or Balbiani body) in *Xenopus* oocytes has several similarities to sponge bodies. The mitochondrial cloud is electron dense, has a granulofibrillar morphology, contains germinal granules, and as its name suggests, numerous mitochondria are found in the structure (Billett and Adam, 1976; Eddy, 1975; Heasman et al., 1984; Fig. 7.3A–C). The mitochondrial cloud also contains several localized maternal mRNAs that are translationally repressed during oogenesis (Heasman et al., 1984; Houston and King, 2000; MacArthur et al., 1999). Some mRNAs such as Xcat-2, Xpat, and DEADSouth localize to, or near, the germinal granules within the mitochondrial cloud (Forristall et al., 1995; Kloc and Etkin, 1995; Kloc et al., 2002; Fig. 7.3D–F). In stage I oocytes, the mitochondrial cloud is large, approximately 40 μ m in diameter, and is located between the nucleus and the future vegetal cortex (Kloc et al., 2002; Fig. 7.3). The germinal granules are uniformly distributed throughout the cloud in the early stage I oocytes and then become aggregated at the vegetal edge of the cloud (Fig. 7.3A; Wilk et al., 2005). The mitochondrial cloud expands in stage II of oogenesis and breaks down into “germ plasm islands” at the vegetal cortex between stages III and IV. Although the function of the mitochondrial cloud has been mysterious for decades, it appears to be linked with the messenger transport organizer (METRO) pathway that localizes RNAs and germinal granules to the vegetal cortex of the oocyte during stages I and II (Kloc and Etkin, 1995; Kloc et al., 1993; Wilk et al., 2005).

2.1.4. Balbiani bodies in zebrafish—The Balbiani body is a structure in oocytes analogous to the mitochondrial cloud and named after the French embryologist E.G. Balbiani who studied these structures in spiders in the late 1800s (Kloc et al., 2004). The Balbiani body is characterized by mitochondria, ER membranes, and Golgi, as well as germ plasm RNAs and germinal granules (Fig. 7.4A–C). Such bodies have been identified in the oocytes of fruit flies, frogs, fish, mouse, and humans (Billett and Adam, 1976; Cox and

Spradling, 2003; Heasman et al., 1984; Hertig, 1968; Hertig and Adams, 1967; Pepling et al., 2007). While relatively little molecular characterization has been done in mammals, it is interesting that the Trailerhitch protein, a component of *Drosophila* sponge bodies and *C. elegans* P granules, localizes to the Balbiani bodies of mouse oocytes (Table 7.2); this suggests a conserved role for these RNP structures in mRNA localization and translation (Pepling et al., 2007). Some differences may exist regarding the role of RNA transport by Balbiani bodies in different species; while RNAs associate with the Balbiani body in flies for only 5–10h, they are localized to the Balbiani body in frogs for several days (Kloc and Etkin, 1995). Similar to the mitochondrial cloud in *Xenopus*, the zebrafish Balbiani body has a role in localizing maternal mRNAs and germinal granules to the vegetal cortex during early oogenesis (Bontems et al., 2009; Kloc et al., 2004; Kosaka et al., 2007; Marlow and Mullins, 2008; Nojima et al., 2010). The assembly of RNAs and proteins into the Balbiani body requires Bucky ball (Buc), a novel vertebrate protein (Bontems et al., 2009; Marlow and Mullins, 2008; Nojima et al., 2010). The underlying mechanisms by which Buc regulates the assembly of the Balbiani body are not yet understood, but studies focused on the function of Buc in mammals may help provide insight into the evolutionary conservation of this important germ granule-containing structure in oocytes.

2.1.5. Oocyte RNP granules, and CBs in male germ cells, of mammals—Until recently, studies of mouse oocytes did not identify RNP granules analogous to germ granules in other species. However, a recent report detected DCP-1A-positive P bodies in full-grown mouse oocytes, with the caveat that P body components were overexpressed in the system (Swetloff et al., 2009). In studies examining endogenous P body components in oocytes, *in situ* hybridization studies detect poly (A) mRNA concentrated in perinuclear foci and foci at the cortex, a pattern similar to that of germ granules in other species (Flemr and Svoboda, 2010; Jud et al., 2008). Moreover, while P bodies seem to disappear during mouse oocyte growth, and levels of some P body components such as DCP1A increase only at later stages during meiotic maturation, CPEB (cytoplasmic poly-adenylation element binding protein), DDX6 (ortholog of the DEAD-box RNA helicase CGH-1/Me31B/Xp54), and YBX2/MSY2 re-localize to subcortical RNP aggregates in germinal vesicle (GV)-stage oocytes (Flemr et al., 2010). The aggregates have been coined SCAs (subcortical aggregates); they contain maternal mRNAs and appear to disperse during meiotic maturation and release maternal mRNAs for translation (Flemr et al., 2010). It has been suggested that the mRNA degradation and storage functions of P bodies are temporally separated in mouse oocytes (Pepling, 2010). Future functional studies should provide further insight as to the relationships among these aggregates and RNP granules in other species.

A final type of germ granule worthy of mention is the chromatoid body (CB), the nuage component of spermatogenic cells. While sperm is not the focus of this review, because the CB shares many characteristics with oocyte RNP granules, it deserves at least brief mention. The CB was first described in the late 1800s (Benda, 1891), and while observed in the male germ cells of a variety of organisms, these RNP granules have been especially well studied in mammals (Kalt, 1977; Kotaja and Sassone-Corsi, 2007; Parvinen, 2005). Like other germ granule RNPs, CB are electron dense sponge-like organelles that lack a membrane (Fig. 7.5B). They share many components including RNA-binding proteins Vasa/MVH, Piwi/MIWI, and Dicer; the P body proteins CAR-1/Trailerhitch, DCAP-1/DCP1a, as well as mRNA and miRNA (Kotaja and Sassone-Corsi, 2007) (Table 7.2). In round spermatids, there is usually a single perinuclear CB per cell. Evidence that contain RNA includes RNase gold staining of isolated rat CBs (Figueroa and Burzio, 1998). The subcellular localization of CBs is dynamic and varies during development (Fig. 7.5A). Similar to the association of germ granules with nuclear pore components in pachytene-stage *C. elegans* oocytes (Patterson et al., 2011; Pitt et al., 2000), during early spermiogenesis, the CBs localizes at the nuclear envelope and associates with nuclear pore complexes (Fawcett et al.,

1970). In secondary spermatocytes, the CBs are distributed throughout the cytoplasm and associate with mitochondria (Russell and Frank, 1978). CBs have also been identified in human spermatids, by electron microscopy (Chemes et al., 1978), and more recently by performing colocalization experiments with Vasa, Nanos, Pumilio, and Gemin3 which localize to discrete cytoplasmic granules (Ginter-Matuszewska et al., 2011). The identification of conserved components in human chromatoid bodies suggests the efforts studying RNP granules in model systems may have direct application for understanding human fertility.

2.2. Processing bodies

P bodies first garnered significant attention after their identification and characterization in yeast (Sheth and Parker, 2003; Teixeira et al., 2005) and have since been identified as conserved RNPs in a myriad of eukaryotic species and cell types, including germ cells (Parker and Sheth, 2007). Based on the localization of mRNA decapping activators, a decrease in P bodies when deadenylation is inhibited, and an increase in P bodies when decapping or 5'–3' exonucleolytic digestion is inhibited, initial data supported a role for P bodies as sites of mRNA decapping and decay (Sheth and Parker, 2003). Subsequent studies made clear that some mRNAs can be translated after localization to P bodies (Bhattacharyya et al., 2006; Brengues et al., 2005; Cougot et al., 2004); thus, P bodies have alternative roles in mRNA storage and translational repression, and several studies demonstrate that mRNAs accumulated in P bodies are not actively translated (Parker and Sheth, 2007). While P bodies are found in many somatic cells, they are also detected in germ cells. Moreover, some components of P bodies are also present in germ granules, for example, Dhh1p and Dcp1p orthologs associate with the Osk mRNP in *Drosophila* (Lin et al., 2006; Nakamura et al., 2001); thus, they are pertinent to a discussion of oocyte RNPs. P body-like RNP granules also assemble in oocytes that are arrested or stressed (Jud et al., 2008; Noble et al., 2008). While these induced granules include a subset of characteristic P body proteins, the granule size is much larger than the size of P bodies in yeast or unstressed eukaryotic cells; thus, it is not clear how the significant remodeling that occurs in these large RNPs affects their function.

2.3. RNP granule remodeling in response to age, meiotic arrest, or environmental stress

We have known for many years that RNP granules can vary in morphology, subcellular localization, size, and composition during development. In *C. elegans*, for example, P granules are distributed throughout the cytoplasm of newly fertilized embryos; they segregate to the posterior cytoplasm prior to cell division such that the posterior daughter cell inherits all of the P granules, and at the four-cell stage, P granules begin to associate with the nuclear envelope and appear larger than in the adult gonad (Strome and Wood, 1982). The P granules remain perinuclear throughout the remainder of embryogenesis, the four larval stages, and in the majority of the adult gonad. In the maturing oocytes, they dissociate from the nuclear envelope and become distributed throughout the cytoplasm. The components of P granules also vary; several components such as MEX-1 and MEX-3 are detected in embryonic P granules, but not in P granules of the oocyte (Draper et al., 1996; Guedes and Priess, 1997).

In addition to the dynamics of RNPs during development, more recent studies have demonstrated that RNP granules also undergo remodeling within oocytes in response to a variety of stimuli, including age, meiotic arrest, starvation, and environmental stresses. In *Drosophila*, sponge bodies have been categorized as either dispersed or reticulated bodies. The dispersed bodies are seen in females fed standard food including dried yeast, while the reticulated bodies are observed when females eat media lacking dried yeast or in virgin females fed either diet (Snee and Macdonald, 2009b). Reticulated bodies are much larger

than dispersed bodies, likely increasing in size as additional components are recruited from the cytoplasm, and they often appear as an interconnected network. In a study of early *Drosophila* egg chambers, protein deprivation reversibly induces Ypsilon schatl (Yps) and other components of the *osk* RNP complex into large P body-like granules in nurse cells (Shimada et al., 2011). The granules are dynamic; they increase in size over time, and time-lapse imaging reveals continuous assembly and disassembly. These granules may be equivalent to the reticulated sponge bodies; however, EM analyses will be required to resolve this question.

