OXFORD GENETICS

GENETICS, 2022, 220(3), iyab195
https://doi.org/10.1093/genetics/iyab195
WormBook

# Germ granules and gene regulation in the *Caenorhabditis elegans* germline

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## Abstract

The transparency of *Caenorhabditis elegans* provides a unique window to observe and study the function of germ granules. Germ granules are specialized ribonucleoprotein (RNP) assemblies specific to the germline cytoplasm, and they are largely conserved across Metazoa. Within the germline cytoplasm, they are positioned to regulate mRNA abundance, translation, small RNA production, and cytoplasmic inheritance to help specify and maintain germline identity across generations. Here we provide an overview of germ granules and focus on the significance of more recent observations that describe how they further demix into sub-granules, each with unique compositions and functions.

Keywords: *C. elegans*; germline; germ granules; P granules; Mutator foci; Z granules; piRNAs; WormBook

## Overview of germ granules

Early studies of animal germline specification noted visibly dense assemblies on the cytoplasmic surface of germline nuclei [reviewed in Eddy (1975)]. These assemblies have been called by various names across species and throughout germline development, but in recent years a consensus has emerged to collectively refer to them as germ granules. In *C. elegans*, the first observed germ granules were called P granules because they segregate with the P cell lineage (germline blastomeres) during embryogenesis [Figure 1, A–D; reviewed in Strome (2005), Hubbard and Greenstein (2005)]. Today, the term "germ granule" defines a collection of dynamic and germline-specific perinuclear assemblies that can be distinguished through high-resolution microscopy. Other cytoplasmic assemblies, such as P bodies, stress granules, and those which form in arrested oocytes will not be covered here. In early embryos, the terms germ granule and P granule are used interchangeably, but as development progresses, germ granules further demix into sub-granules that, along with P granules, include *Mutator* foci, Z granules, SIMR-1 foci, and likely other perinuclear assemblies yet to be defined (Figure 1, C–G). *Caenorhabditis elegans* provide a window to observe the dynamics of these sub-granules, revealing their function in the germline and specific contributions of their individual components. These findings can then be extended to better define the roles of germ-granule proteins in other animals.

Germ granules and their subtypes are heterogeneous ribonucleoproteins. Of the ~90 *C. elegans* proteins currently known to be germ-granule enriched (Table 1), almost all have RNA binding domains. They include RNA (mostly DEAD-box) helicases, mRNA stabilizing and destabilizing proteins, translation initiation factors, small RNA-binding Argonautes, RNA polymerases, mRNA export and nuclear pore complex components, and many proteins harboring KH, CCCH zinc-finger, Tudor, and LOTUS domains. Together, assemblies of these germline proteins at the nuclear periphery position them to regulate mRNA abundance, translation, small RNA production, and cytoplasmic inheritance through cell divisions and across generations. In the proceeding paragraphs, we explore the function of germ granules, and what the composition and modularity of known germ-granule subtypes reveal about their function.

## Organization and function of P granules

### Overview of P granules

The discovery of P granules in *C. elegans* (Strome and Wood 1982) followed the discovery of asymmetric segregation of germ granules that had been previously described during early embryonic cleavages in several invertebrates and amphibians (Ritter 1890; Hegner 1911; Penners 1922; Smith 1966; Mahowald *et al.* 1976). It was recognized that partitioning of P granules in early embryos could be used to understand how asymmetry is established prior to the first cell division [(Kemphues *et al.* 1988; reviewed in Rose and Gonczy (2014)]. Moreover, the accessibility of P granules provided a way to identify their core components and observe their formation, condensation, and dissolution in early embryogenesis (reviewed in Seydoux 2018).

### Structural composition of P granules

The list of P-granule-associated proteins continues to expand (Table 1). Many of these proteins associate transiently with P granules in the early embryo or at other stages of germline

