

P Granule Assembly and Function in *Caenorhabditis elegans* Germ Cells

Review

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ABSTRACT: Germ granules are large, non-membrane-bound, ribonucleoprotein (RNP) organelles found in the germ line cytoplasm of most, if not all, animals. The term *germ granule* is synonymous with the perinuclear nuage in mouse and human germ cells. These large RNPs are complexed with germ line-specific cytoplasmic structures such as the mitochondrial cloud, intermitochondrial cement, and chromatoid bodies. The widespread presence of germ granules across species and the associated germ line defects when germ granules are compromised suggest that germ granules are key determinants of the identity and special properties of germ cells. The nematode *Caenorhabditis elegans* has been a very fruitful model system for the study of germ granules, wherein they are referred to as P granules. P granules

contain a heterogeneous mixture of RNAs and proteins. To date, most of the known germ granule proteins across species, and all of the known P granule components in *C. elegans*, are associated with RNA metabolism, which suggests that a main function of germ granules is posttranscriptional regulation. Here we review P granule structure and localization, P granule composition, the genetic pathway of P granule assembly, and the consequences in the germ line when P granule components are lost. The findings in *C. elegans* have important implications for the germ granule function during postnatal germ cell differentiation in mammals.

Key words: Germ granules, nuage, chromatoid bodies.

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Mammalian germ granules, in their different forms (perinuclear nuage, intermitochondrial cement, chromatoid bodies), are found in the germ line cytoplasm of maturing gametes. As protein components of these germ granules have been identified and subsequently compromised, their crucial role during postnatal male germ cell differentiation has become apparent. To date, mutations in mouse germ granule components result in male sterility (Chuma et al, 2008), so understanding the critical role that germ granules play during spermatogenesis is imperative.

Germ granules are found in the germ line cytoplasm of all animals that have been examined (Eddy, 1975). As a consequence, much of what we now know concerning the composition and function of germ granules has come from work done in invertebrate model systems, such as *Drosophila* and *Caenorhabditis elegans* (Strome and Lehmann, 2007). Here, we focus on the key discoveries during the past 30 years of research on germ granules in *C. elegans* and how these findings comple-

ment research on mammalian germ granules and their essential role in fertility.

Generation of Primordial Germ Cells in C. elegans

Primordial germ cells (PGCs) are established by very different mechanisms during mammalian and *C. elegans* development. In mammalian development, inductive signals from the extraembryonic ectoderm and the visceral endoderm instruct a small number of proximal epiblast cells to become PGCs (Hayashi et al, 2007). In *C. elegans*, maternally contributed germ granules in the 1-cell zygote (P0) are progressively segregated to the germ line blastomeres, or P cells (P1, P2, P3, and P4), through 4 asymmetric cell divisions, resulting in delivery of germ granules to the P4 cell, which is the *C. elegans* PGC (Figure 1; Strome and Lehmann, 2007). Because of the segregation of germ granules to the P lineage, they were termed P granules. P4 divides only once during embryogenesis, generating the PGC daughter cells Z2 and Z3. At the end of the first larval stage, Z2 and Z3 start dividing symmetrically to eventually produce about 1000 germ cells in the adult gonad.

When P granules were first described, over 25 years ago, it was noted that their segregation to the embryonic cells that become germ line could be used to investigate the establishment of asymmetry or polarity within a cell (Strome and Wood, 1982, 1983). Indeed, much of the

