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RNA Granules in Germ Cells

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"Germ granules" are cytoplasmic, nonmembrane-bound organelles unique to germline. Germ granules share components with the P bodies and stress granules of somatic cells, but also contain proteins and RNAs uniquely required for germ cell development. In this review, we focus on recent advances in our understanding of germ granule assembly, dynamics, and function. One hypothesis is that germ granules operate as hubs for the post-transcriptional control of gene expression, a function at the core of the germ cell differentiation program.

"Germ granules" is a general term used to refer to the RNA-rich cytoplasmic bodies of germ cells. By electron microscopy, germ granules appear as compact granulo-fibrillar aggregates with no surrounding membrane (Fig. 1A,D) (Eddy 1975; Guraya 1979). Germ granules contain proteins and RNAs required for germ cell development, and may carry out posttranscriptional regulation specific to germ cells. In some organisms (i.e., *Drosophila*, *Xenopus*, *Caenorhabditis elegans*, and zebrafish), germ granules are present continuously throughout development, with the exception of mature sperm. In those organisms, germ granules are transmitted from oocyte to embryo as part of the germ plasm, a specialized cytoplasm that segregates with the germ lineage and is sufficient to specify germ cell fate. In other organisms, notably mammals, germ granules are not detected in oocytes or early embryos, but are formed de novo in primordial germ cells shortly after their specification. The current view is that, while not all organisms require germ granules to specify germ cell fate, all organisms depend on germ granules for germ cell function. Molecular studies have revealed intriguing similarities between germ granules and RNA granules present in somatic cells. In this review, we focus on recent advances in our understanding of germ granule composition, variety, dynamics, and function.

TYPES OF GERM GRANULES

Cytoplasmic aggregates that stained with RNA dyes were first detected in the germ cells of insects in 1890 (Ritter 1890), and have since been observed by cytochemistry or electron microscopy in over 80 species across the animal kingdom from rotifers to mammals (Eddy 1975). Today, antibodies against the Vasa family of RNA helicases are commonly used to identify

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germ granules. Many other conserved components of germ granules have also been identified (Tables 1 and 2). Some, like Vasa family members, are present in most germ granules, others are found only in specific stages. The morphology and localization of germ granules also changes during development. Here we describe the major classes of germ granules found in immature and differentiating germ cells (nuage), gametes (sponge, balbiani, and chromatoid bodies), and embryos (germ plasm) (Fig. 1) (Eddy 1975; Guraya 1979; also see de Sousa Lopes and Roelen 2010).

Perinuclear Granules

During most of germline development, germ granules appear as rounded fibrillar aggregates (or nuage, from the French for "cloud") that cluster around nuclei. This arrangement has been documented in the primordial germ cells (PGCs) of *Drosophila* (Mahowald 1968, 1971), *C. elegans* (Strome and Wood 1982, 1983), *Xenopus* (Ikenishi et al. 1996), and zebrafish (Knaut et al. 2000). In mice, small granules surrounded by fibrillar matrix and mitochondria become apparent around the nuclei of PGCs two days after their formation at 9–9.5 days of gestation (Spiegelman and Bennett 1973; Clark and Eddy 1975). Perinuclear granules persist after the PGCs become incorporated into the somatic gonad. Electron micrographic studies of *C. elegans* germ cells in meiotic prophase have shown that germ granules overlie dense clusters of nuclear pores, corresponding to the main sites of mRNA export from the nucleus (Fig. 1A) (Strome and Wood 1982; Pitt et al. 2000; Sheth et al. 2010). Close association of the nuage with nuclear pores has also been reported in ultrastructural studies of *Xenopus* (Czolowska 1969), zebrafish (Knaut et al. 2000; reviewed in Kloc et al. 2004), and mouse (reviewed in Chuma et al. 2009).

Balbiani Body

In differentiating gametes, germ granules often adopt unique morphologies. The Balbiani body, or mitochondrial cloud, is a transient structure, containing mitochondria, Golgi, endoplasmic reticulum (ER) and RNA, that forms in the young (previtellogenic) oocytes of insects and vertebrates (Cox and Spradling 2003; Kloc et al. 2004; Wilk et al. 2005; Pepling et al. 2007). In *Drosophila*, ER membranes associated with the Balbiani body are enriched for the ER resident protein reticulon, suggesting that these membranes represent a specialized subset of smooth ER (Roper 2007). The Balbiani body initially forms near the nucleus and spreads to the cytoplasm during oocyte growth. In *Drosophila* and *Xenopus*, the aggregates eventually localize to a subregion of the oocyte cortex, in which they incorporate with other germ granule material to form the germ plasm that will be transmitted to the germline of the next generation (see below). It has been proposed that the Balbiani body selects the best mitochondria for incorporation in the germline (Cox and Spradling 2003). In *Xenopus*, mitochondria in the germ plasm have lower respiratory activity than mitochondria destined for the soma (Kogo et al. 2011). Lower respiration is hypothesized to decrease oxidative stress and mutational damage. A *Drosophila* mutant that disrupts mitochondrial transport and association with the Balbiani body, however, had no apparent effect on fertility (Cox and Spradling 2006).

Balbiani bodies, consisting of ER and mitochondria interspersed with nuage surrounding Golgi stacks, have also been reported in mouse oocytes, which do not form germ plasm (Pepling et al. 2007). The mouse Balbiani bodies are present in the cyst and primordial follicle stages, and disappear during follicular growth (Fig. 1B) (Pepling et al. 2007). Their function is not yet known.

Sponge Bodies

During *Drosophila* oogenesis, each growing oocyte is connected to 15 nurse cells through intercellular bridges or "ring canals" (Fig. 2A). In addition to perinuclear nuage, nurse cells and the oocyte contain sponge bodies, which are stacks of endoplasmic reticulum (ER) cisternae embedded in an electron-dense matrix scattered through the cytoplasm (Fig. 1C)

Table 1. Conserved components of germ granules—listed are components localizing to germ granules in two or more species

	Function	Organism: Protein name and germ granule type	References
Proteins			
Vasa and related DEAD box RNA helicases	RNP remodeling	*Drosophila*: Vasa in nuage and polar granules; *C. elegans*: GLH-1-4 in P granules; *Xenopus*: XVLG1, nuage in oogonia, perinuclear in PGCs; Zebrafish: Vasa, perinuclear in PGCs; Mouse: MVH in chromatoid body; Humans: Vasa, perinuclear in fetal oocytes	Hay et al. 1988; Liang et al. 1994 Gruidl et al. 1996a Ikenishi et al. 1996; Bilinski et al. 2004 Knaut et al. 2000 Toyooka et al. 2000 Castrillon et al. 2000
Argonaute family	Small RNA (miRNA/piRNA) regulation	*Drosophila*: Aubergine in nuage and polar granules, PIWI in polar granules, Ago3 in nuage; Mouse: Miwi, Mili, Ago2, Ago3 in chromatoid body; Zebrafish: Ziwi, Zili in germ granules, perinuclear in PGC and germ cells; *C. elegans*: PRG-1, CSR-1 in P granules	Harris and Macdonald 2001; Megosh et al. 2006; Nishida et al. 2007; Li et al. 2009 Kotaja et al. 2006a,b Houwing et al. 2007, 2008; Batista et al. 2008; Claycomb et al. 2009
Dicer	miRNA production	Mouse: Dicer in chromatoid body; *C. elegans*: DRH-3, DCR-1 in P granules	Kotaja et al. 2006a Claycomb et al. 2009 Beshore et al. 2011
Maelstrom (HMG box)	Chromatin and piRNA regulation	*Drosophila*: Maelstrom in nuage; Mouse: Maelstrom in chromatoid body	Findley et al. 2003 Costa et al. 2006
Tudor domain proteins (methyl-binding modules)	Recruitment of methylated proteins to germ granules; recruitment of mitochondrial ribosomal RNA to polar granules in *Drosophila*	*Drosophila*: Tudor in nuage and polar granules, Krimp, Tejas, and PAPI in nuage; Mouse: Mtr (TDRD1-7;9) in intermitochondrial cement and chromatoid body, Tdrd4/Rnf17 in Rnf17-granule	Bardsley et al. 1993 Arkov et al. 2006; Lim and Kai 2007; Patil and Kai 2010; Liu et al. 2011 Chuma et al. 2003; Pan et al. 2005; Hosokawa et al. 2007; Vasileva et al. 2009 Aravin et al. 2009; Shoji et al. 2009
WD repeat proteins	Cofactors of protein arginine methyltransferase; targeting of methylation?	*Drosophila*: Valois (homolog of MEP50) in polar granules and nuage; Mouse: WDR77 in chromatoid bodies	Anne and Mechler 2005 Vagin et al. 2009b
Sm proteins	Splicing in the spliceosome, germ granule localization and germ cell fate in *C. elegans*	*C. elegans*: Sm in P granules; *Xenopus*: Sm in nuage in oocytes; Mouse: Sm in chromatoid body; *Drosophila*: SmB and SmD3 in polar granules	Barbee et al. 2002 Bilinski et al. 2004 Moussa et al. 1994; Chuma et al. 2003 Anne 2010; Gonsalvez et al. 2010