Received: March 31, 2021. Accepted: October 10, 2021

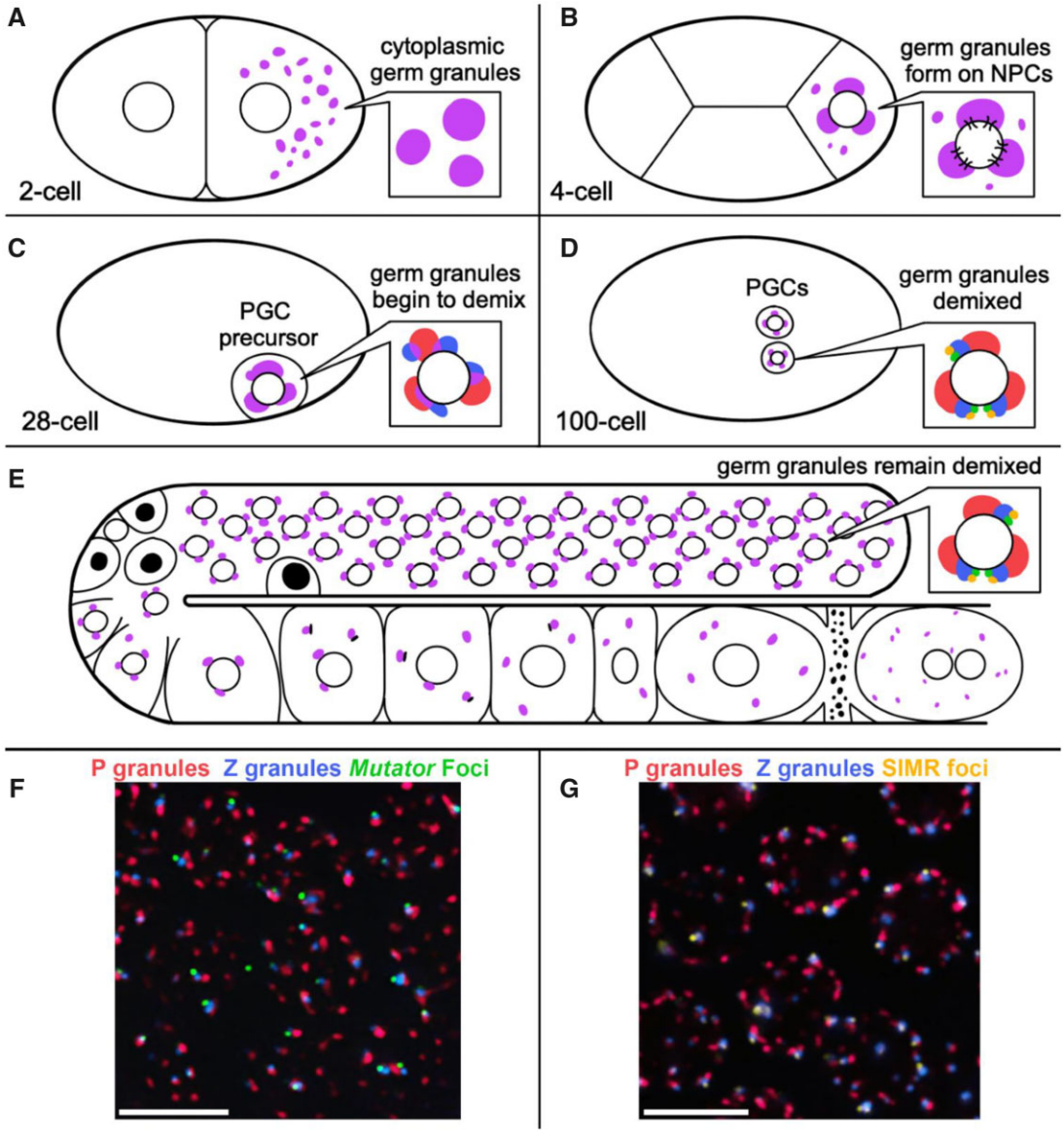


**Figure 1** Germ granule distribution and demixing during development. (A) Posteriorly localized germ granules (purple) in the 2-cell embryo are dispersed in the cytoplasm. (B) Germ granules in the 4-cell embryo begin to adhere to the cytoplasmic surface of the nuclear envelope and cluster NPCs. (C) Germ granule demixing begins at the 28-cell stage in primordial germ cell (PGC) precursors. (D) Germ granules in the PGCs of 100-cell embryos have demixed into adjacent P granules, Z granules, SIMR foci and *Mutator* foci. (E) Germ granules remain demixed in adult germ cells (inset). PGL proteins, but not GLH proteins, are cleared from P granules during physiological apoptosis (black nuclei). P granules disperse into the cytoplasm of oocytes prior to fertilization, initially with part of the nuclear envelope attached. (F, G) Immunofluorescence image of adult germ cells (pachytene) with demixed sub-granules. (images Celja Uebel. scale bars = 5 μm).

development and gametogenesis. A smaller contingent can be described as constitutive proteins that are found in P granules at all stages of the *C. elegans* lifecycle. Among these are a few novel proteins (DEPS-1, PGL-1, and PGL-3), the Vasa DEAD-box germline helicases (GLH-1, GLH-2, GLH-3, and GLH-4), other related DEAD-box helicases (RDE-12, LAF-1, VBH-1), and some of the Argonaute proteins (CSR-1, PRG-1, and worm-specific Argonaute (WAGO)-1). PGL-1 and PGL-3 are scaffolding proteins that interact through two dimerization domains, exhibit RNA endonuclease activity *in vitro*, and have the ability to repress tethered mRNA expression *in vivo* (Aoki *et al.* 2016, 2021). Ectopic expression of PGL-1 and PGL-3, either in the soma of *C. elegans* or in mammalian cell culture, is sufficient to nucleate P-granule-like condensates (Hanazawa *et al.* 2011; Updike *et al.* 2011). Interestingly, different proteins nucleate germ-granule formation in other animals, supporting the idea that germ-granule nucleators evolved through convergent evolution [reviewed in Kulkarni and Extavour (2017)]. Recombinant PGL-3 can self-assemble under physiological conditions *in vitro* (Saha *et al.* 2016). *In vivo*, the posterior condensation of PGL-1 and PGL-3 in the zygote is facilitated by a MEG-3 and MEG-4 scaffold to coat PGL assemblies, and LOTUS-domain MEG-3 interacting proteins MIP-1 and MIP-2 (Wang *et al.* 2014; Chen *et al.* 2016; Smith *et al.* 2016; Putnam *et al.* 2019; Folkmann *et al.* 2021; Price *et al.* 2021; Schmidt *et al.* 2021; Cipriani *et al.* 2021). During spermatogenesis, PGL-1 and PGL-3 are cleared from P granules while GLH proteins are retained, suggesting that nucleators are not always needed to maintain P granules after they are formed [discussed in Updike and Strome (2010)].

The RNA composition of P granules is better defined in embryos than in adults. Early studies demonstrated that embryonic