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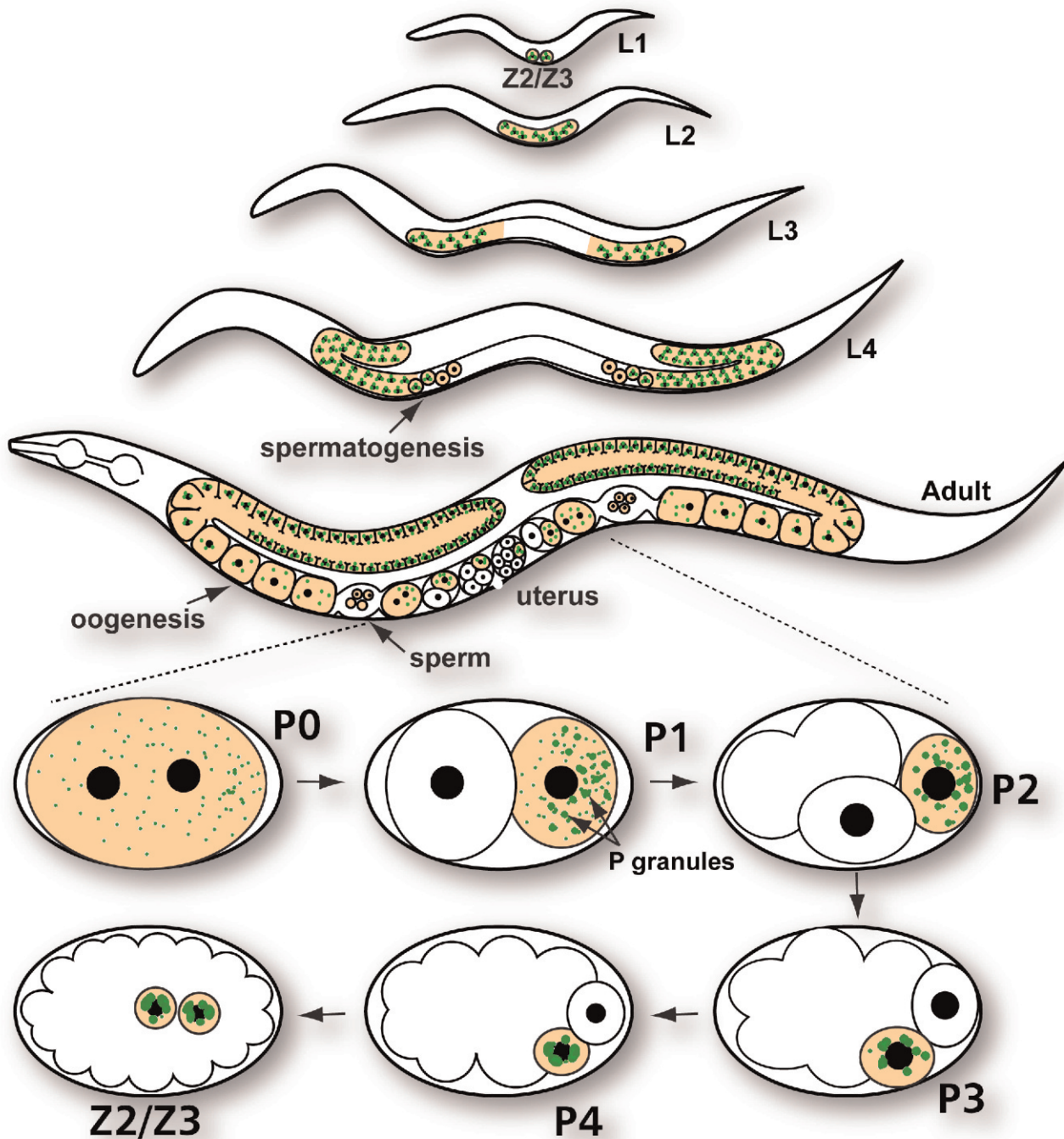


Figure 1. *C. elegans* germ line development. The 2 primordial germ cells (Z2/Z3) begin to proliferate at the end of the first larval stage (L1). During the fourth larval stage (L4) approximately 40 meiotic germ cells undergo spermatogenesis. In the adult hermaphrodite germ line, germ cells switch from making sperm to making oocytes. After fertilization (P0 zygote), P granules are segregated to the germ line blastomeres P1 to P4. At the ~100-cell stage, the primordial germ cell P4 divides into the daughter cells (Z2/Z3). Germ line is in orange. P granules are in green. L2 indicates second larval stage; L3, third larval stage.

focus on P granules over the subsequent decades has been aimed at investigating their segregation during the unequal P cell divisions. Detailed genetic pathways have been worked out to explain cellular asymmetry using P

granules as markers (Gonczy and Rose, 2005). Many of the polarity genes involved are conserved during polarization of many mammalian cell types (Gonczy, 2008). Because establishment of mammalian germ line