Continued

Table 1. *Continued*

	Function	Organism: Protein name and germ granule type	References
Dcp1	Decapping of mRNAs in P bodies, localization of Oskar RNA to the germ plasm in *Drosophila*	*Drosophila*: dDcp1 in sponge bodies; Mouse: Dcp1a in chromatoid body, and perinuclear spermatocyte nuage	Lin et al. 2006 Kotaja et al. 2006a Aravin et al. 2009
Dcp2	Decapping of mRNAs in P bodies	*Drosophila*: dDcp2 in sponge bodies; *C. elegans*: DCP-2 in P granules	Lin et al. 2006 Lall et al. 2005
Dhh1p/Rck (DEAD box RNA helicase)	Repression of translation (also in P bodies)	*Drosophila*: Me31b in sponge bodies, and polar granules; *C. elegans*: CGH-1 in P granules	Nakamura et al. 2001; Thomson et al. 2008 Navarro et al. 2001
Scd6p/Rap55 (Sm-like domain)	Repression of translation (also in P bodies)	*Drosophila*: Trailer Hitch in Balbiani body (early oocyte), nuage and sponge bodies (oogenesis); *C. elegans*: CAR-1 in P granules; mouse: Trailer hitch in Balbiani body (early oocytes), perinuclear granule (spermatocytes)	Wilhelm et al. 2005 Liu et al. 2011 Audhya et al. 2005; Boag et al. 2005; Squirrell et al. 2006 Pepling et al. 2007
Xrn1	Exonuclease degrading mRNA	*Drosophila*: PCM in nuage; Mouse: Xrn1 in perinuclear spermatocyte nuage	Lim et al. 2009 Aravin et al. 2009
Nanos	Repression of translation	Zebrafish: in perinucelar germ granules of PGCs; *C. elegans*: in P granules of embryos	Koprunner et al. 2001 Subramaniam and Seydoux 1999
eIF4F complex	Translational regulation by components or binding partners of eIF4F	*Drosophila*: eIF4A polar granules; eIF4E-binding protein Cup in Babliani body and sponge bodies (oogenesis); *C. elegans*: IFE-1(eIF4E) in P granules	Cox and Spradling 2003; Wilhelm et al. 2003; Nakamura et al. 2004; Thomson et al. 2008 Amiri et al. 2001
RNAs			
Nanos (mRNA)	Repression of translation	*Drosophila*: *Nanos* RNA enriched in germ plasm; *C. elegans*: *nos-2* RNA enriched in P granules; *Xenopus*: *Xcat2* RNA in balbiani body; Zebrafish: *nos1* RNA enriched in germ plasm; Pea Aphid: nanos RNA in nuage-like structure in oocytes	Forrest and Gavis 2003 Subramaniam and Seydoux 1999 Mosquera et al. 1993; Bilinski et al. 2004 Koprunner et al. 2001 Chang et al. 2006
Mitochondrial large and small ribosomal RNAs (noncoding RNAs)	Translation of *germ cell less* (*gcl*) in *Drosophila*	*Drosophila*: on surface of polar granules of syncytial embryo; *Xenopus*: in germinal granules; planaria: on surface of chromatoid bodies	Kashikawa et al. 1999 Kashikawa et al. 2001 Sato et al. 2001
piRNA	Repression of transposons	Zebrafish: oocyte germ granules; mouse: chromatoid body	Houwing et al. 2007 Melkar et al. 2010

Table 2. Components of the mouse chromatoid body

Component	Function	References
MVH	Dead box RNA helicase that is required for spermatogenesis	Chuma et al. 2003; Kotaja et al. 2006a,b
MIWI	Argonaute/PIWI family RNA binding protein that is required for spermatogenesis. MIWI and MILI repress transposone element mediated by piRNA	Deng and Lin 2002; Grivna et al. 2006; Kotaja et al. 2006a,b
RanBPM	RanGTP binding protein that is involved in microtubule nucleation. It associates with MVH	Shibata et al. 2004
Kif17b	Kinesin motor protein that is involved in the transport of the coactivator protein ACT and mRNAs	Kotaja et al. 2006a
Tdrd1	Tudor domain containing protein that is required for spermatogenesis. It interacts with MILI	Hosokawa et al. 2007; Kojima et al. 2009; Wang et al. 2009
Tdrd6	Tudor domain containing protein that is required for spermatogenesis and CB formation	Vasileva et al. 2009
Tdrd7	Tudor domain containing protein	Hosokawa et al. 2007
p48 and p52	Germ cell specific RNA binding proteins	Oko et al. 1996
Ago2 and Ago3	Main catalytic engine of RISC	Kotaja et al 2006b
Dicer	Cytoplasmic RNase that produces siRNA and miRNA from their precursors	Kotaja et al. 2006b
Dcp1a	Decapping enzyme that remove 5'cap of mRNA	van Dijk et al. 2002
GW182	P-body localized RNA binding protein that is required for RNAi	Kotaja et al. 2006b
mRNA	Translation template	Kotaja et al. 2006b
MiRNA	Small RNA that represses complement target mRNA expression	Kotaja et al. 2006b
SnRNP	Essential component of the spliceosomal complex that function in pre-mRNA processing	Paniagua et al. 1985; Moussa et al. 1994; Biggiogera et al. 1990
GRTH	Dead box RNA helicase that is required for spermatogenesis. Deficient of GRTH shows impaired CB formation	Tsai-Morris et al. 2004
GW182	Essential component for si/miRNA induced gene silencing	Kotaja et al. 2006b
H2B	Chromatin formation	Haraguchi et al. 2005
H4	Chromatin formation	Werner and Werner 1995
Acetylated H3	Chromatin regulation	Haraguchi et al. 2005
Acetylated H4	Chromatin regulation	Haraguchi et al. 2005
Actin	Cytoskeletal protein	Walt and Armbruster 1984; Haraguchi et al. 2005
Vimentin	Intermediate filament	Haraguchi et al. 2005
Ubiquitin	Targeting signal for several pathways	Haraguchi et al. 2005
E2	Ubiquitin conjugating enzyme	Haraguchi et al. 2005

Continued

Table 2. *Continued*

Component	Function	References
p52	26S proteasome subunit	Haraguchi et al. 2005
pA700	Proteasome activator	Haraguchi et al. 2005
COX1	Cytochrome C oxidase subunit 1	Haraguchi et al. 2005
HSP70	protein chaperon	Haraguchi et al. 2005
F1α	ATP synthase subunit α	Haraguchi et al. 2005
F1β	ATP synthase subunit β	Haraguchi et al. 2005
LDH	Dehydrogenation of 2-hydroxybutylate	Haraguchi et al. 2005
Enolase	Conversion from 2-phosphogylcerate to phosphoenoylpyruvate	Haraguchi et al. 2005
PHGPx	Reduces lipid hydroperoxides in membranes	Haraguchi et al. 2005
Cytochrome C	Electron transfer component	Hess et al. 1993
Ca^{2+}, Mg^{2+}		Andonov and Chaldakov 1989; Rouelle-Rossier et al. 1993
Histocompatibility antigen	Immune system	Head and Kresge 1985
LAMP1 and LAM2	Lysosome localized glycoproteins	Haraguchi et al. 2005
Acid phosphatase	Lysosome localized phosphatase	Anton 1983
Cathepsins B,D, H, and L	Protease	Haraguchi et al. 2005
LAP	Leucin aminopeptidase	Haraguchi et al. 2005
DNase	hydrolysis of DNA	Haraguchi et al. 2005
RNase	hydrolysis of RNA	Haraguchi et al. 2005
NADPase	hydrolysis of NADP	Tang et al. 1982; Thorne-Tjomsland et al. 1988
CMPase	hydrolysis of monophosphate polysaccharides	Tang et al. 1982; Thorne-Tjomsland et al. 1988 Krimer and Esponda 1980

Many more components have been identified for the individual model organisms, and may later prove conserved. For component lists reflecting component diversity in popular model organisms see: mouse chromatoid body Table 2 in this manuscript; *Drosophila* sponge bodies (Snee and Macdonald 2009); *C. elegans* P granules (Updike and Strome 2009b).