**Table 1** Germ granule proteins (P granules, Z granules, *Mutator* foci, SIMR foci, and unknown)

| Germ granule | Protein | Description | References |
|---|---|---|---|
| P | ALG-3 | Argonaute expressed during spermatogenesis | Conine *et al.* (2010) |
| P | ALG-4 | Argonaute expressed during spermatogenesis | Conine *et al.* (2010) |
| P | ALG-5 | Argonaute associated with miRNAs | Brown *et al.* (2017) |
| P | CAR-1 | Cytokinesis, apoptosis, and RNA-binding 1 TRAL/Lsm14 | Audhya *et al.* (2005), Boag *et al.* (2005), Squirrell *et al.* (2006) |
| P | CCF-1 | CCR4/NOT deadenylase complex | Gallo *et al.* (2008) |
| P | CDE-1 | Uracil nucleotidyltransferase | van Wolfswinkel *et al.* (2009) |
| P | CGH-1 | Dhh1/DDX6 DEAD-box helicase | Navarro *et al.* (2001) |
| P | CSR-1 | Argonaute required for endo-siRNA | Claycomb *et al.* (2009) |
| P | DCAP-1 | mRNA decapping enzyme | Squirrell *et al.* (2006) |
| P | DCAP-2 | mRNA decapping enzyme | Lall *et al.* (2005) |
| P | DCR-1 | Dicer-related RNAse | Beshore *et al.* (2011) |
| P | DDX-19 | DDX19 DEAD-box helicase | Sheth *et al.* (2010) |
| P | DEPS-1 | Defective P granules and Sterile | Spike *et al.* (2008a) |
| P | DRH-3 | Dicer-related DEAD-box helicase | Claycomb *et al.* (2009) |
| P | EGO-1 | RNA-directed RNA polymerase (RdRP) | Claycomb *et al.* (2009) |
| P | ERH-2 | 21U-RNA maturation | Cordeiro Rodrigues *et al.* (2019) and Zeng *et al.* (2019) |
| P | FBF-2 | PUF-domain fem-3 mRNA 3′UTR-binding factor | Voronina (2012) |
| P | GLD-1 | RNA-binding KH domain | Jones *et al.* (1996) |
| P | GLD-2 | Poly(A) polymerase | Wang *et al.* (2002) |
| P | GLD-3 | RNA-binding KH domain | Eckmann *et al.* (2002) |
| P | GLD-4 | Poly(A) polymerase | Schmid *et al.* (2009) |
| P | GLH-1 | Vasa DEAD-box helicase | Gruidl *et al.* (1996) |
| P | GLH-2 | Vasa DEAD-box helicase | Gruidl *et al.* (1996) |
| P | GLH-3 | Vasa DEAD-box helicase | Kuznicki *et al.* (2000) |
| P | GLH-4 | Vasa DEAD-box helicase | Kuznicki *et al.* (2000) |
| P | GLS-1 | GLD-3/4 interacting protein | Rybarska *et al.* (2009) |
| P | HENN-1 | 3′ RNA methyltransferase | Kamminga *et al.* (2012) |
| P | HRDE-2<br>ENRI-3 | Heritable RNAi deficient | Spracklin *et al.* (2017) and Lewis *et al.* (2020) |
| P | IFE-1 | eIF4E mRNA cap-binding | Amiri *et al.* (2001) |
| P | IFE-3 | eIF4E mRNA cap-binding<br>(Perinuclear granules adjacent to PGL-1) | Cordeiro Rodrigues *et al.* (2019), Zeng *et al.* (2019), and Huggins *et al.* (2020) |
| P | IFET-1 | eIF4E transporter | Sengupta *et al.* (2013) |
| P | LAF-1 | DDX3 DEAD-box helicase | Hubert and Anderson (2009) |
| P | MBK-2 | DYRK3 YAK-related kinase | Stitzel *et al.* (2007) |
| P | MEG-1 | Maternal effect germ cell defective | Leacock and Reinke (2008) |
| P | MEG-2 | Maternal effect germ cell defective | Leacock and Reinke (2008) |
| P | MEG-3<br>GEI-12 | Maternal effect germ cell defective GCNA-IDR | Wang *et al.* (2014) |
| P | MEG-4 | Maternal effect germ cell defective GCNA-IDR | Wang *et al.* (2014) |
| P | MEX-1 | CCCH-type zinc-finger protein | Guedes and Priess (1997) |
| P | MEX-3 | RNA-binding KH domain | Draper *et al.* (1996) |
| P | MIP-1 | LOTUS-containing MEG-3 interacting protein | Cipriani *et al.* (2021) |
| P | MIP-2 | LOTUS-containing MEG-3-interacting protein | Cipriani *et al.* (2021) |
| P | NOS-2 | Nanos-related protein | Subramaniam and Seydoux (1999) |
| P | NPP-8 | NUP155 NPC protein | Voronina and Seydoux (2010) |
| P | NPP-10 | NUP98 NPC protein | Voronina and Seydoux (2010) |
| P | NXF-1 | NXF1/TAP-like mRNA export factor | Sheth *et al.* (2010) |
| P | OMA-1 | CCCH-type zinc-finger protein | Shimada et al. (2002) |
| P | OMA-2 | CCCH-type zinc-finger protein | Shimada et al. (2002) |
| P | PAB-1 | Poly(A)-binding protein 1 | Gallo *et al.* (2008) |
| P | PAN-1 | LRRTM4 DEAD-box helicase | Gao *et al.* (2012) |
| P | PARN-1 | Poly(A)-specific 3′–5′-exoribonuclease | Tang *et al.* (2016) |
| P | PATR-1 | Pat1 decapping cofactor | Gallo *et al.* (2008) |
| P | PGL-1 | RGG-containing P granule endoribonuclease | Kawasaki *et al.* (1998) |
| P | PGL-2 | PGL-1 related | Kawasaki *et al.* (2004) |
| P | PGL-3 | RGG-containing P granule endoribonuclease | Kawasaki *et al.* (2004) |
| P | PID-1 | piRNA-induced silencing defective | Cordeiro Rodrigues *et al.* (2019) and Zeng *et al.* (2019) |
| P | PID-3<br>PICS-1 | piRNA-induced silencing defective | Cordeiro Rodrigues *et al.* (2019) and Zeng *et al.* (2019) |
| P | PID-4 | piRNA-induced silencing defective | Placentino *et al.* (2021) |
| P | PID-5 | Aminopeptidase, piRNA-induced silencing defect | Placentino *et al.* (2021) |
| P | PIE-1 | CCCH-type zinc-finger protein | Mello *et al.* (1996) |
| P | PLP-1 | Pur alpha-like protein | Witze *et al.* (2009) |
| P | POS-1 | CCCH-type zinc-finger protein | Tabara *et al.* (1999a) |
| P | PRG-1 | Argonaute required for piRNA synthesis | Batista *et al.* (2008) |
| P | PUF-8 | PUF (Pumilio/FBF) domain 3′UTR-binding factor | Ariz *et al.* (2009) |
| P | RDE-12 | RNAi defective DEAD-box helicase | Sheth *et al.* (2010) |

(continued)

**Table 1** (continued)

| Germ granule | Protein | Description | References |
|---|---|---|---|
| P | RNP-8 | RRM poly(G) RNA binding | Kim *et al.* (2009) |
| P | SIR-2.2 | Sirtuin 4-like protein deacetylase | Jedrusik-Bode *et al.* (2013) |
| P | Sm prot. | Splicing factors | Barbee *et al.* (2002) |
| P | SPN-2 | eIF4E-binding protein | Li *et al.* (2009) |
| P | SPN-4 | RNP-type RNA-binding domain | Ogura *et al.* (2003) |
| P | TIA-1 | TIA-1 RNP-type RNA-binding domain | Gallo *et al.* (2008) |
| P | TOFU-6 | 21U-RNA fouled up | Cordeiro Rodrigues *et al.* (2019) and Zeng *et al.* (2019) |
| P | VBH-1 | Vasa Belle-like DEAD-box helicase | Salinas *et al.* (2007) |
| P | WAGO-1 | Argonaute required for endo-siRNA | Gu *et al.* (2009) |
| P | WAGO-3 | Argonaute present in sperm | Schareier *et al.* (2021) |
| P | Y51F10.2 | TRIM32 E3 ubiquitin-protein ligase | Lee *et al.* (2020) |
| M | MUT-2 RDE-3 | Mutator with predicted nucleotidyltransferase activity | Phillips *et al.* (2012) |
| M | MUT-7 | Mutator with predicted 3′–5′ exoRNAse activity for miRNA end processing | Phillips *et al.* (2012) |
| M | MUT-8 RDE-2 | Mutator RNAi defective | Phillips *et al.* 2012) |
| M | MUT-14 | Mutator resembling the DDX3 DEAD-box helicase | Phillips *et al.* (2012) |
| M | MUT-15 RDE-5 | Mutator RNAi defective | Phillips *et al.* (2012) |
| M | MUT-16 RDE-6 | Mutator RNAi defective | Phillips *et al.* (2012) |
| M | NYN-1 | NYN domain ribonuclease homolog | Uebel *et al.* (2018) |
| M | NYN-2 | NYN domain ribonuclease homolog | Uebel *et al.* (2018) |
| M | RDE-8 | mRNA-binding endo-RNAse that positively regulates RdRP activity | Tsai *et al.* (2015) |
| M | RRF-1 | RNA-directed RNA polymerase (RdRP) | Phillips *et al.* (2012) |
| M | SMUT-1 | Synthetic mutator DDX3 DEAD-box helicase-like | Phillips *et al.* (2014) |
| Z | LOTR-1 | LOTUS and Tudor domain protein | Marnik *et al.* (2021) |
| Z | PID-2 ZSP-1 | piRNA-induced silencing defective | Placentino *et al.* (2021) and Wan *et al.* (2021) |
| Z | WAGO-4 | Argonaute required for RNAi inheritance | Wan *et al.* (2018) |
| Z | ZNFX-1 | NFX1-type zinc finger-containing protein | Ishidate *et al.* (2018) and Wan *et al.* (2018) |
| S | HPO-40 | SIMR-1-like Tudor-domain protein | Manage *et al.* (2020) |
| S | RSD-2 | RNAi spreading defective | Manage *et al.* (2020) |
| S | SIMR-1 | siRNA defective and mortal germline Tudor-domain protein | Manage *et al.* (2020) |
| ? | FBF-1 | PUF-domain fem-3 mRNA 3′UTR-binding factor that docks next to PGL-1 | Voronina *et al.* (2012) |
| ? | MINA-1 | RNA-binding KH protein that docks next to PGL-1 | Sendoel *et al.* (2019) |