In *C. elegans* and *C. remanei* oocytes, similar RNP remodeling occurs in response to extended meiotic arrest, heat shock, osmotic stress, or anoxia (Boag et al., 2008; Jud et al., 2007, 2008; Noble et al., 2008; Schisa et al., 2001). The large RNP granules in meiotically arrested oocytes include the core components of P granules, with the addition of several P body markers, stress granule markers, Dicer, and other RNA-binding proteins that are normally uniformly distributed in oocytes (Table 7.1). The assembly of the RNP granules, also referred to as grP bodies, is reversible and their presence is not deleterious to fertility; after fertilization of oocytes previously containing RNP granules, >90% give rise to viable progeny (Jud et al., 2008). The hypothesis for their function is to maintain maternal mRNA stability or translational repression when fertilization is delayed or a stress is present. The prediction follows that if arrested oocytes cannot assemble the large RNP granules, fertility will decline, and preliminary data support this model (Schisa lab, unpublished results). Additional questions regarding the mechanism of assembly and the effects of RNP granules on mRNA stability and translational regulation remain to be fully answered.

3. Components of RNP Granules in Oocytes

The list of components of RNP granules is extensive, and several reviews include lists of proteins and RNAs that localize to RNPs in various species. However, one limitation to these resources is that most do not distinguish whether a particular protein or RNA localizes to RNP granules in oocytes specifically. Since RNP granules in embryos do not have an identical composition as those in gametes prior to fertilization, here, I have summarized the protein and RNA components that localize to RNPs in oocytes or related cells, such as nurse cells in *Drosophila* and the pachytene germ cells in *C. elegans*; CBs and P bodies are also included (Table 7.1). To more easily appreciate the extensive conservation among the proteins in oocyte RNPs, Table 7.2 summarizes proteins whose localization to visible RNP complexes has been demonstrated in at least two species. Based on recent biochemical studies, this matrix seems likely to be filled to an even greater extent in the near future.

3.1. RNA-binding proteins

3.1.1. DEAD-box RNA helicases—The majority of all proteins identified as components of RNP granules in oocytes contain RNA-binding domains, and several DEAD-box RNA helicases localize to oocyte RNPs. DEAD-box RNA helicases may perform generally in large RNP particles as RNA chaperones or architectural factors that can remodel RNP complexes (Arkov and Ramos, 2010). They can influence many aspects of mRNA metabolism including splicing, export, translation, and mRNA degradation, and also remodel RNP complexes (Linder and Stutz, 2001; Rocak and Linder, 2004). *Drosophila* Vasa is one of the signature proteins found in germ granules, and homologs have been identified in many species including zebrafish, worms, frogs, mice, chicken, and human (Raz, 2000) (Tables 7.1 and 7.2). Vasa is required for oogenesis, associates with the general translation initiation factor eIF5B, and is necessary for the accumulation of Gurken (Grk) (Liu et al., 2009). Vasa also interacts specifically with the 3' untranslated region (UTR) of *mei-P26* mRNA to activate its translation (Liu et al., 2009); to recognize specific target RNAs, the RNA helicases appear to require partner proteins. Several Vasa homologs are

required for germ cell formation and proper oogenesis (Gruidl et al., 1996; Hay et al., 1988a,b; Kuznicki et al., 2000; Salinas et al., 2007). For example, the *C. elegans* *glh* mutants have reduced numbers of germ cells at high temperature and fail to make normal oocytes (Gruidl et al., 1996; Kuznicki et al., 2000). Additional DEAD-box RNA helicases are discussed in Section 3.2 on Processing (P) body components.

3.1.2. P translation initiation complexes—At least two examples of RNP granules contain translation initiation factors. Sponge bodies have high concentrations of eIF4E (Nakamura et al., 2004), suggesting a connection between translational regulation and these large RNP complexes. In *Drosophila*, eight eIF4E-related proteins are predicted that may interact with the cap, eIF4G, and 4E-BPs (4E-binding proteins); thus, this family of regulators likely has widespread impact on the regulation of mRNAs (Hernandez et al., 2005). In *C. elegans*, the eIF4E isoform IFE-1 directly binds PGL-1 and localizes to P granules in early embryos (Amiri et al., 2001). A function for *ife-1* in oocytes is not yet known, but *ife-1* mutants have severe defects in spermatogenesis. In addition to initiation factors, SPN-2/IFET-1, a 4E-BP localizes to P granules in germ cells of the adult gonad; however, intriguingly, its localization to P granules is not required for its function in repressing the translation of *mei-1* mRNA (Li et al., 2009) (Table 7.2). One of the *Drosophila* eIF4E-binding proteins (4E-BPs), Cup, also has a critical role in mediating cap-dependent translational initiation and localizes to both the Balbiani body in early *Drosophila* egg chambers as well to sponge bodies that assemble in later stage egg chambers (Cox and Spradling, 2003; Nakamura et al., 2004; Tanaka et al., 2006; Wilhelm et al., 2003). 4E-BPs compete with eIF4G for binding to eIF4E, interfering in the recruitment of the 40S ribosomal subunit (Gingras et al., 1999). Most 4E-BPs, such as Cup, rely on interactions with additional RNA-binding proteins to target specific messages for repression (Piccioni et al., 2005). One of the targets of Cup is *oskar* mRNA, which is also regulated by the DEAD-box RNA helicase, Me31B (Nakamura et al., 2001). It has been suggested that Me31B contributes to the assembly of the large RNP complexes, and the loss of Me31B results in a failure of the Cup-eIF4E complex to bind with *oskar* RNA (Nakamura et al., 2004). In this view, the close association of the various regulators within a cytoplasmic RNP granule facilitates the tight repression of mRNAs such as *osk*. Additional biochemical evidence comes from *Xenopus*, in which coimmunoprecipitations and *in vivo* pull-down assays reveal a large RNP complex in early oocytes that includes the germ granule components Xp54 and Rap55, and two members of the translation initiation complex: eIF4E1b and the 4E-BP, 4E-T (Minshall et al., 2007). Future studies that include determining the localization of these proteins might be expected to reveal new components of germ granules in the mitochondrial cloud.

3.1.3. Additional RNA-binding proteins—Some RNA-binding proteins in oocyte RNP complexes appear to be less conserved across species (Table 7.2). The Tudor protein localizes to polar granules in late-stage *Drosophila* oocytes, and a Tudor-domain protein is also observed in CBs (Amikura et al., 2001; Chuma et al., 2003; Table 7.2). The molecular function of Tudor-domain proteins is not well understood; however, a Tudor domain in the Survival of Motor Neuron (SMN) protein associates with Sm proteins, and MTR-1/TDRD1 colocalizes with SmB and SmD in the mouse CB (Chuma et al., 2003). No Tudor-domain proteins have yet been identified in oocytes of *C. elegans*, *Xenopus*, or zebrafish. A second less conserved RNA-binding protein is Staufen, which localizes to *Drosophila* sponge bodies in nurse cells, and XStaufen colocalizes with Vg1 and VegT mRNAs in RNPs at the vegetal cortex of stage III–IV *Xenopus* oocytes. XStaufen is also a component of transport RNP particles in stages V–VI oocytes that include Vg1 mRNA, xRAP55, and ElrB (Arthur et al., 2009; Snee and Macdonald, 2009b; Tanaka et al., 2006; Yoon and Mowry, 2004).

3.2. P body components

Several components of P bodies are found in oocyte RNPs, to the extent that some authors have referred to RNP complexes in oocytes as “grP bodies” or “P body-like,” or used terms like P body and sponge body interchangeably (Jud et al., 2008; Lin et al., 2006; Noble et al., 2008). The signature components of P bodies are the decapping proteins first identified in yeast RNPs, Dcp1p, Dcp2p, Pat1p, and Lsm1p (Sheth and Parker, 2003) (Table 7.2). While several of these proteins colocalize with germ granule markers, additional distinct foci can also be observed, suggesting that P body proteins localize to at least two types of RNP complexes. The DEAD-box RNA helicase Dhh1p is also among the core components of P bodies. Studies of Dhh1p homologs Me31B, Xp54, and CGH-1 in flies, frogs, and worms indicate some P body-like RNPs must function to stabilize mRNAs not only to decap and degrade them (Boag et al., 2005; Smillie and Sommerville, 2002; Wilhelm et al., 2005) (Table 7.2). These RNA helicases are also necessary for proper translational regulation of target messages, such as *oskar* (Minshall et al., 2007; Nakamura et al., 2001; Tanaka et al., 2006). Scd6p, an activator of decapping in yeast P bodies, is characterized by its Sm-like domain and clusters of RGG box RNA-binding domains. In flies and worms, the Scd6p orthologs, CAR-1 and Trailerhitch (Trl), associate in RNA-dependent complexes with the Dhh1p orthologs, CGH-1 and Me31B, respectively (Audhya et al., 2005; Boag et al., 2005; Wilhelm et al., 2005). CAR-1 and CGH-1 localize to two types of RNP granules in developing oocytes, P granules, and smaller foci (Audhya et al., 2005; Boag et al., 2005). The overwhelming suggestion from all studies to date is that these proteins bind specific maternal mRNAs and regulate their translation. One unexpected finding has been the physical association of some Scd6p/LSm proteins with the endoplasmic reticulum (ER); this functional association is discussed further in Section 5 of this review. Sm and Sm-like (LSm) proteins are yet additional types of P body proteins that are also widely observed in oocyte RNPs. Sm proteins were first characterized in association with small nuclear RNPs and later shown to function in mRNA degradation in yeast P bodies (Ingelfinger et al., 2002; Kambach et al., 1999; Sheth and Parker, 2003). Sm proteins also colocalize with P granules in *C. elegans* oocytes (Barbee et al., 2002), with Oskar in the posterior pole plasm of *Drosophila* oocytes (Anne, 2010), and with the CB in mouse and rat (Biggiogera et al., 1990; Moussa et al., 1994). One idea for the function of Sm proteins in P granule regulation is that the Sm complex promotes the attachment of P granule components to the nuclear envelope, similar to the proposed function in snRNP nuclear import (Barbee et al., 2002). Alternative possibilities for Sm protein function include roles in mRNP assembly, translation, degradation, or nuclear trafficking.

3.3. Stress granule components

Stress granules are RNPs that assemble in response to environmental stresses such as oxidative stress, heat shock, or osmotic shock. Several proteins that localize to P bodies have also been identified as components of stress granules, and P bodies and stress granules can interact (Anderson and Kedersha, 2009); however, the two types of granules are distinct based on size and ultrastructural criteria (Souquere et al., 2009). In relatively few oocyte RNPs, have stress granule components been detected. In one study, it was concluded that *Drosophila* sponge bodies are not closely related to stress granules based on the absence of poly(A) binding protein and the presence of Dcp1 (Snee and Macdonald, 2009b). However, in a study of arrested or stressed oocytes in *C. elegans*, the localization of GFP-tagged poly(A) binding protein and TIA-1 changed from nuclear and diffuse throughout the cytoplasm, to strong localization to cortical RNP granules (Jud et al., 2008). Since GFP-tags are not entirely reliable, this finding will need to be confirmed using antibodies. In the meantime, examination of the distribution of eIF3 and ribosomal proteins may also be instructive. To date, there is a little clear evidence relating most oocyte RNPs with stress granules.