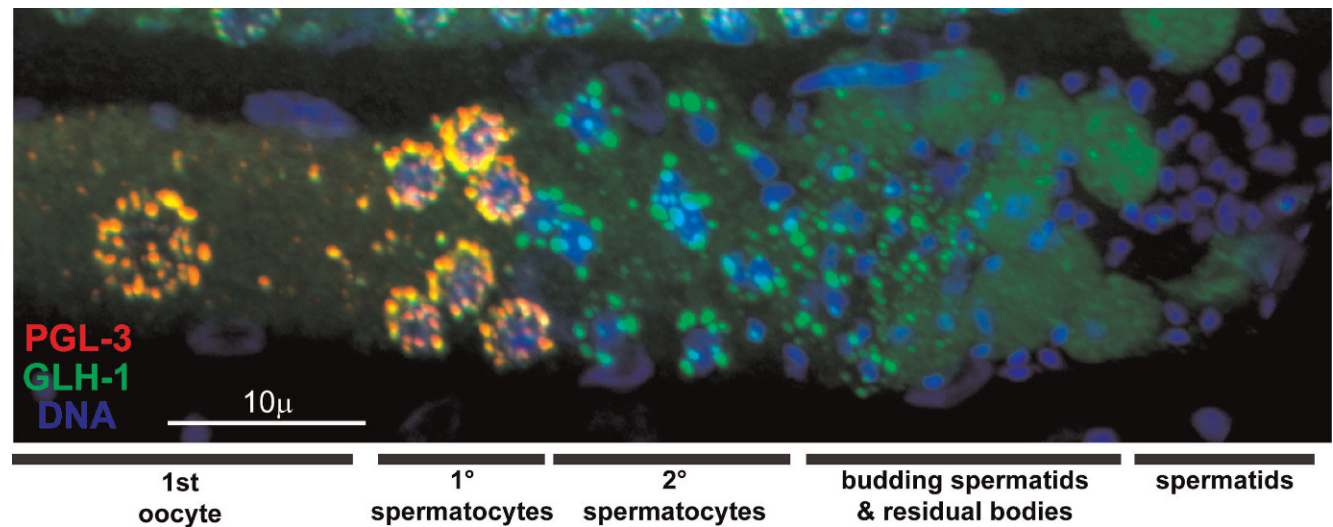


Figure 2. An oocyte and developing spermatocytes and sperm in *C. elegans*. GLH-1 (green) is localized to P granules in the oocyte and in primary and secondary spermatocytes, dispersed throughout the cytoplasm of residual bodies, and undetectable in mature spermatids. PGL-3 (red) is localized to P granules in the oocyte and in primary spermatocytes, and then drops to undetectable at later stages of spermatogenesis. DAPI (4',6-diamidino-2-phenylindole)-stained DNA is in blue.

appears not to depend on asymmetric cell divisions, these pathways are not discussed here. Instead we focus on the many similarities between P granules and the mammalian germ granules that arise during gametogenesis.

P Granule Structure and Localization

P granules and mammalian germ granules are conserved in both structure and localization in the germ line cytoplasm. Ultrastructural studies have demonstrated the electron-dense, fibrillar nature of germ granules in mammals and in *C. elegans* (Eddy, 1975; Krieg et al, 1978; Wolf et al, 1983). These studies and others have shown that cytoplasmic germ granules across species also lack a surrounding membrane. It has also been shown that germ granules reside close to organelles such as mitochondria (Eddy and Ito, 1971; Mahowald, 1968). P granules infrequently contain microtubules, centrioles, or mitochondria, and it is thought that these cytoplasmic components might become engulfed randomly as P granules grow or fuse with neighboring P granules (Pitt et al, 2000; Schisa et al, 2001).