P granules retain both maternally expressed and developmentally regulated mRNAs (Seydoux and Fire 1994; Subramaniam and Seydoux 1999; Schisa *et al.* 2001). More recently, MEG-3 iCLIPs identified approximately 500 specific mRNAs that are enriched in embryonic P granules (Lee *et al.* 2020). *In situ* hybridization studies revealed that nascent mRNAs pass into and through P granules, and that their perinuclear enrichment may be a result of their slowed diffusion during the transit (Sheth *et al.* 2010). In contrast to mRNAs, ribosomal RNAs are not enriched in P granules and even appear excluded (Schisa *et al.* 2001; Marnik *et al.* 2019), suggesting that P granules are devoid of translation. In fact, some P-granule localized transcripts are correlated with low translational status and ribosome coverage, and global translation inhibition directs numerous transcripts to embryonic P granules in a MEG-3 dependent but nonsequence specific manner (Lee *et al.* 2020; Parker *et al.* 2020). These results suggest embryonic P granules are a way to maintain a pool of maternal mRNAs in germline precursors until they resume zygotic transcription.

## Association of P granules with the nuclear pore complex

The association of *C. elegans* P granules on the cytoplasmic surface of the nuclear periphery reflects the general distribution of germ granules across species. In the *C. elegans* germline, P granules cluster nuclear pore complexes (NPCs), and 75% of NPCs are covered by P granules (Figure 1B; Pitt *et al.* 2000). NPCs not covered by P granules are only found where a prominent lobe from the nucleolus contacts the nuclear envelope, suggesting that rRNAs enter the cytoplasm without passing through P granules (Sheth *et al.* 2010). Nuclear export factors, such as NXF-1 and DDX-19, and peripheral nucleoporins, such as NPP-8, NPP-9, and NPP-10, localize to the base of P granules (Sheth *et al.* 2010; Voronina and Seydoux 2010). Upon the cytoplasmic dispersal of P granules during oogenesis, some P granules retain attached NPCs (Pitt *et al.* 2000). In addition, RNAi depletion of a number of nucleoporins cause P granules to detach from the nuclear periphery and disperse into the cytoplasm (Updike and Strome 2009; Voronina and Seydoux 2010). These findings demonstrate the tight association between P granules and NPCs.

P granules and NPC contacts are likely mediated through glycine-rich FG-repeat domains present in several nucleoporins (FG-Nups) and P-granule proteins such as GLH-1, GLH-2, GLH-4, DDX-19, RDE-12 (Sheth *et al.* 2010). Unstructured FG-repeat domains fill up the pore of NPCs to establish the size exclusion barrier between the nucleus and cytoplasm. A current model is that the regularly spaced phenylalanines form weak hydrophobic interactions to create a mesh or smart sieve (Schmidt and Görlich 2016). Proteins under 40 kD can diffuse freely through the sieve, while larger proteins require a karyopherin for import and export. P granules extend the 40 kD size exclusion barrier beyond the pore and into the cytoplasm, and weak concentrations of hexanediol capable of disrupting the hydrophobic interactions within

the pore also disperse P granules (Updike *et al.* 2011). P-granule FG-repeat proteins form hydrophobic tethers with FG-Nups to maximize coverage of NPCs, positioning P granules to receive nascent transcripts that exit the nucleus. Deleting FG-repeats from GLH-1 and GLH-2 increases P-granule size and sphericity as they lose contact with the nuclear periphery (Marnik *et al.* 2019; Chen *et al.* 2020).

## P granules regulate germline apoptosis

Over half of the oogenic germ cells undergo physiological apoptosis (Gartner *et al.* 2008). Germ cells are connected to a cytoplasmic syncytium, and excess germ cells function as nurse cells and dump their mitochondria and other cytoplasmic contents into the shared cytoplasm as apoptosis is initiated (Raiders *et al.* 2018). PGL-1 and PGL-3 disappear from these apoptotic cells, suggesting that P granules and their components not only play a role in the formation of germ cells, but also their preservation (Pitt *et al.* 2000; Sheth *et al.* 2010). In response to UV damage, *pgl-1* and *pgl-3* mutants show elevated levels of apoptosis; the pro-apoptotic factor Apaf1/CED-4 accumulates, and Sirtuin/SIR-2.1, which functions as an antiapoptotic factor in the nucleus, is translocated into the cytoplasm. These findings suggest that P granules (or the presence of PGL proteins in P granules) suppress these pro-apoptotic activities (Min *et al.* 2016). Supporting these findings, somatic programmed cell death is suppressed in synMuvB mutants that express somatic P granules; similarly, ectopic expression of PGL-1 or PGL-3 from transgenes is sufficient to repress apoptosis in the soma in a SIR-2.1-dependent manner (Al-Amin *et al.* 2016). PGL proteins are cleared by autophagy in somatic blastomeres during embryogenesis (Zhang *et al.* 2009, 2018a). Similarly, PGL proteins in the adult germline are cleared by autophagy following UV-induced DNA damage, linking the requirement of autophagy machinery to UV-induced apoptosis in the germline (Min *et al.* 2019).