3.4. RNAi-related proteins and piRNAs

RNAi-related proteins are another group of proteins that localize to oocyte RNP granules. Dicer is the endonuclease that processes precursor RNAs into siRNAs and miRNAs. In worms, DCR-1 (Dicer), CSR-1 (an Argonaute protein with Slicer activity toward secondary-type siRNAs), DRH-3 (a conserved, Dicer-related helicase), and EGO-1 (an RNA-directed RNA polymerase) are seen to varying extents localized to P granules of pachytene-stage nuclei, and all except EGO-1 maintain at least a partial association with P granules in maturing oocytes (Beshore et al., 2011; Claycomb et al., 2009). CSR-1, DRH-3, and EGO-1 function to generate 22G-RNAs, and mutations in *csr-1* result in severe effects on chromosome organization during cell divisions. Thus, an intriguing link exists between RNP granule components, the production of small RNAs, and regulation of chromosome dynamics. This connection is conserved in *Drosophila*, albeit in mitotic cells, as the nuage components Vasa, Aubergine, and SpnE also regulate chromosome dynamics (Pek and Kai, 2011). Interestingly, in *csr-1*, *ego-1*, and *drh-3* mutants, P granules become dissociated from the nuclear envelope. This suggests that the CSR-1 complex and associated mRNA targets promote the perinuclear localization of P granules (Claycomb et al., 2009). Dicer also localizes to CBs in mouse (Kotaja et al., 2006a,b), and in *Drosophila* under certain conditions (Findley et al., 2003). In the absence of Dicer activity, functional oocytes are not produced in mice, worms, or flies (Jin and Xie, 2007; Knight and Bass, 2001; Megosh et al., 2006; Murchison et al., 2007). The biochemical and genetic interactions between Vasa homologs and Dicer-1 homologs further support a role for a Vasa/Dicer complex in germ cell maintenance, as does the requirement for both GLH-1 and DCR-1 in the proper assembly of RNP complexes in the germline (Beshore et al., 2011). Additional evidence linking P granule proteins with the RNAi pathway comes from studies of DEPS-1, a P granule protein with no obvious RNA-binding domains. *deps-1* mutants have decreased levels of *rde-4*, a dsRNA-binding protein essential for RNAi in *C. elegans*, and are resistant to germline RNAi in a similar fashion as *pgl-1* mutants (Robert et al., 2005; Spike et al., 2008; Tabara et al., 2002). The precise basis for RNAi resistance in P granule mutants is not well understood; however, P granule proteins may promote the accumulation of germline endo-siRNAs, in an analogous way that components of *Drosophila* nuage promote the accumulation of rasiRNAs (Lim and Kai, 2007; Pane et al., 2007; Spike et al., 2008).

Small noncoding RNAs have also been identified as components of RNP granules in oocytes. The piRNAs are a large group of noncoding RNAs 24–32nt in length, associated with the PIWI subfamily of Argonaute/Piwi proteins; tens of thousands of piRNA sequences have been discovered in vertebrates and invertebrates (Thomson and Lin, 2009). The piRNAs and Piwi proteins function primarily in the germline with roles in blocking transposition and regulating gene expression posttranscriptionally (Aravin et al., 2007; Houwing et al., 2007; Thomson and Lin, 2009; Vagin et al., 2006). In zebrafish, two Piwi proteins have been characterized, Zili and Ziwi. Zili is present during all stages of oogenesis, and Tdrd1 facilitates its localization to discrete perinuclear granules in stage I oocytes (Houwing et al., 2008; Huang et al., 2011a). The subcellular localization of Ziwi includes enrichment in cortical granules of stage V oocytes, a region where *vasa* RNA and piRNAs are also located (Houwing et al., 2007). Zili and Ziwi are both required for maintenance of the germ cell population and fertility, and both are also detected in developing spermatogonia, along with piRNAs. In contrast to fish, piRNAs are detected solely in the male germ-line in mammals (Kuramochi-Miyagawa et al., 2004). In mouse, piRNAs associate with Argonaute proteins and the CB (Kotaja et al., 2006b), and a role for piRNAs in regulating translation is suggested by their association with MIWI, eIF4E, and Dicer (Grivna et al., 2006).

The components of *Drosophila* pole plasm/polar granules also include members of the PIWI subfamily, Aubergine and Piwi (Harris and Macdonald, 2001; Megosh et al., 2006; Thomson et al., 2008). One theory is that the localization of piRNAs to polar granules in oocytes may function in part to load piRNAs into the early embryo in order to protect the next generation from transposon activity (Brennecke et al., 2008). Both Aubergine and Piwi are required for fertility in females while the function of the third Piwi-type protein in flies, Ago3, is not yet known (Harris and Macdonald, 2001; Lin and Spradling, 1997; Thomson and Lin, 2009; Thomson et al., 2008). In *C. elegans* germlines, the Piwi family member PRG-1 binds 21U-RNAs that appear to be analogous to piRNAs of other species and localizes to germ granules in oocytes and meiotic cells of males (Batista et al., 2008). Some remaining questions are the role piRNAs play in germline development and gene regulation and how PIWI proteins regulate piRNAs. Answers to these questions should provide insight into the localization of these factors to RNP granules in the germline.

3.5. RNA components: mRNA, rRNA, and miRNA

In addition to the various classes of proteins and piRNAs localized to RNP granules, mRNA is a major component of RNP granules in oocytes (Table 7.1). All mRNAs examined to date in RNP granules are translationally repressed, and several become activated after release from the granules (Kloc et al., 2002; Noble et al., 2008; Schisa et al., 2001). This correlative evidence, in addition to experiments summarized in Section 6.3, suggest a role for RNP granules in promoting the translational repression of a subset of maternal mRNAs. In general, rRNA does not appear to be at high levels in RNP granules in oocytes, indicating active translation does not occur in these locations (Kotaja et al., 2006a; Schisa et al., 2001). The presence of Argonaute and other P body proteins in RNP granules in CBs prompted examination of miRNA localization, and several miRNAs are detected in CBs, colocalized with Mouse Vasa Homolog (MVH). These data lend further support for an instrumental role for RNP granules as a central site to promote posttranscriptional regulation of gene expression via the small RNAs pathways (Kotaja et al., 2006a).

3.6. Substructure of RNP granules

Due to improved imaging capabilities, in many recent studies examining the subcellular localization of RNP granule components, distinct sub-domains have been observed for particular proteins or mRNAs within RNP granules. *oskar* mRNA and Staufen protein are two examples where components appear to occupy subdomains within *Drosophila* sponge bodies (Snee and Macdonald, 2009b). In contrast, Me31B::GFP levels appear uniform throughout sponge bodies (Snee and Macdonald, 2009b); therefore, the compartmentalization occurs only for select components. Nonetheless, it suggests that the assembly of the RNP granules is not simply aggregation and is instead more ordered. In *Xenopus*, the *Xcat-2*, *Xlsirts*, and *Xwnt* mRNAs localize to distinct subregions of the Balbiani body with reproducible polarity, such that *Xcat-2* localizes closest to the future vegetal cortex (Kloc and Etkin, 1995). Similar compartmentalization exists in the large RNP granules that assemble in arrested *C. elegans* oocytes. PGL-1 protein and *pos-1* mRNA occupy overlapping domains within these large granules (Schisa et al., 2001), as do the decapping protein, DCP-2, and GFP-tagged PAB-1 (poly(A)-binding protein) (Jud et al., 2008). Although it is not yet well understood, the segregation of RNP granule components into subdomains may reflect differential regulation of RNA degradation or translation.

4. Dynamics of RNP Granules

4.1. Assembly of germ granules during progression through meiosis

Based on the order and substructure of components within RNP granules, the assembly of RNP granules does not appear to be simple aggregation of RNA-binding proteins and RNA.

Several studies have shed light on a variety of mechanisms of assembly that can be considered as three types of pathways. The first assembly pathway implicates RNA as critical for protein–RNA interactions that are necessary for RNP granule maintenance. Inhibiting general transcription results in structural changes of *C. elegans* P granules and CBs in mice (Sheth et al., 2010; Soderstrom and Parvinen, 1976a). When small RNA biogenesis is altered in worms or excess *gurken* RNA is injected in flies, the size and number of P granules or sponge bodies increases (Delanoue et al., 2007; Updike and Strome, 2009b). It has also been suggested that assembly of sponge bodies may be facilitated by Me31B and Exuperantia binding to different regions of the same RNA molecule (Snee and Macdonald, 2009b; Wilhelm et al., 2000). Furthermore, in *Xenopus*, germ granule organization in late-stage oocytes is altered if either VegT or Xlirts RNAs are depleted (Kloc et al., 2007, 2005; Kloc and Etkin, 1994). The localization of RNA export factors is also consistent with a model wherein nuclear RNP complexes undergo remodeling at perinuclear sites near nuclear pores, impacting their assembly. The second assembly pathway involves nucleation of RNP granules by key “seed proteins.” Buckyball is one such example of a protein that is necessary and sufficient to induce assembly of germ plasm components in zebrafish (Bontems et al., 2009; Marlow and Mullins, 2008). A second example is the PGL-1 protein that is sufficient to cause formation of ectopic cytoplasmic P granules (Hanazawa et al., 2011; Updike et al., 2011). The RGG domain is required to recruit additional RNA and RNA-binding proteins to the complex (Hanazawa et al., 2011). Perhaps the best understood example of a “seed” protein is Oskar, which is necessary and sufficient to trigger the assembly of germ plasm in *Drosophila* (Mahowald, 2001). The short form of Oskar appears to interact with Vasa to initiate germ granule formation, and this complex then recruits Tudor and other RNA-binding proteins (Anne, 2010; Breitwieser et al., 1996). The RNP complex then recruits RNAs that are transported from the nurse cells to the oocyte, and the long form of Oskar anchors germ granules to the cell cortex (Becalska and Gavis, 2009; Vanzo and Ephrussi, 2002). The third type of assembly pathway involves the binding of Tudor-domain proteins to methylated arginines. Tudor-domain proteins can have up to 11 Tudor domains, as in *Drosophila* Tudor, or single copies (Arkov and Ramos, 2010). Targets of Tudor-domain proteins with methylated arginines include Vasa, the Sm proteins, and Piwi-family Argonautes (Arkov and Ramos, 2010; Voronina et al., 2011). The assembly of germ granules in flies requires the Aubergine–Tudor interaction that results from sDMA modification of Aubergine (Anne et al., 2007; Kirino et al., 2009; Nishida et al., 2009). Interestingly, FRAP experiments reveal that the movement of proteins as granules assemble is not necessarily unidirectional. For GFP-tagged Staufen, the results are consistent with a random exchange of the protein between the sponge body and the cytoplasm (Snee and Macdonald, 2009b). Photo-bleaching experiments of perinuclear P granules, using GFP::PGL-1 in *C. elegans* also indicate at least some protein components are highly dynamic (Sheth et al., 2010). Further live-imaging analyses should provide important insights for our understanding of RNP granule dynamics and their function.