Another similarity among germ granules across species is their close proximity to the nuclear periphery (Eddy and Ito, 1971; Mahowald, 1971; Eddy, 1974). P granules are no exception. However, their size and distribution change dramatically as early embryogenesis progresses from the 1-cell zygote to the birth of P4 in the ~16-cell embryo (Strome and Wood, 1982; Wolf et al, 1983; Hird et al, 1996; Pitt et al, 2000; Brangwynne et al, 2009). Prior to and immediately after fertilization, approximately 200 small P granules

are dispersed throughout the cytoplasm (Figure 1). During the next 2 cell divisions, these granules begin to coalesce as they are segregated into the germ line blastomeres P1 and P2. The few P granules that are not segregated to the germ line, but remain in the somatic blastomeres, are subsequently degraded (DeRenzo et al, 2003; Spike and Strome, 2003; Zhang et al, 2009). In P2, the cytoplasmic distribution of P granules begins to change as P granules that are dispersed in the cytoplasm begin to attach to the nuclear periphery. The perinuclear association of P granules continues in P3 and P4, resulting in the nucleus of P4 being almost completely surrounded by a handful of large P granules. P granules remain associated with the nuclear envelope throughout germ line proliferation, and detach only during the maturation of male and female gametes. In the *C. elegans* hermaphrodite, gametogenesis starts in the fourth larval stage (L4), when approximately 40 meiotic germ cells near the proximal end of each gonad arm generate sperm (Kuwabara and Perry, 2001). As spermatocytes complete meiosis, P granules are disassembled and remain dispersed in the cytoplasm of the residual body, and spermatids and spermatozoa appear depleted of P granule components (Gruidl et al, 1996; Figure 2). In the adult hermaphrodite, the germ line switches from making sperm to making oocytes, which allows the worm to be self-fertile. During oocyte maturation, P granules detach from the nuclear periphery; they persist as small aggregates during ovulation and fertilization, after which they again coalesce as they are segregated to the germ line blastomeres.

P Granule Composition

RNAs—P granules, like their mammalian counterparts, contain RNA. During *C. elegans* embryogenesis, a class of maternally expressed transcripts (described as class II messenger RNAs [mRNAs]) are selectively degraded in somatic daughter cells after each cell division, but remain protected in the germ line blastomeres (Seydoux and Fire, 1994). Many of the class II transcripts become enriched around the nuclear periphery of P3 and P4 nuclei in a P granule-like distribution. Seydoux and Fire (1994) showed that poly(A)+ and SL1-spliced RNAs are enriched in P granules from the 1-cell zygote to P4, demonstrating that embryonic P granules contain maternal RNAs. In the adult, P granules that surround germ line nuclei also contain RNA (Schisa et al, 2001). The accumulation of RNA in P granules increases when oogenesis is dramatically slowed down by the absence of sperm. This accumulation appears to be primarily mRNA, as probes against multiple ribosomal RNAs showed no enrichment in P granules. Schisa et al (2001) also examined the perinuclear accumulation of specific mRNAs in the adult germ line, and discovered that all 6 of the developmentally regulated transcripts they analyzed because of their role in gonadogenesis or early embryogenesis are enriched in P granules, whereas nondevelopmentally regulated transcripts for actin and tubulin are not enriched. The enrichment of most of the developmentally regulated transcripts with P granule components is observed even after P granules detach from the nuclear periphery in oocytes. In *C. elegans*, transcription is globally repressed during oocyte maturation, and most transcription remains repressed in the germ line throughout embryogenesis (Schaner and Kelly, 2006). One model is that maternal transcripts required in the embryonic germ line are preserved in P granules until transcription resumes during germ line proliferation in hatched larvae.

Proteins—One of the first germ granule protein components identified was the *Drosophila* DEAD-box RNA helicase Vasa (Hay et al, 1988; Lasko and Ashburner, 1988). The *C. elegans* Vasa homologs were identified as P granule components within the next few years (Gruidl et al, 1996). We now know that Vasa is a conserved and essential contributor to germ line development and fertility in all animals in which it has been examined. In *C. elegans*, there are 4 Vasa paralogs, which are named germ line helicase (GLH)-1, GLH-2, GLH-3, and GLH-4 (Kuznicki et al, 2000; Spike et al, 2008a). Like their mammalian counterpart (eg, mouse MVH), the GLHs contain both DEAD-box (GLH-1 to GLH-4) and Gly-rich (GLH1, GLH-2, GLH-4) domains. Interestingly, the Gly-rich domains of the *C. elegans* GLHs consist of FGG repeats instead of the