(Wilsch-Brauninger et al. 1997). Sponge bodies appear to derive from fragments of perinuclear nuage that associate with ER membranes, detach from the nucleus, and intermingle in the cytoplasm with other P-body-like granules (see below) (Wilsch-Brauninger et al. 1997; Jaglarz et al. 2011). Sponge bodies accumulate in the oocyte, apparently by movement along ER membranes that extend from the nurse cells through the ring canals into the oocyte (Jaglarz et al. 2011). Sponge bodies movement has been hypothesized to transport perinuclear nuage material from the nurse cells to the germ (pole) plasm of the oocyte (Nakamura et al. 2001). The composition of sponge bodies, however, is dynamic and differs between nurse cells and oocytes, suggesting that sponge bodies are not simply a mobile version of nuage, but represent a range of heterogeneous RNP assemblies (Snee and Macdonald 2004, 2009).

Membrane-rich structures reminiscent of sponge bodies have also been reported in *C. elegans* oocytes (Fig. 2B). During the oocyte growth phase, the perinuclear germ granules detach from the nuclear envelope, apparently taking with them membrane fragments containing clusters of nuclear pores (Pitt et al. 2000). Stressed and aging oocytes assemble even larger cytoplasmic RNP complexes, whose formation has been correlated with dramatic nuclear membrane blebbing (Jud et al. 2008; Patterson et al. 2011). *Drosophila* sponge bodies also change morphology under different environmental conditions (Snee and McDonald 2009), suggesting a role for these membranous structures in protecting oocytes from stress.

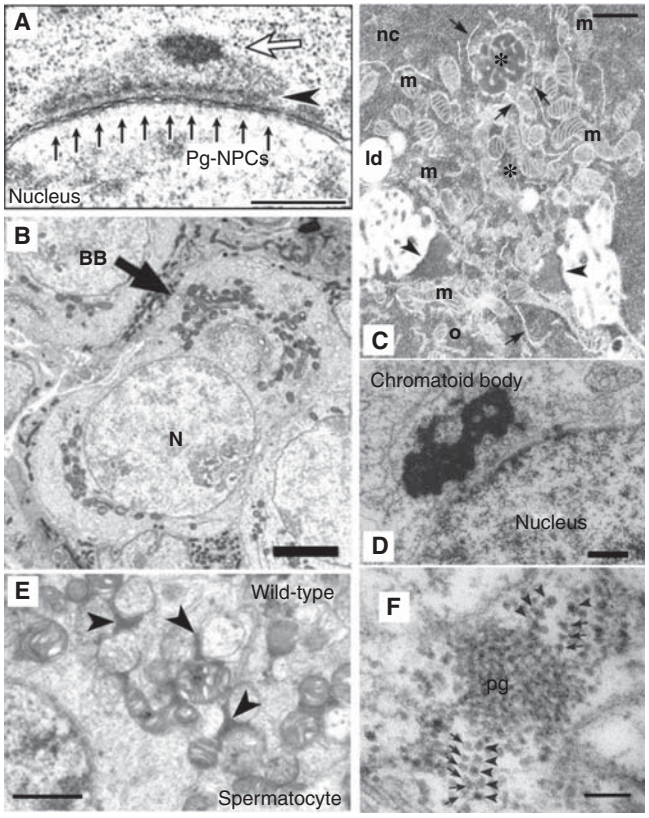

Figure 1. Electron micrographs of the major germ granule classes. (*A*) Perinuclear P granule in a meiotic germ cell of *C. elegans*. Distinct subdomains are visible: the crest (white arrow) and base (arrowhead) overlying a cluster of nuclear pores (black arrows). Scale bar: 500 nm. (Panel *A* is adapted from Sheth et al. [2010] and reproduced, with permission, from The Company of Biologists © 2010.) (*B*) Balbiani body in a mouse oocyte. Black arrow points to mitochondria clustered around Golgi membranes (BB). *N*, nucleus. Scale bar: 5 um. (Panel *B* is adapted from Pepling et al. [2007] and reproduced, with permission, from National Academy of Sciences, USA © 2007.) (*C*) Sponge bodies in the *Drosophila* nurse cell. Sponge bodies (asterisks) intermingled with mitochondria (*m*) and ER cisternae (arrows) near an intercellular bridge (black arrowheads) connecting the nurse cell (*nc*) to the oocyte (*o*). *ld*, lipid droplet. Scale bar: 500 nm. (Panel *C* adapted from Jaglarz et al. [2011] and reproduced, with permission, from Springer © 2011.) (*D*) Perinuclear chromatoid body in a mouse stage I round spermatid. Scale bar: 500 nm. (Panel *D* is adapted from Vasileva et al. [2009] and reproduced here, with permission, from Elsevier © 2009.) (*E*) Intermitochondrial cement in a mouse spermatocyte. IMC (arrowheads) forms in the spaces between clustered mitochondria. Scale bar: 1 um. (Panel *E* is adapted from Chuma et al. [2006] and reproduced, with permission, from National Academy of Sciences, USA © 2006.) (*F*) Polar granules (*pg*) in the cortical cytoplasm of a *Drosophila* embryo. Polysomes (arrows) extend from the surface of the granule. Scale bar: 100 nm. (Panel *F* is adapted from Amikura et al. [2001] and reproduced, with permission, from the Company of Biologists 2001.)

Germ Granules in Germ Plasm

In mature oocytes of animals with "germ plasm," materials transported in the Balbiani and sponge bodies coalesce in a subregion of the oocyte cortex at a position that predicts the site of PGC formation, as seen in *Xenopus* (Czolowska 1969), zebrafish (Knaut et al. 2000), and *Drosophila* (Illmensee and Mahowald 1974). Immediately after fertilization, in *Drosophila*, germ granules appear in small clusters surrounded by ribosomes (Fig. 1F)

(Amikura et al. 2001; Mahowald 2001) near membranes of the endoplasmic reticulum (Thomson et al. 2008). During germ (pole) cell formation, the germ granules are transported on microtubules toward the nuclei of the future pole cells (Lerit and Gavis 2011). In zebrafish embryos, the germ plasm forms on the distal ends of the first and second cleavage furrows, in a complex process involving microtubules, microfilaments, and two distinct RNA segregation pathways (Knaut et al. 2000; Theusch et al. 2006). By the 32-cell stage, the germ plasm aggregates are found in four cells that will give rise to the germline (Knaut et al. 2000).

In *C. elegans*, germ granules detach from nuclei during oogenesis and remain uniformly distributed in the cytoplasm through fertilization. In the zygote, after embryonic polarity is specified, the germ granules become enriched in the posterior cytoplasm and, as a result, are inherited primarily by the posterior daughter, the germline blastomere P_1. This process is repeated for another three asymmetric divisions separating soma from germline. By the time of the birth of the germline founder cells, the germ granules have returned to their perinuclear position (Strome and Wood 1982).

Chromatoid Body

During mouse spermatogenesis (Fig. 2C), germ granules undergo a number of morphological changes (Eddy 1975). Perinuclear nuage and cytoplasmic granules associated with mitochondria ("intermitochondrial cement") are found in early male germ cells (Fig. 1E) (Chuma et al. 2006; reviewed in Chuma et al. 2009). During meiosis, spermatocytes assemble a unique type of germ granule called the chromatoid body (Fig. 1D) (Kotaja and Sassone-Corsi 2007). The chromatoid body arises from intermitochondrial fibers that disperse during the meiotic divisions and consolidate postmeiosis into a single sphere apposed against the nucleus. The site of chromatoid body/nucleus contact is rich in nuclear pores (Fawcett et al. 1970) and labeling of the nucleus with tritiated uridine also labels the chromatoid body, suggesting that RNA synthesized in the nucleus is transferred to the chromatoid body (Soderstrom and Parvinen 1976). Remarkably, the chromatoid body is motile, moving both around the nucleus and into neighboring cells through cytosolic bridges. This movement has been proposed to mediate sharing of RNAs between the haploid spermatids (Ventela et al. 2003). The chromatoid body relocalizes to the base of the flagellum in elongating spermatids (Fig. 2C), and disassembles later during spermiogenesis (Parvinen 2005). No germ granules have been reported in the mature sperm of any organism.

Chromatoid-like bodies have also been described in the neoblasts of planaria; the totipotent stem cells that give rise to both somatic and germ cells (Coward 1974; Hori 1997). Chromatoid bodies are lost when neoblast progeny differentiate into somatic lineages.