4.2. Dissociation of RNP granules

While some of the interactions that lead to the assembly of RNP granules in oocytes are fairly stable such as the Tudor–sDMA interactions, others are lower specificity interactions, including those between RNA and protein. These less stable interactions likely underlie the dynamic nature of RNP granules. Since many of the same components of P bodies and stress granules are found in oocyte RNPs (Table 7.3), it is noteworthy that formation of P bodies occurs within 15min of glucose deprivation or osmotic shock (Teixeira et al., 2005), and stress granules form in human cell lines within 20min of heat shock (Kedersha et al., 1999). Both types of RNP granules also rapidly dissociate upon certain stimuli; P body proteins become delocalized from foci after a 10-min exposure to cyclo-heximide (Teixeira et al., 2005), and disassembly of stress granules occurs within 60–90min of recovery (Kedersha et

al., 2000). In *C. elegans*, large RNP granules form in oocytes within 8h of meiotic arrest. The RNP granules dissociate within 65min of mating, a stimulus that triggers the resumption of meiosis (Jud et al., 2008). Elegant live-imaging studies in *Drosophila* reveal an even faster disassembly of sponge bodies, within 5 min, after inhibition of Dynein (Delanoue et al., 2007). Thus, with seemingly similar kinetics as P bodies and stress granules, the RNP granules in oocytes are extremely dynamic.

4.3. Induction by environmental stresses

While RNP granules are detected in oocytes of many species (Table 7.3), their composition, size, and subcellular localization can be greatly impacted by exposure to environmental stresses. In *C. elegans* that have been heat stressed, large RNP granules assemble in oocytes within 2h (Jud et al., 2008). These granules include novel protein components, relative to P granules, that are generally diffuse throughout the cytoplasm, such as MEX-3. They also include at least a subset of P body and stress granule proteins but differ from the RNP granules induced by extended meiotic arrest in that they lack the core components of P granules (Jud et al., 2008). The heat shock-induced RNP granules dissociate within 2h of recovery.

Analogous responses to environmental stresses appear to occur with *Drosophila* sponge bodies. When less optimal nutrient conditions are present, sponge body components that were previously dispersed throughout the cytoplasm become concentrated in large, reticulated bodies. The reticulated sponge body components include several P body proteins and novel components, Staufen and *oskar* mRNA (Snee and Macdonald, 2009b). A different study tracked the localization of GFP-tagged Yps after nutrient deprivation to demonstrate that RNP aggregates form within 2h of protein deprivation and decrease within 2h of protein-rich food being resupplied (Shimada et al., 2011). The reasons for the increased complexity or size of RNP granules during extended meiotic arrest or after environmental stress are not yet well understood; current hypotheses suggest an increased need for RNA remodeling when fertilization of oocytes is delayed to prevent degradation or precocious translation of mRNA. Recent data support this role for RNP granules, as when they do not assemble properly in arrested *C. elegans* oocytes, the risk of embryonic lethality is increased (Joseph Patterson and Jennifer Schisa, unpublished results; Patterson et al., 2011).

4.4. Intragranule dynamics and subcellular localization changes

In addition to changes in the size and composition of RNP granules during oocyte development, their subcellular localization is also quite dynamic (Table 7.3). Real-time fluorescence imaging studies indicate that while stress granules appear relatively fixed, P bodies are highly motile (Kedersha et al., 2005), and FRAP analyses show differences in the rates of exchange for individual protein components (Andrei et al., 2005). Dynamic movement of proteins between different types of RNP complexes demonstrates yet another type of dynamic movement; the RAP55 protein (a decapping activator with an Sm-like domain) moves between P bodies and stress granules depending on the environment of the cell (Yang et al., 2006). Similar active dynamics have been observed for RNP granules in oocytes. In *Drosophila* oocyte development, live imaging has revealed motile particles of Trailerhitch (the ortholog of RAP55) (Wilhelm et al., 2005), and dynamic movement of sponge bodies through the ring canals connecting nurse cells and the oocyte (Snee and Macdonald, 2009a). In the analogous RNP granule of round spermatids, the CB moves actively through the cytoplasm. It has been hypothesized that while the CB is associated with the nuclear pores, mRNAs and pre-miRNAs are collected from the nucleus, and that it subsequently functions further away from the nucleus to centralize the miRNA posttranscriptional control system (Kotaja et al., 2006a). Similar transient associations with nuclear pores at the nuclear envelope are seen with *C. elegans* P granules; they are

perinuclear in the pachytene-stage meiotic nuclei but diffusely distributed throughout the cytoplasm of diakinesis-stage oocytes (Strome and Wood, 1982). Interestingly, the transport of P granules away from the nuclear envelope in oocytes and early embryos corresponds with the developmental time when transcription is not detected (Schisa et al., 2001; Sheth et al., 2010). The subcellular localization of the Balbiani body or mitochondrial cloud of *Xenopus* and zebrafish oocytes also changes during early development. In stage I oocytes, the Balbiani body is localized along the vegetal side of the nucleus; however, it moves toward the vegetal pole during stages 2–5 (stages Ib–III in zebrafish) such that by stage 6 (stage III in zebrafish), the germ granules are at the vegetal cortex (Kloc et al., 2002, Fig. III). The dynamic localization of RNP granules within oocytes likely parallels their functions, indicating the functions may themselves change over time, or the functions include transport of RNPs. An important goal for future work will be to understand how dynamics are regulated by changes in RNP granule structure, including the trafficking of mRNPs in and out of the larger granules.

5. Role of the Cytoskeleton and Cell Organelles in Regulating RNP Granules

Several studies indicate a requirement for cytoskeletal elements in the assembly of RNP granules. When the cytokeratin or actin networks are fragmented or altered in *Xenopus* oocytes, the distribution of germinal granules and germ cell formation are profoundly affected (Kloc et al., 2007, 2005). Interestingly, the regulation is bidirectional as decreasing the levels of germ granule RNAs, like *Xlsirts*, results in a collapse of the cytokeratin network (Kloc et al., 2007, 2005). In the sections that follow, I focus on our current understanding of the role of cytoskeletal elements and cell organelles in regulating RNP granules.

5.1. Microtubules

Since many localized RNAs move through the cytoplasm in a microtubule or actin-dependent manner, it is not surprising that the microtubule network has a role in regulating RNP granules in oocytes (Table 7.3) (Bertrand et al., 1998; Ferrandon et al., 1994). In zebrafish oocytes, Magellan (microtubule actin crosslinking factor 1) is required both to localize stable microtubules to the oocyte periphery and to localize the Balbiani body to the cortex (Gupta et al., 2010). Interestingly, in Magellan mutants, the Balbiani body is enlarged; thus, the stabilization of the microtubule network is not necessary for the assembly or maintenance of the Balbiani body in zebrafish. Similar effects of increased size of RNP granules in response to disrupting microtubule or the Dynein motor protein, also occur in the PGCs of early zebrafish embryos, where it has been suggested microtubules may play a role in granule fragmentation (Strasser et al., 2008), and in stimulating the formation of P bodies in mammalian cells (Sweet et al., 2007).

A combination of elegant live-imaging analyses and immunoelectron microscopy studies of sponge bodies have been especially helpful in demonstrating the direct role of microtubules and their motor proteins in RNP transport. *gurken* transcripts move from the nurse cells to the oocyte in nonmembrane bound particles that are directly associated with microtubules (Caceres and Nilson, 2005; Clark et al., 2007; Delanoue et al., 2007). *Gurken* is transported in a Dynein-dependent manner, and a second important role for Dynein and the hnRNP Squid is anchoring *gurken* mRNA. To this point, when Dynein is inhibited, but not when microtubules are disassembled, sponge bodies disassemble within 3–5min; this result supports a functional connection between anchoring of RNPs and their structural integrity (Delanoue et al., 2007). Roles for Dynein and Kinesin have also been seen for growth of P bodies in response to stress (Loschi et al., 2009). The transport of *oskar* RNPs from

Drosophila nurse cells to the posterior of the oocyte also occurs along microtubules, and this transport requires the Kinesin motor protein (Becalska and Gavis, 2009). Kinesins also appear to be necessary for the localization of RNAs to the Balbiani body in *Xenopus* (Heinrich and Deshler, 2009). However, the transport of XIsirts RNA to the mitochondrial cloud appears to be microtubule independent (Kloc and Etkin, 1995); thus, microtubules are not the sole means of transport of RNP granule components.

A role for microtubules has also been suggested in regulating the dynamic changes in RNP granules that occur in response to age or stress. Microtubules become cortically enriched in *Drosophila* nurse cells and *C. elegans* oocytes in response to stress or extended meiotic arrest (Harris et al., 2006; Shimada et al., 2011). In both cases, the reorganization of the microtubule network is coordinated with the assembly of large RNP granules (Jud et al., 2008; Shimada et al., 2011), and in the case of *Drosophila*, FLIP imaging studies demonstrate directional transport of the Yps protein along microtubules from the nurse cells to the oocyte (Shimada et al., 2011). A requirement for microtubules is suggested by recent RNAi screens that indicate Tubulin proteins are necessary for the assembly of large RNP granules in arrested *C. elegans* oocytes (J. Schisa, unpublished results). Taken together, in different contexts, either microtubules or the associated Dynein and Kinesin motor proteins appear to play critical roles in regulating the composition, size, and subcellular localization of RNP granules in oocytes.

5.2. Mitochondria

Mitochondria have long been mentioned in descriptions of germ granules in oocytes. They are closely associated with polar granules in *Drosophila* oocytes and often directly contact germ granules in amphibians (Eddy and Ito, 1971; Mahowald, 1968). In other examples, such as *C. elegans*, mitochondria are nearby, but not in direct contact with P granules of pachytene nuclei (Pitt et al., 2000). The mitochondrial cloud is named for the abundance of mitochondria in *Xenopus* oocytes and is composed of approximately 500,000 mitochondria (Marinos and Billett, 1981). Granulofibrillar material accumulates in the mitochondrial cloud and is the precursor of germ granules (Heasman et al., 1984). The mitochondria in the mitochondrial cloud differ in their enzyme activities and replication properties from other mitochondria in the oocyte, and it has been suggested that a subset of germline-specific mitochondria exist that are passed on to gametes and progeny (Kloc et al., 2004; Kogo et al., 2011). Mitochondria are also often associated with sponge bodies in *Drosophila* nurse cells between stages 4 and 9 of oogenesis (Wilsch-Brauninger et al., 1997) and with P bodies in a microtubule-dependent manner, though neither seems to be required for the other's function in the case of P bodies (Huang et al., 2011b). Interestingly, the disruption of mitochondrial activity results in decreased recruitment of Ago2 to P bodies and a failure of active RISC complexes to assemble, a function normally carried out by P bodies. One corollary potentially relevant to human disease is that in various pathological conditions caused by mitochondrial dysfunction, derepression of miRNA-controlled genes may occur (Huang et al., 2011b).