RGG repeats found in most Vasa orthologs. RGG repeats are an established RNA-binding domain (Kiledjian and Dreyfuss, 1992), whereas FGG repeats are associated with nuclear pore components (Suntharalingam and Went, 2003). P granules not only localize to the nuclear periphery, but 75% of nuclear pores in the *C. elegans* germ line are associated with P granules (Pitt et al, 2000). The association of P granules at the nuclear periphery can be disrupted by depleting specific nuclear pore components by RNA interference (RNAi; Updike and Strome, 2009). It is likely that the FGG domains of the GLHs contribute to the localization of P granules at the nuclear periphery. In fact, after depletion of *glh-1* and *glh-2*, only very small foci of electron-dense germ granule material persist at the nuclear periphery; they appear to completely lack the fibrillar-granular matrix of wild-type P granules (Schisa et al, 2001). It is possible that the GLHs serve both localization and structural roles in P granules. In other organisms in which Vasa does not contain FG repeats, it is possible that RGG domains or even hydrophobic repeats found in other germ granule components serve these roles.

The list of germ granule components continues to grow, but the majority of germ granule components across species, and all P granule components in *C. elegans*, are associated with RNA metabolism (Strome, 2005). A current list of P granule components is shown in the Table. Some of these components are constitutive, that is, they are found in P granules throughout the life cycle of the worm. Other components show a more transient association with P granules at only specific stages in the life cycle. The list includes factors that have roles in mRNA splicing, translation initiation, poly(A) polymerization, deadenylation, decapping, degradation, slicing, Piwi-interacting RNA (piRNA) and endogenous RNAi (endo-siRNA) synthesis. Included in the list are nematode-specific components like DEPS-1 and the PGL proteins. The PGLs all contain RGG motifs that are thought to bind RNA, much like the RGG motifs in Vasa, but their other domains seem to be nematode specific. Taken together, these components emphasize the role of P granules in RNA metabolism and posttranscriptional regulation.

P Granule Assembly Pathway

Some P granule components have been placed into a P granule assembly pathway: DEPS-1→GLH-1→PGL-1→IFE-1. At the top of this pathway is DEPS-1, a nematode-specific novel protein with a serine-rich C-terminal domain. A genome-wide analysis of mRNA levels in germ lines from *deps-1* mutants identified only 13 genes that are down-regulated, including *deps-1* and *glh-1* (Spike et al, 2008b). In *deps-1* mutants, *glh-1*