## P granules promote germline gene expression

Given the complex composition of P granules and the redundancy involved in their nucleation, depleting single P-granule components does not clear P granules from the adult germline. However, core P-granule proteins and electron-dense P-granule assemblies are no longer detected in *mex-3 gld-1* double mutants, and this absence correlates with germ-to-soma transdifferentiation (Ciosk *et al.* 2006). The simultaneous RNAi depletion of multiple core P-granule proteins inhibits fertility and also causes germ-to-soma transdifferentiation (Updike *et al.* 2014), and an increase in somatic expression in the germline of older adults (Knutson *et al.* 2017). These results suggest that P granules safeguard germline development through mRNA surveillance mechanisms that repress the accumulation and translation of somatic transcripts that become stochastically expressed. The analysis of *deps-1*, *glh-1*, *glh-2*, *glh-4*, *pgl-1*, and *pgl-3* single mutants has revealed underproliferated germlines and defects in gametogenesis at restrictive temperatures, while sterility in double mutants can make it difficult to distinguish primary from secondary effects on gene expression (Kawasaki *et al.* 2004; Spike *et al.* 2008a, 2008b). Before the onset of these defects in *deps-1* and *glh-1* single mutants, expression profiling reveals only subtle germline expression changes (Spike *et al.* 2008a, 2008b). The same is the case in healthy germlines of young adults after simultaneous RNAi depletion of multiple transcripts (*pgl-1, pgl-3, glh-1, glh-4*), except for a global increase of spermatogenic transcripts in proximal germ cells slated to undergo oogenesis (Campbell and Updike 2015; Knutson *et al.* 2017).

Mechanisms used by P granules to recognize and suppress somatic and spermatogenic expression in the adult germline are areas of continuing focus. During spermatogenesis PGL proteins are cleared from secondary spermatocytes (Amiri *et al.* 2001), while GLH proteins, which are necessary for the completion of spermatogenesis (Kuznicki *et al.* 2000), are retained in P granules until they are deposited in the residual body near the completion of spermatogenesis (Gruidl *et al.* 1996). The expression of P-granule associated Argonaute proteins like CSR-1, WAGO-1 and WAGO-3 is accompanied by transient expression of Argonautes ALG-3 and ALG-4 during spermatogenesis; while all of these Argonautes are implicated in paternal inheritance, CSR-1, WAGO-1 and WAGO-3 persist in sperm while ALG-3 and ALG-4 become deposited in residual bodies (Conine *et al.* 2010, 2013; Schreier *et al.* 2021). How the temporospatial expression of each these factors impact spermatogenesis still needs to be resolved. Recent studies have shown that CSR-1 has both long (a) and short (b) isoforms, but the long CSR-1a isoform is selectively expressed during spermatogenesis in L4 hermaphrodites, where it primarily targets spermatogenic genes (Nguyen and Phillips 2021; Charlesworth *et al.* 2021). Dimethylarginine modifications to the CSR-1a isoform are necessary for this target specificity (Nguyen and Phillips 2021). Translational initiation may also be involved; for example, the PGL-associated isoform of the m7G cap-binding eIF4E initiation factor IFE-1 promotes sperm translation—an activity likely repressed with the occurrence of PGLs in the first wave of oogenesis (Amiri *et al.* 2001; Henderson *et al.* 2009; Friday *et al.* 2015). In contrast, a second isoform of the m7G cap eIF4E, IFE-3, associates with eIF4E transporter IFET-1 to drive oocyte translation and the sperm-to-oocyte switch but is not required for spermatogenesis (Sengupta *et al.* 2013; Huggins *et al.* 2020).

## P granules as sites of mRNA surveillance

The tight association with the NPC positions P granules to survey transcripts for foreign or somatic sequences as they exit the nucleus. This epigenetic memory of germline expression is conferred through the small RNA machinery within P granules. The PIWI-class Argonaute, PRG-1, is a constitutive P-granule component that associates with more than 10,000 distinct Piwi-interacting RNAs (piRNAs), also known as 21U RNAs in *C. elegans* due to their 21-nt length and 5′ bias for uracil, to form piRNA-induced silencing complexes (piRISCs). These piRISCs use imperfect complementarity to engage and surveil the entire germline transcriptome, including mRNAs, non-coding RNAs, and transposable elements (Ruby *et al.* 2006; Wang and Reinke 2008; Batista *et al.* 2008; Lee *et al.* 2012; Bagijn *et al.* 2012; Shen *et al.* 2018; Zhang *et al.* 2018b). piRNA targeting can initiate heritable epigenetic silencing that, in many cases, bypasses the need for piRNAs to maintain that silencing in subsequent generations (Shirayama *et al.* 2012; Ashe *et al.* 2012; Luteijn *et al.* 2012). This heritable silencing is mediated by WAGO 22G-RNAs, 22-nt siRNAs with a 5′ guanosine bias, that are bound by a class of WAGO proteins. WAGO 22G-RNAs are synthesized by RNA-dependent RNA polymerases (RdRPs) associated with the *mutator* complex (described in more detail below; Gu *et al.* 2009; Phillips *et al.* 2012).

The biogenesis of piRNAs requires both 5′ and 3′ processing. First, the 5′ piRNA end maturation is carried out by the PETISCO/PICS complex, made up of PID-1, PID-3/PICS-1, ERH-2, TOFU-6, and IFE-3 (Cordeiro Rodrigues *et al.* 2019; Zeng *et al.* 2019; Perez-Borrajero *et al.* 2021). This maturation involves decapping and removal of two nucleotides from the 5′ end of the piRNA precursor (Gu *et al.* 2012). The PETISCO/PICS complex is also P-granule associated, though the co-localization of this complex with

known P-granule factors may be imperfect (Cordeiro Rodrigues *et al.* 2019; Zeng *et al.* 2019; Perez-Borrajero *et al.* 2021). For example, perinuclear IFE-3 granules appear to dock next to PGL-1 labeled granules (Huggins *et al.* 2020). 5′ end maturation is followed by loading of the piRNA precursor into PRG-1 where the 3′ end is trimmed by the P granule-localized exonuclease PARN-1 (Tang *et al.* 2016). Finally, the 3′ end of the piRNA is 2′O-methylated by the methyltransferase HENN-1, which may also localize to P granules (Billi *et al.* 2012; Montgomery *et al.* 2012; Kamminga *et al.* 2012). While initially the mobilization of transposable elements was assumed to cause the transgenerational decline in fertility in *prg-1* mutants, *prg-1* sterility correlates more with perturbed P-granule structure and may be independent of genomic stability (Spichal *et al.* 2021). Also correlating with the progressive loss of fertility in *prg-1* mutants, is the accumulation of 22G-RNAs antisense to replicative histone genes and ribosomal RNA genes and a corresponding reduced expression of histone mRNAs (Barucci *et al.* 2020; Montgomery *et al.* 2020; Reed *et al.* 2020; Wahba *et al.* 2021). Whether reduced expression of rRNAs and histones, disruption of P granules, or another unknown factor is underlying cause of transgenerational sterility in *prg-1* mutant animals is a matter that will need further investigation.