5.3. Endoplasmic reticulum

Associations of RNP granules with the ER have been observed in oocytes of multiple species. The organelles localized to the Balbiani body (or mitochondrial cloud) of *Xenopus*, *Drosophila*, zebrafish, and mouse oocytes include ER in addition to the numerous mitochondria and Golgi (Kloc et al., 2004; Pepling et al., 2007); sponge bodies in *Drosophila* nurse cells consist of ER-like cisternae amidst an electron dense material (Wilsch-Brauninger et al., 1997). Live-imaging analyses of GFP-Trailerhitch in nurse cells reveal a subset of Tral particles tethered to an ER-like structure, and biochemical analyses combined with colocalization studies of KDEL with Trailerhitch, Cup, and Me31B

demonstrate that the three *Drosophila* proteins are part of an RNP complex that localizes to the borders of ER exit sites (Wilhelm et al., 2005). Thus, the Trailerhitch RNP complex appears to have an unexpected role in regulating protein trafficking through the secretory pathway. Tral also colocalizes with Me31B in the *Drosophila* oocyte and in early germarium stages and has been hypothesized to “hitchhike” on specialized, Rtn1-containing ER that is transported into the growing *Drosophila* oocyte (Roper, 2007). Support for a conserved connection between mRNA processing in RNP complexes and regulation of the secretory pathway comes from studies in *C. elegans* showing a change in the subcellular localization and morphology of the ER in arrested and stressed oocytes in which large RNPs have assembled (Patterson et al., 2011). However, in *Drosophila*, no similar changes in the ER are observed concomitant with the assembly of the large, reticulated sponge bodies (Snee and Macdonald, 2009b). Further studies are needed to better describe the close association between the ER membranes and RNP granules in oocytes.

5.4. Nuclear envelope

5.4.1. Close associations of RNP granules with nuclear pore complexes—

Several types of RNP granules localize near the oocyte nucleus at least transiently, and in some cases, contacts occur with nuclear pores in the nuclear envelope. Over 40 years ago, electron dense material in early amphibian oocytes was shown to extend between the nuclear pores and germ granules (Eddy and Ito, 1971). From more recent ultrastructural studies, we know that sponge bodies are enriched on the cytoplasmic face of the nuclear membrane in stages 7–10 of *Drosophila* oogenesis (Wilsch-Brauninger et al., 1997). Furthermore, *C. elegans* P granules are associated with clusters of nuclear pore complexes on the envelope of pachytene-stage nuclei where they interact with RNA export factors and newly synthesized RNA (Pitt et al., 2000; Sheth et al., 2010). Further evidence suggesting some functional significance of the association between germ granules and nuclear pore complexes comes from mouse spermatocytes in which the CB is connected to electron dense material within the nuclear pore complexes (Soderstrom and Parvinen, 1976b). Molecular studies indicate some RNP granule components, such as the *Xenopus* RNA helicase Xp54, shuttle between the nucleus and cytoplasm through nuclear pores (Smillie and Sommerville, 2002), and some yeast P body proteins interact with mRNA export factors (Fischer and Weis, 2002). The close proximity of RNP granules to nuclear pores may ensure that subsets of mRNA are properly targeted to ensure proper translational control.

5.4.2. Increase of nuclear membrane blebbing and formation of annulate lamellae concomitant with RNP granule assembly—Recent ultrastructural evidence has revealed a novel response involving nuclear pore complexes in heat-stressed and meiotically arrested *Caenorhabditis* oocytes. Increased numbers of nuclear membrane blebs form along the nuclear envelope of these oocytes and detach from the envelope, trafficking toward the cell cortex (Patterson et al., 2011). The detached nuclear membrane blebs near the nuclear envelope are closely associated with P granules in the cytoplasm, and the increase in nuclear blebbing coincides with the formation of large RNP granules in the oocyte cortex. The nuclear membrane blebs appear to be distinct from any previously described nuclear membrane extension. For example, at step 3 of spermiogenesis, cytoplasmic CBs have been seen transiently located at “outpocketings of the nuclear envelope”; however, these extensions do not appear to actively bleb away from the envelope, and they have different morphological characteristics (Parvinen and Parvinen, 1979).

Perhaps related to the nuclear membrane blebs, the formation of annulate lamellae in arrested and heat-stressed *Caenorhabditis* oocytes has recently been detected (Patterson et al., 2011). Structures resembling nuclear pores on cytoplasmic membranes, and termed

annulate lamellae, have been observed in the oocytes of many animals and in a variety of other cell types, particularly those with translationally repressed mRNAs (Kessel, 1992). These lamellae can occur in stacks of up to 100 aligned membranes. In human round spermatids, annulate lamellae-nuclear pore complexes are found at the nuclear envelope at the site of CBs (Chemes et al., 1978). The function of these membranes is not yet understood; however, one speculation is that the annulate lamellae facilitate the recruitment of novel components to large RNP granules. Consistent with this model, RNAi knockdown of a subset of nuclear pore complex proteins prevents the normal assembly of RNP granules (Patterson et al., 2011).

6. Function of RNP Granules in Oocytes

6.1. mRNA localization

Numerous localized maternal mRNAs associate with RNP complexes in oocytes of *Drosophila*, *Xenopus*, zebrafish, and *C. elegans* (Table 7.1; Beshore et al., 2011; Bontems et al., 2009; Kloc and Etkin, 1995; Kloc et al., 2002; Kosaka et al., 2007; Nakamura et al., 2001; Schisa et al., 2001), and several components of RNP granules have been identified that are required for the recruitment of the mRNAs. Buckyball, for example, is required for both Balbiani body aggregation and localization of mRNAs to the vegetal cortex of zebrafish oocytes (Bontems et al., 2009; Marlow and Mullins, 2008). For some mRNAs in some species, the process of RNA localization appears to begin in the nucleus with specific elements in the 3' UTR interacting with RNA-binding proteins. The actual movement of RNA to regions of the germ plasm or to germ granules often involves motor-driven transport along micro-tubules or microfilaments, differential stabilization of RNA, or entrapment. Once at its destination, the localized RNA within the germ plasm is often anchored to the cell cortex. Throughout the localization process, translation must be regulated to avoid ectopic expression (Zhou and King, 2004). In fact, mRNA localization is often tightly coupled with translational regulation, as this can result in activation of translation in specific subcellular regions of a cell. How RNP granules may contribute to the regulation of translation is described below; in this section, I will focus on a few classic studies as well as more recent advances in our understanding of RNA localization that stem from elegant live imaging and high-resolution microscopy studies.

In *Xenopus*, two pathways have been defined that localize oocyte RNAs: the early or messenger transport organizer (METRO) pathway localizes RNAs such as *Xcat-2* as to the vegetal region of the oocyte in stage I oocytes, and the late pathway localizes RNAs such as *Vg1* during later oogenesis (King et al., 1999; Kloc and Etkin, 1995). In the METRO pathway, the mitochondrial cloud is the location where several mRNAs as well as the germinal granules are concentrated (Kloc and Etkin, 1995). The mitochondrial cloud moves from a perinuclear position toward the vegetal cortex, and between stages II and VI, the MC fragments into small islands that continue to localize germ granules and mitochondria. The islands eventually become anchored at the vegetal cortex, likely via actin filaments (Kloc and Etkin, 1995). Detailed electron microscopy *in situ* hybridization studies have illustrated that some METRO RNAs localize to the germ granules, while others are at the periphery of the granules, and still others do not localize to the granules (Kloc et al., 2002; Fig. 7.3D–F).

The localization of mRNAs to the mitochondrial cloud in early *Xenopus* oocytes or in sponge bodies of the *Drosophila* nurse cells may serve as a means for coupled transport of multiple localized mRNAs and RNA-binding proteins within discrete particles (Macdonald, 2011). Indeed, microtubules and *Drosophila* hnRNP Squid (Sqd) have been proposed to facilitate first the transport, and ultimately the anchoring, of mRNAs such as *gurken* to their ultimate destination in sponge bodies (Delanoue et al., 2007). Interestingly, the motor protein Dynein is present in sponge bodies and is required for the anchoring of *grk* mRNA

in the RNPs, but the anchoring does not require Egl or BicD, two other motor proteins needed for RNA transport. Other mechanisms of RNA localization include “trapping” of RNA by germ granules, as indicated for *nanos* (Rongo et al., 1997). Several RNA-binding proteins in *Drosophila* sponge bodies are required to direct *oskar* RNA (Exu, Yps, Rap55/Trailerhitch, Hrp48) (Wilhelm and Smibert, 2005; Wilhelm et al., 2000; Yano et al., 2004), and two recent studies also point to a role for polypyrimidine tract binding protein (PTB) in the assembly of large *osk* RNPs concomitant with *osk* RNA localization and translational repression. An intriguing model is suggested wherein the PTB-dependent assembly of *osk* mRNA into large RNPs facilitates translational regulation *in trans* (Besse et al., 2009; Reveal et al., 2010). One aspect of the model, discussed by Macdonald (2011), requires that the RNPs exclude mRNAs not translationally regulated, and the observation that *osk* mRNA localizes to specific subdomains of sponge bodies is consistent with the idea of specificity and exclusion. Future studies will continue to test this model and determine if similar *trans* mechanisms occur in other systems. Additional questions remain as to whether mRNAs stably associate with large RNP complexes or actively transit in and out of them, and whether a subset of mRNAs functions as scaffolding to promote the assembly or maintain the structure of RNP granules.

6.2. mRNA stability

Early embryonic development relies on the contribution of maternal mRNAs from the oocyte, yet late-stage oocytes are often transcriptionally quiescent (Schisa et al., 2001; Walker et al., 2007); thus, the regulation of maternal mRNA stability is critical in oocytes. Since many oocyte RNP complexes contain P body proteins with established roles in mRNA decapping and decay in somatic cells, it was surprising to find that in *C. elegans*, the DDX-like DEAD-box RNA helicase CGH-1 associates with approximately 300 mRNAs that are maintained during oogenesis (Boag et al., 2008). In the absence of CGH-1, several mRNAs including *pos-1*, *nos-2*, *oma-1*, and *glp-1* are destabilized, and sterility results (Boag et al., 2008; Noble et al., 2008). Thus, CGH-1 appears to have a role in protecting a subset of maternal mRNAs from degradation. Interestingly, loss of CGH-1 does not result in premature translation of *pos-1* mRNA (Boag et al., 2008). In meiotically arrested oocytes, CGH-1 is localized to large RNPs where it may play similar roles in regulating mRNA stability. It also appears to control aspects of RNP granule composition and dynamics (Noble et al., 2008). Since DEAD-box helicases generally bind RNA nonspecifically (Rocak and Linder, 2004), it is still unclear how CGH-1 protects particular mRNAs. However, one model suggests that the RNA-dependent association of poly (A) binding protein, PAB-1, with CGH-1 may promote the interaction of PAB-1 with mRNAs, thereby resulting in inhibited deadenylation and degradation (Boag et al., 2008; Mangus et al., 2003).