Table. *C. elegans* P granule proteins

Protein	Description	Reference
CAR-1	Cytokinesis, apoptosis, and RNA-binding 1 TRAL/Lsm14	Audhya et al, 2005; Boag et al, 2005; Squirrell et al, 2006
CCF-1	CCR4/NOT deadenylase complex	Gallo et al, 2008
CDE-1	Uracil nucleotidyltransferase	van Wolfswinkel et al, 2009
CGH-1	Dhh1/DDX6 DEAD-box helicase	Navarro et al, 2001
CSR-1	Argonaute required for endo-siRNA	Claycomb et al, 2009
DCAP-1	mRNA decapping enzyme	Squirrell et al, 2006
DCAP-2	mRNA decapping enzyme	Lall et al, 2005
DEPS-1	Novel defective P granules and sterile	Spike et al, 2008b
DRH-3	Dicer related DEAD-box helicase	Claycomb et al, 2009
EGO-1	RNA-directed RNA polymerase	Claycomb et al, 2009
GLD-1	RNA-binding KH domain	Jones et al, 1996
GLD-2	Poly(A) polymerase	Wang et al, 2002
GLD-3	RNA-binding KH domain	Eckmann et al, 2002
GLD-4	Poly(A) polymerase	Schmid et al, 2009
GLH-1	Vasa DEAD-box helicase	Gruidl et al, 1996
GLH-2	Vasa DEAD-box helicase	Gruidl et al, 1996
GLH-3	Vasa DEAD-box helicase	Kuznicki et al, 2000
GLH-4	Vasa DEAD-box helicase	Kuznicki et al, 2000
GLS-1	Novel GLD-3/4 interacting protein	Rybarska et al, 2009
IFE-1	eIF4E mRNA cap-binding	Amiri et al, 2001
LAF-1	DDX3 DEAD-box helicase	Hubert and Anderson, 2009
MEG-1	Novel maternal effect germ cell defective	Leacock and Reinke, 2008
MEG-2	Novel maternal effect germ cell defective	Leacock and Reinke, 2008
MEX-1	CCCH-type zinc-finger protein	Guedes and Priess, 1997
MEX-3	RNA-binding KH domain	Draper et al, 1996
OMA-1	CCCH-type zinc-finger protein	Shimada et al, 2002
OMA-2	CCCH-type zinc-finger protein	Shimada et al, 2002
PAB-1	Poly(A)-binding protein 1	Gallo et al, 2008
PATR-1	Pat1-decapping cofactor	Gallo et al, 2008
PGL-1	Novel RGG domain	Kawasaki et al, 1998
PGL-2	Novel PGL-1 related	Kawasaki et al, 2004
PGL-3	Novel RGG domain	Kawasaki et al, 2004
PIE-1	CCCH-type zinc-finger protein	Mello et al, 1996
POS-1	CCCH-type zinc-finger protein	Tabara et al, 1999
PRG-1	Argonaute required for piRNA synthesis	Batista et al, 2008
Sm proteins	Splicing factors	Barbee et al, 2002
SPN-2	eIF4E-binding protein	Li et al, 2009
SPN-4	RNP-type RNA-binding domain	Ogura et al, 2003
TIA-1	TIA-1 RNP-type RNA-binding domain	Gallo et al, 2008
VBH-1	Vasa Belle-like DEAD-box helicase	Salinas et al, 2007
WAGO-1	Argonaute required for endo-siRNA	Gu et al, 2009

Abbreviations: endo-siRNA, endogenous RNAi synthesis; mRNA, messenger RNA; piRNA, Piwi-interacting RNA; RNP, ribonucleoprotein.

mRNA and protein are reduced 5- to 10- fold. GLH-1 is subsequently required for the localization of the RGG-containing protein PGL-1 to P granules (Kawasaki et al, 1998). So in the absence of either DEPS-1 or GLH-1, PGL-1 (as well as PGL-2 and PGL-3) is dispersed throughout the cytoplasm. At the end of the known pathway is the P granule component IFE-1. IFE-1 is 1 of the 5 *C. elegans* homologs of eIF4E, which is the mRNA cap-binding subunit of the translation initiation complex. IFE-1 was identified as a PGL-1 interacting protein in a yeast 2-hybrid screen (Amiri et al, 2001). In the absence of PGL-1, IFE-1 fails to localize to P granules. The association of IFE-1 with P granules

supports the view that these granules are involved in translational regulation. In addition to the current list of known P granule assembly components, other potential components in this pathway continue to be identified through genome-wide screens for disrupted PGL-1 localization (Spike et al, 2008b; Updike and Strome, 2009).

What are the developmental consequences of loss of P granule components? Mutant alleles of *glh-2*, *glh-3*, *glh-4*, *pgl-2*, and *pgl-3* do not cause dramatic phenotypes on their own, but animals with null or strong loss-of-function mutations in *deps-1*, *glh-1*, *pgl-1*, and *ife-1* have temperature-sensitive defects in germ line proliferation

and gametogenesis (Amiri et al, 2001; Kawasaki et al, 2004; Spike et al, 2008a,b). For *glh-1* and *pgl-1*, the absence of sterility at low temperatures is due to functional redundancy with their paralogs. For example, *glh-1;glh-4* and *pgl-1;pgl-3* double mutants exhibit sterility even at low temperatures. Currently, a mechanism to explain the temperature sensitivity of sterility observed in *deps-1*, *glh-1*, *pgl-1*, and *ife-1* mutants is unknown. Interestingly, elevated temperature enhances the null or strong loss-of-function phenotypes of many germ line–required genes, suggesting that the germ line is inherently sensitive to elevated temperature (Spike et al, 2008a). An intriguing possibility is that the stability and function of P granules and other germ line complexes are compromised at higher temperatures, such that the loss of otherwise redundant components results in sterility. This model could potentially explain the inherent sensitivity of germ line development across multiple species.