P-Bodies

Two main classes of cytoplasmic RNA granules have been described in somatic cells: processing bodies and stress granules (Balagopal and Parker 2009). Processing bodies (P bodies) and stress granules share components implicated in RNA storage, turnover, and translational regulation. Each granule type also possesses unique features: P bodies contain components of the mRNA decay machinery, and stress granules contain TIA proteins, poly-A binding protein, and stalled translation initiation complexes (Anderson and Kedersha 2009). Several P body and stress granule components have been detected in germ granules (Tables 1 and 2). This overlap has generated some speculation as to whether germ granules might correspond to P bodies and/or stress granules. Although functional parallels are likely, a direct correspondence remains unclear. So far, stalled initiation complexes have not been reported in germ granules, suggesting that germ granules may not be directly equivalent to stress granules. In contrast, most components of the RNA decay machinery (DCP1, DCP2, CGH-1/Me31B/Rck, CAR-1/Tral/RAP55, and PCM/Xrn1) have been detected in the perinuclear nuage, sponge bodies, germ plasm, and/or chromatoid bodies, suggesting a strong connection to P

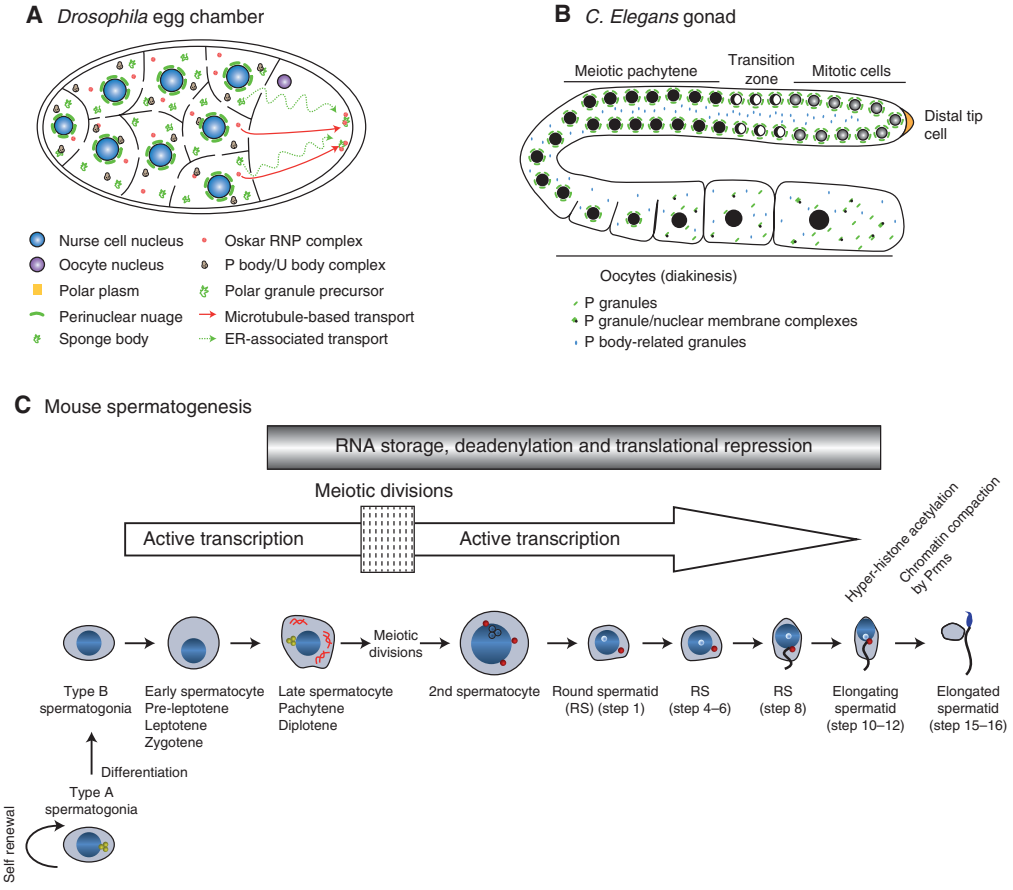

Figure 2. Germ granules during gametogenesis. (*A*) Transport of germ granule material in a *Drosophila* egg chamber. Various germ granule RNPs intermingle in the nurse cells' cytoplasm. Green dashed lines represent migration of sponge bodies, red lines indicate the *oskar* RNP transport pathway. (Panel *A* adapted from Jaglarz et al. [2011] and reproduced, with permission, from Springer © 2011.) (*B*) Germ granules in the germline of an adult *C. elegans* hermaphrodite. Germ cells progress along an "assembly line" of development from mitotic stem cells at the distal end to the oocytes at the proximal end. Germ cells share a common cytoplasm, and are individualized late in oogenesis. P granules (green) are docked at the germ cell nuclei until diplotene, and transition to the cytoplasm along with nuclear pore complexes (green/black). P body-related granules (blue) accumulate in the shared cytoplasm (rachis) and in oocytes. (*C*) Mammalian spermatogenesis. Intermitochondrial cement (yellow dots) is present in type A spermatogonia, and disappears in type B spermatogonia until meiosis. The chromatoid body (CB; red filaments then becoming dots) appears in late pachytene cells as thick cytoplasmic fibers. After the first meiotic division, the CB starts condensing as one or two round foci. Except during meiotic divisions, transcription is active until the elongating spermatid stage, and many transcripts are translationally silenced and located in the CB. In elongated spermatids, transcripts are released from translational arrest.

bodies (Nakamura et al. 2001; Navarro et al. 2001; Audhya et al. 2005; Boag et al. 2005; Lall et al. 2005; Wilhelm et al. 2005; Kotaja et al. 2006a; Lin et al. 2006; Squirrell et al. 2006; Kotaja and Sassone-Corsi 2007; Pepling et al. 2007; Thomson et al. 2008; Aravin et al. 2009; Lim et al. 2009; Yabuta et al. 2011). Careful examination of the distribution of P body proteins in germ cells has revealed, however, that these factors also accumulate in foci that do not contain core germ granule proteins. For example, mature mouse oocytes, which lack

VASA-positive granules, contain foci positive for the decapping enzyme Dcp1a (Swetloff et al. 2009). Mouse spermatocytes also contain canonical P bodies distinct from the germ granules (Aravin et al. 2009; Shoji et al. 2009). In *C. elegans*, several types of P-body related granules have been observed in pachytene germ cells and in maturing oocytes (Navarro et al. 2001; Audhya et al. 2005; Boag et al. 2005; Noble et al. 2008). In embryos, granules containing the decapping activator PATR-1 and other P body proteins, but lacking the germ granule component PGL-1, dock around the germ granules (Audhya et al. 2005; Lall et al. 2005; Gallo et al. 2008). Unlike germ granules, the PATR-1 granules are partitioned symmetrically during mitosis and recruit additional P body proteins after segregation into somatic blastomeres (Gallo et al. 2008). Similarly, in the sponge bodies of *Drosophila* nurse cells, structures resembling the P body/U body complexes of somatic cells (Liu and Gall 2007) can be distinguished from nuage (Lim et al. 2009; Jaglarz et al. 2011). Together, these observations suggest that germ cells contain bona fide P-bodies, or P body-related granules, which interact and may even exchange components with germ granules, but are maintained as distinct RNP assemblies. Whether germ granules represent specialized P-bodies, P-body/stress granule hybrids, or a distinct granule type unique to the germline, remains to be determined.

DYNAMICS

In electron micrographs, germ granules appear as dense, fibrillar structures that in some cases appear to have distinct subcompartments (Fig. 1A,D) (Fawcett et al. 1970; Sheth et al. 2010). In live recordings, perinuclear germ granules in *Drosophila* nurse cells (nuage) and in *C. elegans* meiotic cells (P granules) have been observed to maintain their shape and relative position for up to 40 minutes, the maximum recording time (Snee and Macdonald 2004; Sheth et al. 2010). Despite this apparent stability, experiments using fluorescence-recovery after photobleaching (FRAP) have revealed that individual components are in fact highly dynamic. GFP::Vasa is replaced in the *Drosophila* nuage with a half-time of 60 sec (Snee and Macdonald 2004). Similarly, GFP::PGL-1 diffuses within seconds inside the perinuclear granules of *C. elegans* (Brangwynne et al. 2009), and whole perinuclear clusters recover from photobleaching with a half-time of 20 sec (Sheth et al. 2010). Whether germ granules also contain stable components that form static scaffolds is not known.