Additional examples of P granule components having roles in RNA stability are the GLD-4 and GLS-1 proteins that together form a functional poly (A) polymerase (PAP) (Rybarska et al., 2009; Schmid et al., 2009). The PAP complex prevents the degradation of the *gld-1* mRNA, which is otherwise mediated by two PUF proteins and CCR, a likely deadenylase. In contrast, some RNP granule proteins appear to have opposing roles in promoting mRNA degradation. Three proteins with roles in the 22G-RNA pathway, Dicer-related helicase, DRH-3, RNA-dependent RNA polymerase, EGO-1, and the Argonaute protein, CSR-1, localize to P granules (Claycomb et al., 2009). In *ego-1* mutants, small triphosphorylated antisense RNAs are not made, resulting in their target mRNAs being overexpressed (Maniar and Fire, 2011).

In *Drosophila*, bicoid stability factor (BSF) is required for stabilizing *bcd* transcripts and appears to be localized in discrete RNPs in nurse cells that appear to be distinct from sponge bodies (Mancebo et al., 2001; Snee and Macdonald, 2009b). Importantly, the authors were

able to separate the actions of RNA localization and RNA stability, as mutants that abrogated *bcd* localization did not impact RNA stability. In *Xenopus*, Xp54 localizes to germinal granules of the Balbiani body and regulates mRNA stability in an mRNA-specific manner. When Xp54 is overexpressed, the levels of histone H4 decrease; however, the levels of FRGY1 and cyclin-B1 increase. And likewise, when Xp54 expression is downregulated, histone H4 levels increase and FRGY1 levels decrease (Weston and Sommerville, 2006). Experiments performed to decouple the localization of BSF or other localizing factors from RNPs may reveal important answers regarding the functional relationship between the RNP structure and the regulation of RNA stability.

6.3. Translation repression

Translational control in oocyte development has been a hotbed of active investigation over the past few decades. One recent review comprehensively describes the advances in understanding the repression and activation of maternal mRNAs in oocytes in *Drosophila* and *Xenopus* (Richter and Lasko, 2011); therefore, this review includes only a few highlights of those advances and focuses on them in the context of RNP granules. Findings from *C. elegans* are also included to illustrate conservation of several mechanisms as well as some differences.

The regulation of *oskar* mRNA translation is regulated by at least 14 different proteins, several of which localize to discrete RNPs (Herget et al., 2000; Wilhelm and Smibert, 2005). Within sponge bodies, *osk* mRNA is repressed by Bruno and Cup. Bruno contains three RNA recognition motifs (RRMs) that bind Bru-response elements in the 3'UTR of *osk* to repress its translation (Kim-Ha et al., 1995; Lie and Macdonald, 1999; Snee et al., 2008; Webster et al., 1997). This is thought to occur via the recruitment of Cup, where Cup competes with eIF4G for binding to eIF4E (Filardo and Ephrussi, 2003; Nakamura et al., 2004; Nelson et al., 2004; Wilhelm et al., 2003; Zappavigna et al., 2004), and *oskar* is precociously translated if Cup binding to eIF4E is disrupted (Nakamura et al., 2004). Bruno also functions in a Cup-eIF4E-independent manner, as it is required for *osk* RNA to be recruited to very large "silencing particles" that contain multimers of RNA that are inaccessible to the translational machinery (Chekulaeva et al., 2006). The relationship between these biochemically described particles in ovaries and the cytologically studied sponge bodies of nurse cells and oocytes has not been addressed; however, it seems likely that these studies are describing the same type of particle or granule, as Bruno is known to be at high levels in sponge bodies of nurse cells (Snee and Macdonald, 2009b).

Both silencing particles and sponge bodies contain the DEAD-box RNA helicase Me31B, which appears to be required for translational silencing of *osk* and *BicD* as they are transported from nurse cells to the oocyte (Nakamura et al., 2001). How an RNA helicase represses translation is not obvious, though one possibility may be to unwind target mRNAs to allow for loading of the repression machinery. This may be an oversimplification, however, as Me31B appears to be a fairly stable component of the complex (Nakamura et al., 2001), and therefore it may have additional regulatory functions including a possible switch between repression and active translation. Other negative regulators of *osk* translation include Bicaudal-C (Bic-C), another RNA-binding protein found in sponge bodies that recruits the CCR4 deadenylase complex to target mRNAs (Saffman et al., 1998; Snee and Macdonald, 2009a). Deadenylation often plays a role in repressing translation of mRNAs, and the regulation of deadenylation is emerging as an increasingly important mechanism (Richter and Lasko, 2011). Broad themes of translational control also include the coupling of RNA localization and translational regulation, and the location of translational regulation elements in 3'UTRs of target mRNAs, as mentioned above for *osk*. Many details on the mechanisms of translational repression of maternal mRNAs and the localization of

translational regulators to sponge bodies in *Drosophila* oocytes have been elucidated. What is less clear is the precise, mechanistic advantage of accumulating upward of 18 proteins and 8 mRNAs (Table 7.1) into extremely large RNP complexes. Outstanding questions also include whether localization of mRNAs to sponge bodies is necessary for translational repression,

From a biochemical standpoint, the model of *osk* translational repression in *Drosophila* is often compared to that for *cyclin-B1* in *Xenopus*. In the case of *Xenopus* oocytes, the Maskin protein mediates repression of the *cyclin-B1* mRNA (Cao and Richter, 2002; Stebbins-Boaz et al., 1999). Maskin, like Cup, binds eIF4E preventing the binding of eIF4G with eIF4E. This also prevents eIF4G from recruiting the 40S ribosomal subunit to the 5' end of the mRNA. However, one important difference is that Maskin is localized at the animal pole of stage 5 and 6 oocytes and is not observed in the mitochondrial cloud in the vegetal half of oocytes at earlier stages of development (Groisman et al., 2000). Other pertinent proteins have been identified biochemically, but their localization within the oocyte is not yet known. Thus, perhaps more relevant to the function of the germinal granules in the mitochondrial cloud are the following two observations. One is the localization of the shuttling protein Xp54, an RNA helicase homologous to Me31B (Table 7.3), to RNP particles in the mitochondrial cloud (or Balbiani body) (Ladomery et al., 1997; Smillie and Sommerville, 2002). Xp54 binds nascent transcripts in the nucleus and appears to be exported with them into the cytoplasm; it continues to associate with the masked messages until they are translationally activated. The second set of observations includes several of the mRNAs listed in Table 7.1, including *Xdazl* and *Xcat2*, that are translationally repressed at the time the mRNAs are localized to the mitochondrial cloud (Houston and King, 2000; MacArthur et al., 1999). Future studies should bolster the current, largely correlative evidence suggesting that RNA localization to the mitochondrial cloud is functionally linked with translational regulation. The relationship between mechanisms of translational repression involving the germinal granules in the mitochondrial cloud of early *Xenopus* oocytes and the RNP particles observed in later stages of *Xenopus* oogenesis should also continue to be explored. In addition to the repression mediated by Maskin in the animal half of stages V–VI oocytes, the *Xenopus* homolog of the ELAV RNA-binding protein family member HuB, ElrB, binds the 3'UTR of the vegetally localized *Vg1* mRNA to repress its translation (Colegrove-Otero et al., 2005). ElrB localizes at the vegetal cortex with XStaufen and appears in discrete RNP particles (Arthur et al., 2009).

Evidence that proteins within RNP granules regulate translational repression is also found in *C. elegans*. The evidence for RNP-localized eIF4E-binding proteins inhibiting translation by competing with eIF4G for binding to eIF4E is somewhat mixed. On the one hand, the first homolog of an eIF4E-binding protein, SPN-2 (also known as PQN-45 and IFET-1), was determined to localize to P granules in oocytes and early embryos, and its binding to the RNA-binding protein OMA-1 results in OMA binding to the *mei-1* 3'UTR and repressing its translation during the first mitotic division (Li et al., 2009). Interestingly, SPN-2 does not repress the translation of *mei-1* in oocytes, and more intriguingly, SPN-2 does not need to be localized to P granules in order to effectively repress *mei-1* translation in the early embryo. Thus, the need for repressors to localize to RNP granules is not universal; however, more recent data with a deletion allele of *spn-2* indicate that *spn-2* is required for oogenesis as well as P granule formation. In addition, SPN-2 appears to be necessary for translational regulation of at least two mRNAs in the gonad (*puf-5* and *rme-2*) (P. Boag, unpublished results).

Translationally repressed mRNAs accumulate to high levels in the large RNPs of arrested or stressed *C. elegans* oocytes (Noble et al., 2008; Schisa et al., 2001), similar to the abundant mRNAs in *Xenopus* mitochondrial clouds/Balbani bodies and *Drosophila* sponge bodies

that are also typically repressed while associated with granules but activated after release from the granules (Kloc et al., 2002). Moreover, in mouse spermatids, translation of Brd2 and GCNF occurs as the mRNAs exit from the CB (Nguyen Chi et al., 2009). Studies in worms bolster the view from *Drosophila* and *Xenopus* experiments that proteins concentrated in RNP granules play essential roles in repressing translation. Two such proteins, CAR-1 and PUF-5, are required to repress the translation of *glp-1* mRNA, and as for *osk* mRNA, a defined element in the *glp-1* 3'UTR is the target of PUF-5 binding (Lublin and Evans, 2007; Noble et al., 2008). Loss of *car-1* results in a failure of granules to assemble normally, concomitant with derepression of the *glp-1* mRNA. Interestingly, despite the many indicators linking RNP complexes with translational regulation, the formation of P bodies in yeast and *Drosophila* appears to be a consequence, not a cause of repression for at least some mRNAs (Chu and Rana, 2006; Eulalio et al., 2007). Likewise, the repression of mRNA translation in *C. elegans* does not strictly require the assembly of large RNPs. For example, in *car-1* mutants when CGH-1 fails to assemble into large RNPs in oocytes in extended meiotic arrest, *glp-1* mRNA remains repressed (Noble et al., 2008). This could mean the role of CAR-1 is to promote the association of target mRNAs to RNP granules or maintain the structure of RNP granules. Alternatively, some type of RNP granule lacking CGH-1 may form and be sufficient to repress *glp-1* translation. Since *car-1* mutants have relatively mild phenotypes, it has been suggested that other related RNA-binding factors more directly control translational repression, thus separating granule structure from repression (Noble et al., 2008). CGH-1, in contrast, is not required for the translational repression of at least one of its target mRNAs, *pos-1* (Boag et al., 2008). Instead, CGH-1 appears to have more critical roles in stabilization of maternal messages. An unresolved issue in this area is a clear understanding of the contribution of individual proteins, and the impact of RNP granule architecture on translational repression.