Opposing piRNA-mediated silencing, 22G-RNAs bound by the P granule-associated Argonaute CSR-1 (CSR-1 22G-RNAs) license protein-encoding transcripts for germline expression (Claycomb *et al.* 2009; Gu *et al.* 2009; Seth *et al.* 2013; Wedeles *et al.* 2013; Cecere *et al.* 2014; Tu *et al.* 2014). CSR-1 22G-RNAs are produced through the activity of the EGO-1 RdRP (Claycomb *et al.* 2009), yet the mechanism by which EGO-1 activity is initiated on CSR-1 target transcripts has been a bit of an enigma. While WAGO 22G-RNAs can be initiated by piRNAs or other classes of primary siRNAs, there has been no known primary siRNA class in the CSR-1 pathway. However, recent work has demonstrated that CSR-1 slicer activity is necessary to trigger biogenesis of CSR-1 22G-RNAs within the coding region of CSR-1 target genes. Interestingly, CSR-1 slicer activity is not required for initiation of EGO-1 RdRP activity in CSR-1 target 3′UTRs. Thus, while it is unknown what triggers the recruitment of EGO-1 to target 3′UTRs, these new data indicate that within target gene bodies EGO-1 22G-RNA synthesis may be initiated following cleavage by 22G-RNA-bound CSR-1, independent of any primary siRNAs (Singh *et al.* 2021). Furthermore, it appears that CSR-1 22G-RNA biogenesis occurs on actively translated mRNAs in the cytoplasm, in contrast to other 22G-RNAs which are mostly synthesized in germ granules (Singh *et al.* 2021). Lastly, CSR-1 22G-RNAs can be uridylated by the P granule-localized nucleotidyl transferase CDE-1, to restrict their accumulation (van Wolfswinkel *et al.* 2009).

Through CSR-1 22G-RNA/WAGO 22G-RNA opposition, P granules have the capacity to retain a memory of germline expression. How CSR-1 promotes the expression of its 22G-RNA targets is unclear; however, one model is that CSR-1 may secure the passage of its targets through P granules and into the cytoplasm where they can ultimately be translated. Compromising CSR-1 and select components upstream of CSR-1 22G-RNA synthesis cause a very distinct enlarged P-granule phenotype (Vought *et al.* 2005; Claycomb *et al.* 2009; Updike and Strome 2009; Campbell and Updike 2015; Andralojc *et al.* 2017). This could reflect the pooling of CSR-1 22G-RNA target transcripts that can no longer make their way through and into the cytoplasm. Interestingly, while CSR-1 22G-RNA target mRNA levels change very little in dissected germlines and whole worm lysates, expression profiling in early embryos suggest a P-granule independent role for the slicer activity of CSR-1 in clearing its maternally deposited targets from somatic blastomeres (Quarato *et al.* 2021). Determining whether germ granules in other animals confer a similar mRNA surveillance system to retain an epigenetic memory of germline expression will be a critical next step.

## Organization and function of *Mutator* foci

### Overview of *Mutator* foci

The term "mutator" was first used to describe spontaneous mutations caused by insertions of the transposon Tc1 (Eide and Anderson 1985; Collins *et al.* 1987). Later experiments linked the phenomenon of transposon activation to the disruption of RNA interference pathways via two parallel genetic screens, one for defects in RNAi and the other for germline mobilization of transposons (Ketting *et al.* 1999; Tabara *et al.* 1999b). The overlap of these two screens provided some of the first evidence that the RNAi pathway is required for transposon silencing. Since then, nearly a dozen *mutator* genes have been identified, with their protein products found to interact to form the *mutator* complex and function in the production of WAGO 22G-RNAs and ERGO-1 26G-RNAs (see Table 1; Ketting *et al.* 1999; Tabara *et al.* 1999b; Tijsterman *et al.* 2002; Vastenhouw *et al.* 2003; Tops *et al.* 2005; Chen *et al.* 2005; Grishok *et al.* 2005; Robert *et al.* 2005; Kim *et al.* 2005; Phillips *et al.* 2012; Tsai *et al.* 2015). Consequently, loss of the *mutator* complex results in defects in the production of WAGO 22G-RNAs and ERGO-1 26G-RNAs but not other classes of small RNAs such as piRNAs, microRNAs (miRNAs), CSR-1 22G-RNAs, and ALG-3/4 26G- and 22G-RNAs (Gu *et al.* 2009; Zhang *et al.* 2011; Lee *et al.* 2012; Phillips *et al.* 2014; Tsai *et al.* 2015). Interestingly, the loss of ERGO-1 26G-RNAs in *mutator* mutants can be attributed to the disruption of a homeostatic feedback loop mediated by *mutator*-dependent 22G-RNAs at the *eri-6/7* gene locus rather than the direct involvement of the *mutator* proteins in ERGO-1 26G-RNA biogenesis (Rogers and Phillips 2020b). Therefore, the *mutator* complex is thought to function primarily for the amplification of WAGO 22G-RNAs through the activity of RdRPs.

Mutations in genes from this group not only have active germline transposition and defects in exogenous RNAi, but they also are temperature-sensitive sterile and have more male progeny, suggestive of chromosome segregation defects (Ketting *et al.* 1999; Tabara *et al.* 1999b; Zhang *et al.* 2011; Wallis *et al.* 2019). The fertility defects at elevated temperature are present in spermatogenic and oogenic cells, though more severe during spermatogenesis, manifesting within a single generation at elevated temperature (Rogers and Phillips 2020a). In contrast, the oogenic defect accumulates over several generations, becoming progressively sterile over 2–3 generations at elevated temperature (Rogers and Phillips 2020a). While the spermatogenic defect has not been fully characterized, the oogenic defect can be attributed to changes in germline chromatin accessibility resulting in the aberrant expression of somatic and spermatogenic genes in oogenic nuclei (Rogers and Phillips 2020a).