Under some circumstances, whole granules can rapidly change size or position. During the asymmetric cleavages of the *C. elegans* embryo, germ granules in cytoplasm destined for the germline grow and fuse into larger granules, whereas germ granules in cytoplasm destined for the soma shrink in size until no longer visible (Brangwynne et al. 2009). These dynamics have been compared to those of liquid droplets undergoing condensation and dissolution (Brangwynne et al. 2009). In zygotes, a subset of germ granules have also been observed to be move directionally over several microns (Hird et al. 1996). This movement is microtubule-independent (Hill and Strome 1990) and may be mediated by cytoplasmic flows that develop in response to actomyosin contractions in the zygote's cortex (Marston and Goldstein 2006).

Microtubules have been implicated in germ granules dynamics in *Drosophila* and zebrafish. During *Drosophila* oogenesis, transport of *oskar* RNP particles from nurse cell to the posterior pole occurs along microtubules in kinesin-dependent manner (reviewed in Becalska and Gavis 2009). In *Drosophila* embryos, translocation of VASA-containing granules from the cortex to nuclei occurs along astral microtubules in a process that requires dynein (Lerit and Gavis 2011). In zebrafish, microtubules promote germ granule aggregation in early embryos (Theusch et al. 2006), and have been implicated in germ granule partitioning during mitosis in primordial germ cells (Strasser et al. 2008). Disruption of microtubules or dynein causes the germ granules to coalesce into larger granules, suggesting a role in regulating both granule dynamics and size (Strasser et al. 2008).

In mouse spermatids, the chromatoid body is highly mobile, tracking around the nucleus

and shuttling from cell to cell via cytoplasmic bridges (Fawcett et al. 1970; Ventela et al. 2003). Microtubule-depolymerizing drugs disrupt these movements and cause the chromatoid body to disperse (Ventela et al. 2003). The testis-specific kinesin KIF17b is a possible candidate for mediating chromatoid body movement. KIF17b was first identified as a nucleus-cytoplasm shuttling protein that transports ACT (activator of CREM in testis), a transcriptional coregulator of postmeiotic transcription (Fimia et al. 1999). KIF17b also contributes to the transport of TB-RBP, Testis-brain RNA-binding protein, which associates with CREM regulated mRNAs (Chennathukuzhi et al. 2003a,b). Importantly, KIF17b is also found in the chromatoid body (Kotaja et al. 2006b), where it may participate in RNA transport and/or mediate chromatoid body movement.

ASSEMBLY OF GERM GRANULES

Unlike other cellular organelles, germ granules are not bound by membranes, yet maintain distinct boundaries and shapes. What molecular interactions underlie germ granule form and integrity? Although no single assembly pathway accounts for the wide range of germ granule morphologies, several have been documented.

Nucleation by Seed Proteins

Genetic analyses in *Drosophila* have led to a hierarchical model for the organization of germ plasm: a single protein, Oskar, is necessary and sufficient to initiate a cascade of interactions that assemble germ plasm at the site of Oskar localization (Mahowald 2001). Oskar exists in two forms: a short form required for germ granule assembly (Markussen et al. 1995), and a long form required to anchor the granules to the cell cortex (Vanzo and Ephrussi 2002). Short Oskar initiates granule formation likely through direct interactions with Vasa, which in turn recruits Tudor (Breitwieser et al. 1996; Anne 2010), Valois, and Piwi-family Argonautes (Kirino et al. 2009; Nishida et al. 2009). Germ granule mRNAs (i.e., *nanos*, *germ-cell-less*, and *polar granule component*) are synthesized in the nurse cells and transferred to the oocyte during nurse cell cytoplasm "dumping" (reviewed in Becalska and Gavis 2009). Localization to the germ plasm depends on sequences in the 3′UTR and an actin-dependent, cortex-anchoring mechanism initiated by Oskar (Becalska and Gavis 2009; Rangan et al. 2009). How the long form of Oskar anchors the germ plasm to the cortex is not fully understood, but it involves recruitment of endosomal proteins and stimulation of endocytosis, which eventually reorganizes F-actin in the oocyte cortex (Tanaka and Nakamura 2008).

Oskar homologs have not been found outside of insects, but proteins with similar germ plasm "seed" functions have been identified in zebrafish and *C. elegans*. In zebrafish, the Balbiani body component Bucky Ball is necessary and sufficient to induce germ plasm aggregation (Bontems et al. 2009). Bucky ball is conserved among vertebrates but does not possess recognizable functional domains; its mode of action remains unknown (Bontems et al. 2009). In *C. elegans*, the RGG-domain proteins PGL-1 and PGL-3 are required for germ granule integrity in embryos, and can form cytoplasmic granules when ectopically expressed in tissue culture cells or in intestinal cells (Hanazawa et al. 2011; Updike et al. 2011). Ectopic PGL granules recruit RNA and other germ granule components. Granule formation depends on a domain in the PGL proteins that mediates self-association, whereas recruitment of RNA and RNA-binding proteins requires the RGG domain (Hanazawa et al. 2011). PGL-1 and PGL-3 also form transient granules in the somatic blastomeres of early embryos, but in those cells, granule formation depends on the autophagy machinery, which ultimately targets the PGL granules for degradation (Zhang et al. 2009).

Nucleation by Organelles

Germ granules often form near mitochondria, raising the possibility that nucleating factors also exist on the membranes of these organelles. Two recent reports implicate such mechanism in generation of the intermitochondrial cement

(IMC) in mouse spermatocytes. Mutants lacking the mitochondrial phospholipase D MitoPLD do not form IMC and mislocalize IMC components (Huang et al. 2011; Watanabe et al. 2011). MitoPLD generates the signaling lipid phosphatidic acid. Remarkably, a deficiency in the phosphatidic acid-metabolizing enzyme, Lipin 1 causes hyper-aggregation of electron-dense nuage around mitochondria (Huang et al. 2011). Phosphatidic acid may therefore function as the organizing signal for IMC.

Binding of Tudor Domains to Methylated Arginines

The Tudor domain is a small (~55 amino acid) protein–protein interaction module that recognizes methylated arginines or lysines. Tudor domains have been found in several proteins localized to perinuclear nuage, germ plasm, and/or chromatoid bodies (see Table 1 for references). The founding member of the family, *Drosophila* Tudor, has 11 Tudor domains, but Tudor domains can also be found in single copy or combined with other conserved motifs, such as in SpnE/TDRD9 (reviewed in Arkov and Ramos 2010).

The Tudor domain forms an aromatic cage that interacts directly with the methylated amino acid (reviewed in Arkov and Ramos 2010; Vourekas et al. 2010). Crystallographic studies of Tudor proteins complexed with methyl-arginine-containing peptides show that the Tudor module is embedded in a larger 170 amino-acid domain that contacts the peptide at other sites (Liu et al. 2010; Vourekas et al. 2010). These additional interactions may help Tudor-containing proteins discriminate among potential partners.

Piwi-family Argonautes, Vasa helicases and Sm proteins in *Drosophila*, *Xenopus*, and mouse all contain methylated arginines and thus are potential targets for Tudor domain-containing proteins (Kuramochi-Miyagawa et al. 2004; Gonsalvez et al. 2006; Thomson et al. 2008; Chen et al. 2009; Kirino et al. 2009; Kojima et al. 2009; Nishida et al. 2009; Reuter et al. 2009; Shoji et al. 2009; Vagin et al. 2009a,b; Wang et al. 2009; Kirino et al. 2010a,b; Patil and Kai 2010; Liu et al. 2011). Symmetrical dimethylation of arginines (sDMA) is performed in flies and mice by the methyltransferase Capsuleen/PRMT and its cofactor Valois/MEP50/WDR77 (Gonsalvez et al. 2006; Anne et al. 2007). In *Drosophila*, sDMA modifications of the Argonaute Aubergine are recognized by Tudor, and are necessary for Aubergine-Tudor interaction and germ granule assembly (Anne et al. 2007; Kirino et al. 2009; Nishida et al. 2009). sDMA modifications of the spliceosomal Sm proteins SmB and SmD3 also depend on Capsuleen/Valois, and are required for their localization to germ granules; however, the relevant Tudor-domain partners of these proteins have not been identified (Anne 2010; Gonsalvez et al. 2010). In the mouse, several Tudor domain proteins interact with the sDMA-modified Piwi family Argonautes and are required for spermatogenesis (reviewed in Arkov and Ramos 2010; Vourekas et al. 2010). Lack of interactions causes both the Tudor domain proteins and Piwi-family Argonautes to become mislocalized, suggesting a mutual dependency for localization.