6.4. Translation activation

While the majority of studies of RNP granules suggest functions in repressing translation of maternal mRNAs in oocytes, a few experiments, mainly in *Drosophila*, also point to roles in translation activation. In the sponge body, *gurken* mRNA is initially, translationally repressed by Cup, Bruno, and Squid and then activated after localization to the dorsal anterior of oocytes. Poly(A) binding protein, PABP55B, contributes to the activation of *grk* mRNA, likely via its RRM(s) binding the poly(A) tail (Clouse et al., 2008). Once PABP55B binds a target RNA, it binds eIF4G and its affinity is increased for binding to eIF4E, outcompeting Cup binding to eIF4E (Richter and Sonenberg, 2005). The DEAD-box helicase Vasa also positively regulates *grk* translation (Styhler et al., 1998; Tomancak et al., 1998), likely by recruiting eIF5B (Johnstone and Lasko, 2004). A second example of an activator protein localized to RNP granules is Orb. Orb is the *Drosophila* homolog of CPEB and is a sponge body component (Snee and Macdonald, 2009b). The Orb protein and extension of the *oskar* mRNA poly (A) tail are required for its efficient derepression at the posterior pole of the oocyte (Castagnetti and Ephrussi, 2003; Chang et al., 1999). The CPEB protein also associates with the RNP subcortical aggregates in mouse oocytes (Flemer et al., 2010). A recent study in *C. elegans* also illustrates the positive role of the P granule-associated PAP proteins GLD-3/GLD-2 and GLD-4/GLS-1 in activating the translation of the *gld-1* mRNA (Schmid et al., 2009). A key question in this area will be to determine how changes in the composition of RNP granules alter the balance between translational repression and translational activation.

6.5. Fertility

The functions of RNP granules in regulating mRNA localization and metabolism are beginning to become understood; however, a larger question is their effect on germ cell function and fertility. A role for germ granules in specifying the germline was first

suggested a long time ago. Irradiation experiments and transplantation experiments tested the necessity and sufficiency of germ granules to direct the germ cell fate (Bounoure, 1934; Geigy, 1931; Illmensee and Mahowald, 1974; Smith, 1966). When key RNP granule proteins are missing, germ cell development is often disrupted. For example, *Drosophila vasa* mutants lack pole cells and are infertile (Schupbach and Wieschaus, 1986), and *C. elegans glh* mutants have germlines with reduced numbers of germ cells and no oocytes (Gruidl et al., 1996). That RNP granules do not have obvious toxic or deleterious effects on oocyte viability is demonstrated by the outcomes when extra-large RNP complexes are induced in oocytes. When reticulated sponge bodies assemble, viable embryos result with no obvious morphological defects (Snee and Macdonald, 2009b). Similarly, in the case of arrested *C. elegans* oocytes that have assembled large RNP granules, greater than 90% of the resulting embryos are viable (Jud et al., 2008).

7. Conclusions and Future Perspectives

It is clear that the past decade has brought immense progress in the field of germline RNP granules. With the ongoing identification of RNP granule components, the conserved nature of these RNP complexes becomes even more clear (Table 7.2), and we can expect additional RNA and protein components to be confirmed as *bona fide* granule components based on results from biochemical and cell culture experiments. Considerable efforts have been made toward understanding the regulation of RNP granule dynamics. These questions have been greatly aided by advances in live-imaging technology. The microtubule cytoskeleton and associated motor proteins appear to play important roles in the assembly of RNP granules in oocytes. In the future, we can anticipate progress in understanding the regulation of specific components trafficking to and from RNP granules, and whether mRNA degradation affects this process. Additional key questions for the field include understanding the kinetics of protein movement in and out of granules and the regulation that shifts the balance toward granule assembly or dissociation. The formation of extremely large RNP complexes in oocytes that are induced by extended meiotic arrest, protein deprivation, and environmental stresses such as heat shock may suggest remodeling of existing RNP granules as cellular needs change. Indeed, several groups speculate the various types and sizes of RNP granules represent related hierarchies of RNP complexes.

A major goal remains to determine the function of RNP complexes in oocytes and their functional relationship to RNA stability, localization, and translation. Further work is needed to understand the minimal functional size of an RNP granule. For example, when RNPs are not detected cytologically, and translational repression persists, one interpretation might be that the RNP components have no role in regulating translation. An alternative model is that small mRNPs, that are not detectable at the light microscopic level, are capable of controlling translation, and the larger, visible aggregates of RNPs have additional properties. For example, one could imagine that assembly effectively compartmentalizes mRNAs away from the translation machinery. Further detailed investigations into these questions will be needed to fully explain whether RNP granules have any required role in regulating translation or if they are instead a consequence of repressed mRNAs.

Acknowledgments

The author thanks Peter Boag, Florence Marlow, and Melissa Pepling for critical comments on the chapter and the members of the Schisa laboratory for helpful discussions and suggestions. This work was supported by NIH Grant 1 R15 GM93913-01 to J. Schisa.

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Figure 7.1.

C. elegans P granules. (A) Schematic showing one of two arms that form the U-shaped *C. elegans* gonad. Note cells are in mitosis at distal end, and in meiosis in the medial and proximal regions of the gonad. Oocytes mature at the proximal end where they will be ovulated and fertilized by the hermaphrodite's sperm (sp). (B) Transmission electron micrograph shows granulo-fibrillar, electron dense P granules (Pg) in the cytoplasm of the oocyte. Mitochondria (mt) are often in close proximity to P granules. Nu is nucleus; no is nucleolus. Scale bar is 1 μm . (C and D) Oocyte P granules are marked by anti-PGL-1 (green) and are in close association with nuclear pore complex proteins (red). Scale bar is 5 μm . Panels A–D are reprinted from Pitt et al. (2000), Copyright 2000, with permission from Elsevier. (E) Large RNP granules (RNPg) assemble at the cortex of *C. remanei* oocytes after heat shock (also true for *C. elegans*, not shown). Pm is plasma membrane; Mt is mitochondria. Arrowheads indicate cross-sections of ER membrane. Panel E is reprinted from Patterson et al. (2011), Copyright 2011, with permission from Elsevier.

Figure 7.2.

Drosophila sponge bodies, polar granules, and Balbiani bodies. (A) Schematic of the developing posterior germarium and oogenesis, showing early stages through stage 7. Oocyte is the right-most cell in which the nucleus moves to a dorsal anterior position by stage 7 and is surrounded by nurse cells. Panel A is reprinted from Bastock and St Johnston (2008) with permission from Elsevier. (B and B') Transmission electron micrographs of granulofibrillar, electron dense, and membrane-rich sponge bodies in a stage-9 nurse cell (B) and stage-9 oocyte (B'). Panels B and B' ©1997 Rockefeller University Press. Originally published in Wilsch-Brauninger et al. (1997). (C–H) Sponge bodies are marked Trailerhitch::GFP (green) and anti-Bruno (red). Panels C–E show dispersed sponge bodies; Bruno is restricted to sponge bodies of nurse cells mainly. Panels F–H show reticulated sponge bodies which have larger granules of Tral and Bru. Panels C–H adapted from Snee and Macdonald (2009b) and reproduced with permission from John Wiley and Sons. (I) Transmission electron micrograph shows mitochondria entering the oocyte via a ring canal to form the Balbiani body (arrow). Panel I adapted and reproduced with permission from *Development*, Cox and Spradling (2003). (J and K) Transmission electron micrographs of electron dense polar granules in the posterior of a stage-10 oocyte (arrows). Panel K shows a high-magnification view of polar granules in a stage-14 mature oocyte. Several polar granules are associated with mitochondria (m). Panels J and K adapted and reproduced with permission from *Development*, Hay et al. (1988b).

Figure 7.3.

Xenopus germ granules and the mitochondrial cloud. (A) Schematic of early *Xenopus* development. Mitochondria (green) are clustered in the mitochondrial cloud, which moves from a perinuclear position in stage-1 oocytes to the vegetal cortex by stage 5. (B and C) Transmission electron micrographs show mitochondrial cloud in previtellogenic *Xenopus* oocyte. GV labels the germinal vesicle; MC labels the mitochondrial cloud. Note smooth endoplasmic reticulum (ER) in panel C. Panels B and C adapted from Fukui et al. (2003) and reproduced with permission from John Wiley and Sons. (D–F) Immunoelectron micrographs of stage-1 oocytes show Xcat2 mRNA (D), Xpat mRNA (E), and DEADSouth mRNA (F) localized inside, concentrated at the periphery, or at a low level at the periphery of germ granules, respectively. Panels A and D–F are reprinted from Kloc et al. (2002), Copyright 2002, with permission from Elsevier.

Figure 7.4.

Balbiani body in zebrafish oocyte. (A) The Balbiani body (Bb) is detected in the future vegetal region of the oocyte (red box). (B) Note mitochondria (arrows) are abundant and clustered within the Bb. Panels A and B are reprinted from Marlow and Mullins (2008), Copyright 2008, with permission from Elsevier. (C) Stage-1 oocyte stained with DiOC6 to label ER and mitochondria. Nucleus is marked by arrowhead, and Bb is marked with arrow at future vegetal side of the oocyte. WT indicates wild-type oocyte. Panel C is adapted from Gupta et al. (2010) and reproduced in accordance with the Creative Commons license.

Figure 7.5.

Chromatoid body in mouse spermatid. (A) Schematic of the chromatoid body during spermatogenesis. Note the condensed shape is detected after meiosis is completed (arrows). Panel A is reprinted by permission from Macmillan Publishers Ltd: (Kotaja and Sassone-Corsi, 2007), copyright 2007. (B, C) Immunoelectron micrographs showing spermatids labeled with anti-mouse Vasa homolog (MVH) antibody at two different magnifications. N is nucleus; mt is mitochondria. Panels B and C are reprinted from Toyooka et al. (2000), Copyright 2000, with permission from Elsevier.