### Composition and assembly of *Mutator* foci

Assembly of *mutator* complex and accumulation of the *mutator* complex into visible perinuclear germline foci, referred to as *Mutator* foci, depend on the scaffolding properties of MUT-16 (Figures 1F and 2, A and C; Phillips *et al.* 2012; Uebel *et al.* 2018). Both structured and unstructured/disordered regions of MUT-16 are required for *Mutator* foci assembly and for the recruitment of other *mutator* complex members (Uebel *et al.* 2018). Specifically, the most C-terminal region of MUT-16 (~275 amino acids), is both necessary and sufficient for the germline *Mutator* foci

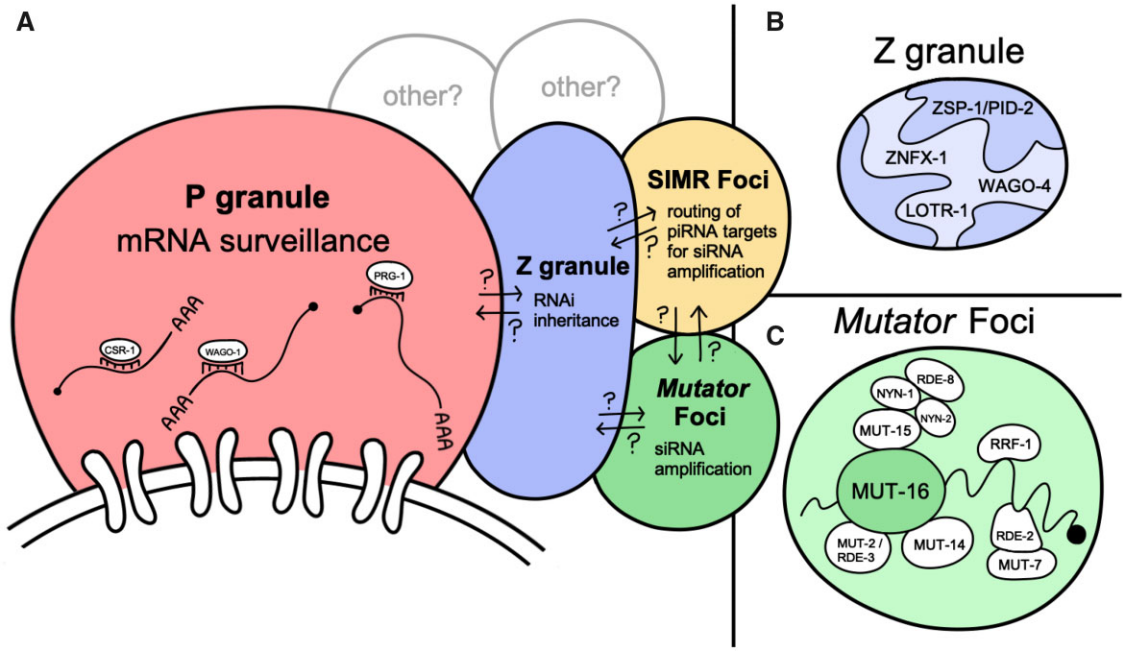


**Figure 2** Physical and functional compartmentalization of germ granules. (A) P granules are positioned to receive and scan nascent transcripts as they exit the nucleus. This is accomplished through CSR-1, PRG-1, and WAGO-1 Argonautes and their associated small RNAs, which can distinguish foreign transcripts from those licensed for germline expression. Z granules promote amplification of siRNAs for memory storage and transgenerational silencing. SIMR foci route piRNA targets for siRNA amplification. *Mutator* foci are sites of siRNA amplification. The trajectory of small RNAs and mRNAs through the granules is still unknown and possible routes are indicated by arrows. (B) Substructure within Z granules is mediated through ZSP-1/PID-2. (C) *Mutator* foci are nucleated by MUT-16, which contains both structured and disordered regions. Additional *mutator* complex proteins are recruited to *Mutator* foci through either direct or indirect interactions with MUT-16.

assembly (Phillips *et al.* 2012). MUT-2/RDE-3, a nucleotidyltransferase that adds untemplated poly(UG) tails (pUG tails) to the 3′ termini of cleaved siRNA-targeted mRNAs, is recruited to the complex through a structured region near the N-termius of MUT-16 (Chen *et al.* 2005; Phillips *et al.* 2012; Uebel *et al.* 2018; Shukla *et al.* 2020). The same region also recruits a DEAD-box helicase MUT-14, which acts redundantly with its paralog SMUT-1 in the initiation of WAGO 22G-RNA amplification (Tijsterman *et al.* 2002; Phillips *et al.* 2014), and MUT-15, a protein with no known domains that recruits NYN-1 and NYN-2 to *Mutator* foci (Uebel *et al.* 2018). NYN-1 and NYN-2, two NYN-domain proteins, in turn recruit to *Mutator* foci the NYN-domain endoribonuclease RDE-8, which associates with MUT-2/RDE-3 and cleaves siRNA-targeted mRNAs for MUT-2 pUGylation (Tsai *et al.* 2015; Uebel *et al.* 2018).

The disordered central region of MUT-16 recruits the RdRP RRF-1 and RDE-2, a protein with no known domains, which then recruit the 3′–5′ exonuclease MUT-7 to *Mutator* foci (Ketting *et al.* 1999; Tops *et al.* 2005; Phillips *et al.* 2012; Uebel *et al.* 2018). The RNAs targeted by the MUT-7 exonuclease are currently unknown. It is also worth noting that the RdRPs EGO-1 and RRF-1 are redundantly required for *mutator* complex-dependent small RNA amplification; however, only RRF-1 localizes to *Mutator* foci and co-IPs with *mutator* complex proteins (Gu *et al.* 2009; Phillips *et al.* 2012; Manage *et al.* 2020). Perhaps the distinct localization of the two RdRPs can be attributed to their activity in two distinct 22G-RNA pathways: EGO-1 is also required for biogenesis of CSR-1 22G-RNAs (Claycomb *et al.* 2009), suggesting that EGO-1 may act primarily with CSR-1 in P granules or the cytoplasm for CSR-1 22G-RNA biogenesis, whereas RRF-1 acts with the *mutator* complex for WAGO 22G-RNA biogenesis. Perhaps then, only in the absence of RRF-1, EGO-1 can compensate for the loss by also generating WAGO 22G-RNAs with the *Mutator* complex. Despite the distinction, both RdRPs, EGO-1 and RRF-1, and MUT-16 co-IP with the Dicer-related helicase DRH-3 and the Tudor-domain protein EKL-1 (Gu *et al.* 2009; Thivierge *et al.* 2011; Manage *et al.* 2020). DRH-3 and EKL-1 are required for both CSR-1 22G-RNA and WAGO 22G-RNA biogenesis making them likely members of the *mutator* complex (Claycomb *et al.* 2009; Gu *et al.* 2009), though it has not been demonstrated that they colocalize with *mutator* complex proteins at *Mutator* foci.