Low Specificity Interactions

Tudor-sDMA interactions have the potential to create high affinity/specificity complexes that are stable and long-lived. A different type of interaction, however, may be needed to bring these complexes into higher-order dynamic structures. Studies in *C. elegans* has led to the proposal that germ granules show liquid-like behaviors (Brangwynne et al. 2009). In response to pressure, germ granules in the nuage of meiotic germ cells appeared to "drip" off nuclei and fuse into large drops; germ granules also "condense" and "dissolve" during the asymmetric divisions of the embryo (Brangwynne et al. 2009). Liquid behaviors are thought to be mediated by weak, fast-rearranging molecular interactions, as could arise from low-affinity protein–RNA interactions. Consistent with a role for RNA in maintaining germ granule integrity, depletion of the VegT and Xlsirts RNAs disrupts the organization of germ granules in the vegetal cortex of *Xenopus* oocytes

(Kloc and Etkin 1994; Kloc et al. 2005, 2007). Inhibition of transcription causes loss of PGL-1 and GLH-2 from the *C. elegans* nuage (Sheth et al. 2010) and loss of the electron-dense core of the mouse chromatoid body (Soderstrom 1977). Mutations in genes implicated in the biogenesis of small RNAs (*ego-1*, *csr-1*, *drh-3*) cause a dramatic increase in the size and number of germ granules in meiotic germ cells (Updike and Strome 2009a).

Other studies in *C. elegans* have implicated weak hydrophobic interactions involving phenylalanine-glycine (FG)-repeats. FG-repeats are naturally unfolded and flexible, and thus well suited for dynamic binding to multiple partners, as in the liquid droplet model above. FG-repeats are found in nucleoporins that mediate active transport across the nuclear pore and prevent passive diffusion of molecules greater than >30 kD (Elad et al. 2009; Terry and Wente 2009). Perinuclear germ granules exclude dextrans larger than 40 kDa and are sensitive to treatments predicted to disrupt FG-repeat interactions (Updike et al. 2011). Consistent with a role for FG-repeats, three *C. elegans* VASA family members, GLH-1, 2, and 4, contain FG repeats (Kuznicki et al. 2000), and the FG-containing nucleoporin Nup98 and mRNA export factor DDX-19 colocalize with germ granules (Sheth et al. 2010; Voronina and Seydoux 2010). RNAi screens have also identified several nucleoporins required for germ granule integrity in embryos (Updike and Strome 2009a; Voronina and Seydoux 2010) and for the assembly of sponge body-like aggregates in stressed oocytes (Patterson et al. 2011).

Recently, FG-repeat proteins have been recognized as a subtype of prions (Ader et al. 2010). Remarkably, overexpression in mouse neuroblastoma N2a cells of the cytosolic form of the prion PrP causes the formation of a large RNP aggregate near the cell nucleus, resembling a chromatoid body (Beaudoin et al. 2009). The "PrP body" associates with mitochondria, and is able to recruit mRNAs, FG-repeat nucleoporins, Dicer, Dcp1a, DDX6, and SmB proteins reminiscent of germ granules (Beaudoin et al. 2009; Roucou 2009). Nucleation of stress granules in somatic cells also depends on a prion-domain containing protein, TIA-1 (Gilks et al. 2004). An intriguing possibility is that prion-like templating properties give germ granules not only their unique structural characteristics, but also the ability to propagate from germ cell to germ cell, and from generation to generation in animals with germ plasm.

HYPOTHESES FOR FUNCTION OF GERM GRANULES

Germ Cell Fate Specification and Differentiation

The first proposed function for germ granules was to support the specification of the germline in embryos where germ granules are inherited maternally as a part of the germ plasm. In *Xenopus* and *Drosophila* embryos, irradiation of a specific region of the cytoplasm (germ plasm) results in embryos that lack germ cells (Geigy 1931; Bounoure 1937; Smith 1966). In *Drosophila*, transplantation of germ plasm to an ectopic site is sufficient to reprogram cells to become germ cells (Illmensee and Mahowald 1974). In fact, ectopic localization of the germ plasm component Oskar is sufficient to induce ectopic germ granules (see above) and form ectopic germ cells (Ephrussi and Lehmann 1992). Similarly, overexpression of the Balbiani body protein Bucky ball in zebrafish embryos induces extra germ cells (Bontems et al. 2009). These experiments suggest that germ plasm, and perhaps the granule granules therein, contain the factors that specify germ cell fate.

In *Drosophila*, mothers with mutations in *vasa* generate embryos that lack germ cells (Schupbach and Wieschaus 1986; Lasko and Ashburner 1990; Styhler et al. 1998). In *Xenopus* embryos, morpholino treatments that reduce Vasa protein levels decrease germ cell numbers (Ikenishi and Tanaka 1997; Ikenishi et al. 2006). Genetic analyses have identified several other germ granule proteins like Vasa that are required for germ cell specification, but none (besides Oskar) is sufficient to induce germ cell fate on its own (Rongo and Lehmann 1996; Harris and Macdonald 2001; Megosh

et al. 2006; Anne 2010; Gonsalvez et al. 2010). Germ granules in germ plasm associate with ribosomes, raising the possibility that translation of mRNAs stored in the germ granules is the critical factor in germ cell fate specification (Mahowald 2001). Consistent with this hypothesis, mRNAs enriched in germ granules encode proteins (i.e., Nanos and Pgc) that are required for germ cell development (see below). Again, however, no single RNA was found to be sufficient to induce germ cell fate.

Recent data has challenged the simple model that germ granules are sufficient to specify germ cell fate. Mutants that mis-express multiple germ granule proteins in larvae have been identified in *Drosophila* and *C. elegans* (Unhavaithaya et al. 2002; Wang et al. 2005; Janic et al. 2010; Petrella et al. 2011). These mutants show somatic defects, but do not develop extra germ cells. Mutants that mispartition germ granules in embryos also do not make extra germ cells (Strome et al. 1995; Gallo et al. 2010). In fact, a *C. elegans* mutant, in which germ granules disassemble at each mitosis and components segregate symmetrically, is still viable and fertile (Gallo et al. 2010). These observations suggest that germ granules are not essential organizers of germ plasm, and that other factors maintain the distinction between soma and germline in embryos. Consistent with this view, germ granules are not present at the time of germ cell specification in mammals.

In contrast, an essential role for germ granules during germ cell differentiation is well supported by genetic analyses in several animals. For example, loss of Nanos causes PGCs to fail to reach the somatic gonad and eventually die in *Drosophila*, *C. elegans*, and zebrafish embryos (Kobayashi et al. 1996; Subramaniam and Seydoux 1999; Koprunner et al. 2001). Nanos is also required for PGC viability in mice (Tsuda et al. 2003; Suzuki et al. 2007), for maintenance and regeneration of the germline in planaria (Wang et al. 2007), and for the in vitro differentiation of human germ cells from embryonic stem cells (Julaton and Reijo Pera 2011). Members of the Vasa family of RNA helicases and the PIWI family of Argonautes have also been implicated in several aspects of germ cell proliferation and differentiation in *Drosophila*, *C. elegans*, zebrafish, and mouse (Lin and Spradling 1997; Cox et al. 1998; Kuznicki et al. 2000; Tanaka et al. 2000; Deng and Lin 2002; Kuramochi-Miyagawa et al. 2004; Carmell et al. 2007; Houwing et al. 2007; Batista et al. 2008; Houwing et al. 2008; Spike et al. 2008; Li et al. 2009a; Unhavaithaya et al. 2009). Phenotypes include failure to maintain germline stem cells, germ cell death before meiosis onset, reduced germ cell proliferation, and meiotic defects. The challenge now is to identify the molecular mechanisms that underlie this wide range of phenotypes. In this respect, much progress has been made recently in flies and mice, in which meiotic failure has been traced back to specific defects in chromatin and transposon regulation.

Production of piRNAs

Transposition must be under tight regulation in germ cells to prevent spreading of deleterious DNA rearrangements to gametes and the next generation. Recent studies in several organisms have shown that the PIWI subfamily of Argonautes blocks transposition by producing small RNAs, called piRNAs, against transposon sequences (Vagin et al. 2006; Aravin et al. 2007; Houwing et al. 2007; reviewed in Thomson and Lin 2009). The PIWI Argonautes function in a two-step "ping-pong" amplification to generate sense and antisense piRNAs from piRNA precursor loci and expressed transposon sequences (Aravin et al. 2006, 2007; Grivna et al. 2006; Brennecke et al. 2007; reviewed in Senti and Brennecke 2010).