Table 7.1

Diversity of RNP granules in oocytes

Type of RNP granule	Species	Components	
		RNA	Protein
P granule	<i>C. elegans</i>	<i>nos-2</i> (Schisa et al., 2001) <i>pos-1</i> (Schisa et al., 2001) <i>mex-1</i> (Schisa et al., 2001) <i>gld-1</i> (Schisa et al., 2001) <i>skin-1</i> (Schisa et al., 2001) <i>par-3</i> (Schisa et al., 2001)	GLH-1-4 (Gruidl et al., 1996; Kuznicki et al., 2000) PGL-1-3 (Kawasaki et al., 1998; 2004) CGH-1 (Navarro et al., 2001) CAR-1 (Boag et al., 2005) VBH-1 (Salinas et al., 2007) OMA-1-2 (Shimada et al., 2006) GLD-2-3-4 (Eckmann et al., 2002; Schmid et al., 2009; Wang et al., 2002) Sm proteins (Barbee et al., 2002) WAGO-1 (Gu et al., 2009) SPN-2 (Li et al., 2009) PRG-1 (Batista et al., 2008) GLS-1 (Rybarska et al., 2009) EGO-1, CSR-1, DRH-3 (Claycomb et al., 2009) DEPS-1 (Spike et al., 2008) DCAP-2 (Lall et al., 2005) DCR-1 (Beshore et al., 2011)
RNP granule (grP body) in arrested or stressed oocytes	<i>C. elegans</i>	<i>pos-1</i> (Schisa et al., 2001) <i>gld-1</i> (Schisa et al., 2001) <i>skin-1</i> (Schisa et al., 2001) <i>nos-2</i> (Schisa et al., 2001) <i>par-3</i> (Schisa et al., 2001) <i>glp-1</i> (Noble et al., 2008)	PGL-1, GLH-1, GLH-2, MEX-1 (Jud et al., 2007; Schisa et al., 2001) MEX-3, DCAP-2 (Noble et al., 2008; Schisa et al., 2001) CGH-1 (Boag et al., 2008; Jud et al., 2008; Noble et al., 2008) CAR-1 (Boag et al., 2008; Jud et al., 2008) PAB-1, TIA-1 (Jud et al., 2008) PUF-5, MEX-5 (Noble et al., 2008) DCR-1 (Beshore et al., 2011)
P body	Yeast/ mammalian or other cells*	Several	Dep1p, Dep2p (Ingelfinger et al., 2002; Lykke-Andersen, 2002; Sheth and Parker, 2003; van Dijk et al., 2002) Dhh1p, Pat1p, Ccr4p, Xrm1p, Edc3p (Sheth and Parker, 2003) Lsm1p (Andrei et al., 2005; Ferraiuolo et al., 2005; Ingelfinger et al., 2002; Sheth and Parker, 2003) Scd6p (Anantharaman and Aravind, 2004) RAP55* (Yang et al., 2006) Ago1, Ago2 (Liu et al., 2005; Sen and Blau, 2005) HuR* (Bhattacharyya et al., 2006) Siaufen* (Barbee et al., 2006)
Polar granule (or n = nuage)	<i>Drosophila</i>	Nos (Wang and Lehmann, 1991)	Oskar, Siaufen, Vasa, n (Breitwieser et al., 1996) Tudor, n (Hay et al., 1988a) Aubergine, n (Amikura et al., 2001; Harris and Macdonald, 2001) Piwi (Megosh et al., 2006) Maelstrom, n (Thomson et al., 2008) Smb (Anne, 2010; Findley et al., 2003)
Sponge body (or starvation-induced P body-like granule*)	<i>Drosophila</i>	Bcd, Nos, Orb, Pgc, Gcl, BicD, Osk* (Nakanura et al., 2001; Shimada et al., 2011) Grk (Delanoue et al., 2007)	Exuperantia (Wilsch-Brauninger et al., 1997) Trailerhitch (Wilhelm et al., 2005) Me31B* (Nakanura et al., 2001; Shimada et al., 2011) Dep1*, Dep2 (Lin et al., 2006; Shimada et al., 2011)

Type of RNP granule	Species	Components	
		RNA	Protein
Balbani body Balbani body (germ granules*)	<i>Drosophila</i> Zebrafish	Osk, Orb (Cox and Spradling, 2003) Vasa, nanos1, dazl (Kosaka et al., 2007) Buc, foxH1, yglRBP (Bontems et al., 2009) Syntaxin (Nojima et al., 2010) wnt8a (Lu et al., 2011)	Staufen, Hrb27C, Bru, Orb (Snee and Macdonald, 2009b) Yps* (Nakamura et al., 2001; Shimada et al., 2011; Wilhelm et al., 2000) eIF4E*, Btz (Shimada et al., 2011; Wilhelm et al., 2003) Cup (Nakamura et al., 2004; Wilhelm et al., 2003) dGe-1 (Fan et al., 2011) Gus (Styhler et al., 2002) Dhc, Sqd (Delanoue et al., 2007) Bic-C (Snee and Macdonald, 2009a) Cup, Orb (Cox and Spradling, 2003) GASZ (Yan et al., 2004) Hermes (Kosaka et al., 2007) Actin (Marlow and Mullins, 2008) Buc (Bontems et al., 2009; Marlow and Mullins, 2008) Zili* (Houwling et al., 2008) Ziwi* (Houwling et al., 2007)
Mitochondrial cloud/ Balbani body (germ granules in cloud*) (granules in late-stage oocytes#)	<i>Xenopus</i>	Xcat-2* (Forristall et al., 1995; Kloc and Etkin, 1995; Kloc et al., 2002) Xisirts (Kloc and Etkin, 1995; Kloc et al., 1993) Xwnt11 (Kloc and Etkin, 1995; Ku and Melton, 1993) Xdazl (Kloc et al., 2002; Houston et al., 1998) Xpat* (Kloc et al., 2002; Hudson and Woodland, 1998) DEADSouth* (Kloc et al., 2002; MacArthur et al., 2000) Fingers, XFACS (Kloc et al., 2002) Xotx1 (Pannese et al., 2000) FatVg (Kloc et al., 2002; Chan et al., 2001) Hermes, mtrRNA, mtrRNA (Zearfoss et al., 2004) centroid* (Kloc and Chang, 2007) Vgl (Yoon and Mowry, 2004)	Hermes (Kloc and Etkin, 1998) Spectrin (Kloc and Etkin, 1998) EF-1 alpha (Viel et al., 1990) XALK4 (Fukui et al., 2003) GASZ (Yan et al., 2004) Xp54 (Smillie and Sommerville, 2002) xRAP55 (Tanaka et al., 2006) Xpat (Machado et al., 2005) ElrB#, XStaufen# (Arthur et al., 2009) Actin (Gard et al., 1997)
SCA (subcortical aggregate) or Balbani body*	Mouse		DDX6, CPEB, YBX2/MSY2 (Flemer et al., 2010) Trailerhitch* (Pepling et al., 2007)
Chromatoid body	Mouse/human*	mRNA, miRNA (Kotaja et al., 2006a) RNA (Soderstrom and Parvinen, 1976b; Walt and Armbruster, 1984)	MVH/Vasa* (Noce et al., 2001; Toyooka et al., 2000) MIWI/Piwi, Dicer, GW182, DCP1a, Ago2, Ago3 (Kotaja et al., 2006a) KIF17b (Kotaja et al., 2006b) GRTH/Ddx25 (Tsai-Morris et al., 2004) P48/p52 (Oko et al., 1996) RanBPM (Shibata et al., 2004) Mtr1/Tdrd (Chuma et al., 2003) snRNP Sm proteins (Biggogera et al., 1990; Moussa et al., 1994)

Type of RNP granule	Components		
	Species	RNA	Protein
			Actin (Walt and Ambruster, 1984) Histone H4, Cytochrome c (Hess et al., 1993) MAEL (Soper et al., 2008) MIL1 (Wang et al., 2009) GEMIN3 [*] , NANOS1 [*] , PUMILIO2 [*] (Ginter-Matuszewska et al. 2011)

^{*} indicates proteins that are found in P bodies of mammalian or other cells (in contrast to yeast).

[#] indicates proteins that are found in germ granules in late-stage *Xenopus* oocytes (in contrast to germ granules in the mitochondrial cloud of early-stage oocytes).

Table 7.2

Conserved RNP granule proteins in oocytes

Type of proteins	<i>C. elegans</i> P granule	<i>Drosophila</i> sponge body/polar granule*	<i>Xenopus</i> mitochondrial cloud/late granule [#]	Zebrafish Balbiani body/germ granule*	Mouse subcortical aggregate/Balbani body [^]	Mouse/human* Chromatoid body	Yeast/mammalian* P body
RNA binding							
• Vasa RNA helicase	GLH family	Vasa*			Vasa (in fetal oocyte)	MVH-1, Vasa*	
• Staufen		Staufen	XStaufen [#]				Staufen*
• Hu/ELAV			ElrB [#]			HuR	HuR*
• Hermes			Hermes	Hermes			
• Cytoplasmic polyadenylation related Y box	GLD-2, -4 GLS-1	Orb			CPEB		
mRNA degradation		Ypsilon			YBX2/MSY2		
• Decapping enzymes	DCAP-2	dDcp1 dDcp2				DCP1a	Dcp1p Dcp2p
• Decapping activator/RNA helicase	CGH-1	Me31B	Xp54		DDX6	RCK	Dhh1p
• Decapping activator/Sm-like domain	CAR-1	Trailerhitch	xRAP55 [#]		Trailerhitch [^]	RAP55/Lsm14*	Scd6p
• Sm proteins of snRNP	Sm proteins	SmB*				Sm proteins	Lsm1p
• Tudor domain eIF4F complex	SPN-2	Tudor eIF4E, Cup				Mtr-1/Tdrd1	4E-T*
RNA interference							
• Dicer	DCR-1, DRH-3					Dicer	
• Argonaute family	CSR-1, PRG-1	Aubergine, Piwi		Ziwi*, Zili*		Ago2, Ago3, Miwi, Mili	Ago1*, Ago2*
Transposon related/piRNA		Maelstrom				Mael	

Table 7.3

Regulation of RNP granule dynamics in oocytes

Type of RNP granule	Species	Movement or subcellular change	Microtubule regulated or associated	Association with mitochondria
P granules	<i>C. elegans</i>	Perinuclear to cytoplasm		Yes
RNP granules (grP bodies) in arrested or stressed oocytes	<i>C. elegans</i>	Become enriched perinuclear and cortical	MT regulated	Yes
P bodies	Yeast/all eukaryotes	Highly motile	MT regulated; dependent on MT motor proteins	Yes
Polar granules	<i>Drosophila</i>	More stable than nuage		Yes
Sponge bodies	<i>Drosophila</i>	From nurse cells to oocyte	MT associated; dependent on MT motor proteins	Yes
Balbiani bodies	<i>Drosophila</i>		Yes	Yes
Balbiani bodies	Zebrafish	From nucleus to vegetal cortex	Yes	Yes
Mitochondrial cloud/germinal granules	<i>Xenopus</i>	From nucleus to vegetal cortex	No ^a	Yes
Subcortical aggregates	Mouse	ND	No	ND
Chromatoid bodies	Mouse	Highly motile		Yes

^a Acetylated microtubules were not disrupted in these *Xenopus* experiments, and acetylated microtubules are organized in the Balbiani body (Gard, 1991).