The Roles of P Granule Components During Gametogenesis

Analysis of *deps-1*, *glh-1*, *pgl-1*, and *ife-1* mutants has revealed the germ line processes that those P granule components participate in regulating. *deps-1*, *glh-1*, and *pgl-1* mutants have underproliferated germ lines, and the majority of mutants fail to make oocytes and sperm at restrictive temperatures (Kawasaki et al, 2004; Spike et al, 2008a,b). Therefore, these components are involved in germ cell proliferation and gametogenesis. *ife-1* mutants make oocytes, but the production of mature sperm is delayed, causing the majority of hermaphrodites to be spermless. Thus, it is thought that IFE-1 is specifically required during spermatogenesis (Amiri et al, 2001; Henderson et al, 2009). After the pachytene stage of spermatogenesis, PGL-1 and PGL-3 disappear, whereas GLH-1 persists and is deposited in the residual body (Figure 2). It has been speculated that the association of IFE-1 with P granules reduces its availability until it is needed during postpachytene stages of spermatogenesis, when PGL-1 is absent (Amiri et al, 2001).

The mutant phenotype of *ife-1* is much like that of another constitutive P granule component, *prg-1*. PRG-1 is an argonaute required for *C. elegans* piRNA synthesis, which, when mutated, results in a temperature-sensitive sterile phenotype (Batista et al, 2008; Das et al, 2008; Wang and Reinke, 2008). The *prg-1* phenotype arises, in part, from a defect in late spermatogenesis. *prg-1* mutants raised at the restrictive temperature possess differentiated spermatocytes, but relatively few mature spermatids, each of which fails to produce a normal pseudopod (Wang and Reinke, 2008). How PRG-1 regulates genes preferentially expressed

during spermatogenesis, most likely through piRNAs, is a matter of debate (Batista et al, 2008; Wang and Reinke, 2008). Perhaps a similar mechanism to that proposed for IFE-1 is being used for PRG-1 to allow it to regulate targets that act relatively late in spermatogenesis, after P granules dissociate.

Conclusions

In *C. elegans*, P granules occupy a significant portion of the germ line cytoplasm, consistent with the critical role these granules play in germ line identity, maintenance, and fertility. We have a much better idea of that role now that more P granule components have been identified. Each of the 41 known P granule-associated factors listed in the Table highlights the importance of RNA metabolism in germ cells. So far, predicted levels of RNA regulation include mRNA splicing, translation initiation, poly(A) polymerization, deadenylation, decapping, degradation, slicing, and mRNA turnover. Although many of the P granule factors are associated with RNA destabilization and degradation, others, like PAB-1, can potentially serve to protect transcripts. Recent studies investigating live germ cells have demonstrated that P granules and P granule composition are dynamic, showing that P granules have domains that other cytoplasmic ribonucleoproteins, like P bodies, dock or merge with (Boag et al, 2008; Gallo et al, 2008; Noble et al, 2008). Protein composition could vary in each aggregate. Some aggregates may facilitate mRNA storage, whereas others may direct mRNA degradation or promote gene expression.

Although some functions and components of P granules appear to be nematode specific (DEPS-1, the PGLs, and the MEGs), there are many similarities in structure and composition between germ granules in *C. elegans* and mammals. Future studies include investigating the significance of the proximity of germ granules to the nuclear periphery, how germ granules cluster nuclear pores, and how mRNAs are distributed and processed as they exit the nucleus and become enriched in germ granules. Do germ granules extend the nuclear pore environment and act as a scaffold for posttranscriptional activities? Do germ granules activate or inhibit their components and release them at specific times during development when their function is needed? These questions will be best answered by a combinatorial approach using mammalian, *C. elegans*, and other model systems.

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