In *Drosophila* ovaries, the Argonautes Aubergine and Ago3 colocalize with Vasa and other factors required for piRNA production in the perinuclear nuage of nurse cells (reviewed in Senti and Brennecke 2010). In *vasa* mutant ovaries, piRNA-pathway proteins do not concentrate in the nuage and transposon mRNAs are up-regulated (Snee and Macdonald 2004; Vagin et al. 2004). Genetic and biochemical analyses suggest that Tudor-domain proteins Tudor, Tejas, and PAPI are important for

recruitment of Aub and Ago3 to the nuage (Nishida et al. 2009; Patil and Kai 2010; Liu et al. 2011). Additionally, Aubergine/Tudor and Ago3/PAPI complexes interact with P-body proteins Dhh1/Me31B and Rap55/Trailer Hitch implicating the mRNA degradation machinery in transposon silencing (Thomson et al. 2008; Liu et al. 2011). Consistent with this view, the P-body proteins CCR4/TWIN, DCP1, SKI3, and Rap55/TRAL are all required for transposon silencing (Lim et al. 2009; Liu et al. 2011).

piRNA pathway proteins are also found in the germ plasm of *Drosophila* oocytes and embryos, raising the possibility that piRNAs are transmitted maternally with the germ plasm to the next generation. Consistent with this possibility, piRNAs targeting I-elements are found in the daughters of mothers with I-elements, but not in the daughters of fathers with I-elements. The latter, but not the former, are sterile (hybrid dysgenesis) because of I-element activation (Brennecke et al. 2008).

As in *Drosophila*, zebrafish Piwi family Argonautes Zili and Ziwi localize to nuage and are required for piRNA biogenesis and repression of transposons in germ cells (Houwing et al. 2007, 2008). In both species, as well as in mouse (see also below), piRNAs are modified at their 3′ end by 2′-hydroxyl methylation (Vagin et al. 2006; Horwich et al. 2007; Houwing et al. 2007; Kirino and Mourelatos 2007b; Ohara et al. 2007). The enzyme responsible for this modification is Hen1 (Horwich et al. 2007), which interacts with Piwi proteins (Horwich et al. 2007; Kirino and Mourelatos 2007a; Saito et al. 2007). Hen1 is a component of nuage in zebrafish, and functions in *Drosophila* and zebrafish to stabilize piRNAs and maximize transposon repression (Horwich et al. 2007; Kamminga et al. 2010).

In the mouse, piRNAs are most abundant in spermatocytes in which they associate with Piwi family Argonautes (Aravin et al. 2007, 2008) and accumulate in the chromatoid body (Meikar et al. 2010). The Argonautes MILI and MIWI2 and Tudor proteins TDRD1, TDRD5, and TDRD9 are all required for piRNA production, methylation of transposon promoters, and repression of transposon transcription (Aravin et al. 2007; Carmell et al. 2007; Reuter et al. 2009; Shoji et al. 2009; Vagin et al. 2009b; Yabuta et al. 2011). piRNA production and transposon silencing also require Vasa (MVH), and the germ granule assembly factors MAEL, GASZ, and MitoPLD (Soper et al. 2008; Ma et al. 2009; Kuramochi-Miyagawa et al. 2010; Watanabe et al. 2011). Spermatocytes lacking MitoPLD or MVH do not form intermitochondrial cement, the germ granule precursor to the chromatoid body. In contrast, intermitochondrial cement is made in MILI-deficient spermatocytes, which lack most piRNAs, indicating that piRNAs are not required for IMC formation. Unlike in *Drosophila*, the piRNA pathway in mice functions primarily in the male germline: mutations in this pathway block spermatogenesis, but do not affect oogenesis. Repression of transposons is mediated by a different group of small RNAs in oocytes: the Dicer-dependent endo-siRNAs formed from double-stranded RNA precursors (Murchison et al. 2007; Tam et al. 2008; Watanabe et al. 2008).

A spermatogenesis-specific function for Piwi-family Argonautes is also observed in *C. elegans*. Piwi-family member PRG-1 is a component of germ granules that binds to 21U-RNAs, analogous to piRNAs of other species (Batista et al. 2008). A subclass of 21U-RNAs targets the transposon Tc3. *prg-1* mutants lack 21U-RNAs and have higher Tc3 RNA levels (Batista et al. 2008) and Tc3 transposition (Das et al. 2008). Transposon regulation in *C. elegans* also depends on a greatly expanded subgroup of worm-specific argonautes (WAGOs) that produce a different class of small RNAs, termed 22G-RNAs (Batista et al. 2008; Das et al. 2008; Gu et al. 2009). PRG-1 and WAGOs are hypothesized to function in a two-step mechanism, in which primary 21U-RNAs trigger secondary 22G-RNA production required for efficient silencing of transposons and pseudogenes (Batista et al. 2008; Das et al. 2008).

Mutations in the piRNA pathway lead to both transposon activation and meiotic defects, suggesting that the two may be linked (reviewed

in Ollinger et al. 2010). Transposition could cause meiotic defects by inducing DNA damage, interfering with homolog recognition, and/or cytotoxicity of retrotransposon-encoded proteins. Another possibility, however, is that the piRNA pathway regulates transposons and meiosis in parallel.

Mitosis and Chromatin Organization

The 22G-RNAs of *C. elegans* target not only repetitive elements, but also unique sequences, including many annotated genes (Gu et al. 2009). 22G-RNAs targeting unique genes associate preferentially with the Argonaute CSR-1 (Claycomb et al. 2009). CSR-1 is a component of the nuage in which it functions with the Dicer-related helicase DRH-3, the RNA-dependent RNA polymerase EGO-1, and the Tudor domain protein EKL-1 to generate 22G-RNAs. Surprisingly, mutations in *csr-1* do not affect the expression of most genes targeted by 22G-RNAs, but cause dramatic defects in chromosome organization during meiosis and mitosis (Claycomb et al. 2009). In embryos, CSR-1 and its partners associate with mitotic chromosomes and are required for the proper distribution of kinetochore proteins (Claycomb et al. 2009). These observation suggest that CSR-1 could function in the delineation of centromeres, as has been shown for Ago1 in *Schizosaccharomyces pombe* (Verdel et al. 2004).

A role for nuage components in chromosome dynamics has also been reported in *Drosophila* (Pek and Kai 2011a). *vasa* mutant germ cells display lagging chromosomes at anaphase that have reduced levels of type I condensins. Vasa coimmunoprecipitates with type I condensins and localizes to perichromosomal foci during mitosis. *aubergine* and *spnE* mutants lack Vasa-positive perichromosomal foci and show mitotic defects similar to those seen in the *vasa* mutants (Pek and Kai 2011b). These findings suggest that small RNAs produced in the nuage also regulate chromatin compaction during mitosis (Pek and Kai 2011b). Interestingly, in the sea urchin, Vasa is also required for chromosome segregation but this function does not appear to be specific to the germline: Vasa associates with mitotic spindles in all early embryonic blastomeres (Yajima and Wessel 2011). These observations suggest that Vasa may have an ancestral role in mitosis, distinct from its role in germ cell development.

Regulation of mRNAs

As germ granules are enriched in RNA-binding proteins and developmentally regulated mRNAs, they have been hypothesized to function as hubs of posttranscriptional regulation; specifically to regulate mRNA localization, levels, and translational activity (Kotaja and Sassone-Corsi 2007; Rangan et al. 2009).

mRNA Localization

Germ granules have been implicated in RNA localization during oogenesis in several organisms (for details see reviews by Kloc et al. 2004; Zhou and King 2004; Becalska and Gavis 2009). Localized mRNAs associate with Balbiani and sponge bodies in the oocytes of *Drosophila*, *Xenopus*, and zebrafish (Ephrussi et al. 1991; Kim-Ha et al. 1991; Wang and Lehmann 1991; Kloc et al. 2002; Cox and Spradling 2003; Wilk et al. 2005; Kosaka et al. 2007). The Balbiani body protein Bucky Ball is required for the Balbiani body aggregation and localization of several mRNAs to the vegetal cortex of zebrafish eggs (Dosch et al. 2004; Marlow and Mullins 2008; Bontems et al. 2009). Several RNA-binding proteins associated with sponge bodies are required for the directed transport of *oskar* to the posterior pole, including Exuperantia, Yps, Rap55/Trailer Hitch, and the hnRNP Hrp48 (Wilhelm et al. 2000, 2005; Yano et al. 2004). *nanos* localization to the germ plasm, in contrast, appears to depend on the trapping of a minority of mRNA molecules in the germ (polar) granules (Wang et al. 1994; Rongo et al. 1997). The Argonaute Aubergine is a candidate tether for *nanos* in the polar granules (Becalska et al. 2011). Aub is in a complex with *nanos* RNA, and *aub*/+ embryos show defects in *nanos* RNA localization but no defect in Oskar localization. Remarkably Aubergine's function in *nanos* localization appears to be

independent of its role in the piRNA pathway (Becalska et al. 2011).

mRNA Translation

Germ granules have been implicated in both the repression and activation of translation. Granules implicated in RNA transport such as in Balbiani and sponge bodies typically contain mRNAs that are translationally repressed and become activated after release from the granules or after transition to another granule type.

Several mechanisms of translation repression have been described for germ granule associated mRNAs. In *Drosophila* nurse cells, repressed mRNAs are often found in sponge bodies (Nakamura et al. 2001). Repression of *oskar* mRNA in sponge bodies depends on the sequence-specific translational repressor Bruno (Kim-Ha et al. 1995). Bruno binds to Bruno-response elements in the *oskar* 3′UTR and promotes the formation of large (up to 80S) *oskar* RNA-containing complexes that exclude ribosomes (Chekulaeva et al. 2006). Bruno additionally represses *oskar* translation through interactions with Cup. Cup is a component of sponge bodies that forms a complex with the translation initiation factor eIF4E and the DEAD-box helicase Dhh1/Me31B (Nakamura et al. 2001, 2004; Wilhelm et al. 2003). eIF4E-binding proteins (4E-BPs) such as Cup are thought to modulate translational initiation by blocking the interaction between eIF4E and eIF4G in a reversible manner (Gingras et al. 1998). Mutations in Cup that disrupt binding to eIF4E cause premature translation of *oskar* mRNA (Nakamura et al. 2004). A similar mechanism has been proposed for the *C. elegans* germ granule component SPN-2 (Li et al. 2009b). SPN-2 is a 4E-BP protein that is recruited to the *mei-1* mRNA by interaction with the sequence-specific RNA-binding proteins OMA-1 and OMA-2 (Li et al. 2009b).

During *C. elegans* oogenesis, translationally-repressed mRNAs are targeted to P-body like granules (grP bodies), that also contain the sequence-specific RNA-binding protein and translational repressor PUF-5 (Noble et al. 2008). These granules become larger in arrested oocytes and may correspond to the membrane-associated, sponge body-like structures characterized by electron microscopy (Jud et al. 2008; Patterson et al. 2011). Loss of the P-body protein CAR1/Tral/RAP55 disrupts grP bodies, and causes ectopic translation of *glp-1* mRNA in oocytes, as is observed upon loss of PUF-5 and related PUFs (Noble et al. 2008).

Unlike the repressive sponge bodies, germ granules in the *Drosophila* germ (pole) plasm have been implicated in translational activation. For example, *nanos* mRNA in the cytoplasm is maintained translationally repressed by mechanisms that block translation both before and after initiation (Andrews et al. 2011; Jeske et al. 2011). Only mRNA localized with the germ (polar) granules is translated. Electron microscopy studies have shown that polyribosome chains extend from the surface of germ granules (Mahowald 1968, 1971; Amikura et al. 2001), suggesting that the granules could deliver mRNAs directly to the translational machinery. Translational activation of mRNAs in the germ plasm occurs in discrete temporal patterns dictated by specific sequences in the 3'UTR (Rangan et al. 2009), and may involve disassembly of silencing complexes. Translation of *nanos* in the germ plasm requires Oskar, which binds directly to the repressor Smaug and is sufficient in vitro to prevent assembly of a Smaug-dependent silencing complex on the *nanos* 3′UTR (Ephrussi and Lehmann 1992; Zaessinger et al. 2006; Jeske et al. 2011). Another key player is likely to be Vasa. Vasa binds to the initiation factor eIF5B and this interaction is required for germ cell formation in embryos (Carrera et al. 2000; Johnstone and Lasko 2004). Several germ plasm mRNAs bind to Vasa (Liu et al. 2009). Translation of at least one of them, *mei-p26*, is dependent on Vasa, and requires the Vasa-eIF5B interaction (Liu et al. 2009).

In *Xenopus* and mouse, translational activation has been correlated with exit from germ granules. In *Xenopus*, *Xcat2* mRNA is maintained in germ granules in the Balbiani body and in the germ plasm through oogenesis and early embryogenesis (Kloc et al. 2002). Release of *Xcat2* from germ granules correlates with its

translational derepression (Kloc et al. 2002). Similarly, in mouse spermatids, translation of *Brd2* and *GCNF* correlates with their exit from the chromatoid body (Nguyen Chi et al. 2009). When in the chromatoid body, the mRNAs cofractionate with Vasa (MVH) in ribonucleoprotein complexes away from polysomes (Grivna et al. 2006; Nguyen Chi et al. 2009). In stage V and older spermatids, mRNAs exit the chromatoid body, associate with polysomes, and become translated (Nguyen Chi et al. 2009). Remarkably, at that time, MVH also becomes associated with polysomes, consistent with a possible role in translational activation (Nguyen Chi et al. 2009).

mRNA Stability

Germ granules have also been implicated in the regulation of mRNA levels, by promoting either mRNA storage or mRNA turnover at specific developmental stages. In the mouse, genes required for spermiogenesis are transcribed several days before the corresponding proteins are needed (Kleene 1993). The untranslated transcripts are stored stably without degradation for up to a week. Some of these transcripts localize to chromatoid body, suggesting that this organelle provides a stable storage site for mRNAs (Soderstrom and Parvinen 1976; Kotaja et al. 2006a; Nguyen Chi et al. 2009). A role for germ granules in mRNA storage has also been proposed in *C. elegans*, based on the function of the DEAD-box RNA helicase Dhh1/CGH-1 (Boag et al. 2008; Noble et al. 2008). In somatic cells, Dhh1/CGH-1 functions with PATR-1 to promote mRNA decapping and degradation in P bodies. In contrast, in germ cells, Dhh1/CGH-1 functions independently from PATR-1, and associates with ~300 mRNAs that are stably maintained during oogenesis and passed on to the embryo (Boag et al. 2008). Loss of Dhh1/CGH-1 causes destabilization of many of these mRNAs and sterility (Boag et al. 2008; Noble et al. 2008).

Other germ granule proteins in contrast have been implicated in mRNA turnover. The *C. elegans* Argonaute family members ALG-3 and ALG-4 generate 26G-RNAs that are required for meiosis and spermiogenesis, especially at elevated temperatures (Conine et al. 2010). Most 26G-RNAs target mRNAs are expressed during spermatogenesis, and 27% of stringently defined targets are up-regulated by twofold or more in *alg-3/4* mutant worms (Conine et al. 2010). Similarly, the RNA-dependent RNA polymerase EGO-1 is required to produce a population of small triphosphorylated antisense RNAs that target hundreds of germline transcripts expressed at different stages of germline development (Maniar and Fire 2011). In *ego-1* mutants, the small RNAs are not made and their target mRNAs are moderately overexpressed (Maniar and Fire 2011). One possibility is that *alg-3/4* and *ego-1* provide temporal fine-tuning of gene expression during germline development by targeting specific mRNAs for degradation.

CONCLUDING REMARKS

Germ granules are remarkable not only for their morphological diversity but also for the many functions they perform during germ cell development. This complexity can be dizzying, but the studies summarized here suggest two important principles and questions for future investigations.

First, many germ granule proteins have been shown to play fundamental roles in RNA regulation. Control of gene expression at the RNA level is common in germ cells, and may be essential to preserve the plasticity of the germline genome (Seydoux and Braun 2006). In fact, Vasa, PIWI, and Nanos homologs have been proposed to form a "conserved multipotency program" used not only by germ cells, but also by multipotent progenitors that retain the ability to generate both somatic and germline tissues (Juliano et al. 2010). An important challenge will be to determine whether specific steps of RNA regulation occur within germ granules, or whether germ granules function primarily as sorting stations where RNAs and regulators meet up before transitioning to the cytoplasm.

Second, unlike other organelles, germ granules are maintained in the cytoplasm without

the help of surrounding membranes. The molecular interactions that give germ granules their unique combination of macro-level stability and micro-level dynamics are only beginning to be elucidated. An understanding of the unique microenvironment of germ granules is likely to yield fundamental new insights into the physical principles that organize the flow of genetic information in cells.

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