## Regulation of *Mutator* foci

Several variables have been shown to modulate the presence and intensity of *Mutator* foci. Early observations of *Mutator* foci indicated that they are present in germ cells but not somatic cells, which was initially perplexing due to the necessity of the *mutator* complex to promote RNAi and WAGO 22G-RNA production in somatic cells (Chen *et al.* 2005; Kim *et al.* 2005; Gent *et al.* 2010; Phillips *et al.* 2012; Uebel *et al.* 2018). Interestingly, increasing the concentration of MUT-16 in somatic cells can lead to the formation of ectopic *Mutator* foci that are capable of nucleating other *mutator* complex proteins (Uebel *et al.* 2018). This result suggests that somatic cells are not lacking any factors required for *Mutator* foci formation, but rather that somatic cells do not possess MUT-16 at high enough concentration to nucleate visible *Mutator* foci. In addition to protein concentration, *Mutator* foci are also regulated by environmental temperature, as elevated temperatures promote their dissolution (Uebel *et al.* 2018). Examination of *Mutator* foci suggested that their intensity is greatest in the mitotic region, transition zone, and late pachytene/diplotene (Phillips *et al.* 2012; Uebel *et al.* 2020). This association of foci intensity with germ cell progression can be monitored through the examination of mitotic and meiotic mutants, demonstrating that

extended regions of mitosis but not the transition zone can expand the region of intense *Mutator* foci and establishing that foci intensity is modulated by germline cell cycle stage (Uebel *et al.* 2020). Finally, similar to work demonstrating that RNA is integral to P-granule stability (Sheth *et al.* 2010), injection of the transcriptional inhibitor α-amanitin results in dispersal of *Mutator* foci and indicating that RNA is a critical component of this granule (Uebel *et al.* 2020).

## Organization and function of Z granules

Z granules are defined by the localization of ZNFX-1, a superfamily one (SF1) RNA helicase and zinc-finger domain protein, and WAGO-4, a WAGO-clade Argonaute protein (Ishidate *et al.* 2018; Wan *et al.* 2018). Loss of ZNFX-1 and WAGO-4 disrupt RNAi inheritance from one generation to the next but do not disable the response to double-stranded RNA within a single generation (Ishidate *et al.* 2018; Wan *et al.* 2018; Xu *et al.* 2018). Curiously, in a transgene expression assay, mutations in *znfx-1* do not simply disrupt the inheritance of silencing signals but rather alter the stability of transgene expression, with the transgenes switching back and forth between silencing and expressed states across generations (Ishidate *et al.* 2018). Furthermore, ZNFX-1 co-immunoprecipitates with Argonaute proteins in activating and silencing pathways, including PRG-1, WAGO-1, and CSR-1 (Ishidate *et al.* 2018). These data suggest that Z granules may not solely be required for RNAi inheritance of silencing signals but instead for the inheritance of both expressed and silent epigenetic states.

There is some discrepancy regarding the small RNA that associates with WAGO-4, with separate reports indicating that it primarily associates with CSR-1 22G-RNAs or WAGO 22G-RNAs (Xu *et al.* 2018; Wan *et al.* 2021). However, these data, along with data indicating that WAGO-4 binds small RNAs antisense to genes targeted by exogenous RNAi, suggest that, like ZNFX-1, WAGO-4 may act with both activating and silencing small RNA pathways (Xu *et al.* 2018). Yet, precisely how ZNFX-1 and WAGO-4 promote balanced epigenetic inheritance is still unclear. One clue comes from the sequencing of small RNAs in a *znfx-1* mutant. This mutant shows a shift in the distribution of both CSR-1 and WAGO 22G-RNAs across target mRNAs and toward the 5′ end, indicating that one role for ZNFX-1 may be the redistribution of RdRPs from the 5′ end to the 3′ end of target mRNAs (Ishidate *et al.* 2018). Another Z-granule protein called LOTR-1 contributes to this function, as *lotr-1* mutants exhibit a similar loss of small RNAs from the 3′ ends of WAGO and *mutator* targets and impacts transgenerational RNAi inheritance (Marnik *et al.* 2021). The Tudor domain of LOTR-1 positions ZNFX-1 and LOTR-1 in perinuclear Z granules, while the LOTUS domain of LOTR-1 appears to associate with cytoskeletal and 3′UTR-binding components. While the LOTUS-domain proteins MIP-1 and MIP-2 interact with the helicase GLH-1 to help nucleate P-granule assembly, LOTR-1 functions similarly by interacting with the helicase ZNFX-1 to help nucleate Z-granule assembly (Marnik *et al.* 2021; Cipriani *et al.* 2021).

More recently, PID-2/ZSP-1, a protein with intrinsically disordered regions (IDRs), has been shown to localize to the surface of Z granules where it regulates Z-granule size, number, and fluidity (Figure 2B; Placentino *et al.* 2021; Wan *et al.* 2021). PID-2/ZSP-1 is required for germline RNAi and heritable silencing downstream of piRNAs, and, similarly to *znfx-1*, mutations in *pid-2/zsp-1* display only modest alterations in overall small RNA levels or levels of small RNAs mapping to previously defined categories of 22G-RNAs (Ishidate *et al.* 2018; Placentino *et al.* 2021; Wan *et al.* 2021). Furthermore, like *znfx-1*, *pid-2/zsp-1* mutants alter the distribution of both WAGO 22G-RNAs and CSR-1 22G-RNAs along mRNA targets (Ishidate *et al.* 2018; Placentino *et al.* 2021). However, *pid-2/zsp-1* mutants have reduced small RNAs at the 5′ end of target mRNA transcripts, which is opposite to the effect of a *znfx-1* mutation and suggests a possible role for PID-2/ZSP-1 in the processivity of the RdRPs (Placentino *et al.* 2021). Nonetheless, the fact that most small RNA target genes still produce small RNAs, including germline genes targeted by RNAi, yet *pid-2/zsp-1* mutants have strong desilencing of a piRNA sensor and fail to silence RNAi-targeted germline mRNAs, suggests that PID-2/ZSP-1 may somehow fail to couple siRNA production to gene silencing (Placentino *et al.* 2021; Wan *et al.* 2021). Altogether, there are clearly still questions to be answered regarding the precise role Z granules play in RNA silencing and RNA inheritance.

## Organization and function of SIMR foci

SIMR-1, an extended Tudor domain protein, was initially identified through a MUT-16 immunoprecipitation followed by mass spectrometry of interacting proteins (Manage *et al.* 2020). Loss of *simr-1* has no RNAi-defective (Rde) or Enhanced RNAi (Eri) phenotypes, though the mutation causes a depletion of piRNA-dependent but not piRNA-independent *Mutator* targets. Further, *simr-1* mutants can desilence a piRNA sensor and prevent sterility after reestablishing WAGO-class 22G-RNA production, a phenotype previously only associated with piRNA pathway mutants (de Albuquerque *et al.* 2015; Phillips *et al.* 2015; Manage *et al.* 2020). The piRNAs themselves are unaffected in a *simr-1* mutant, so together, this data suggests that SIMR-1 acts in the piRNA pathway, downstream of piRNA biogenesis but upstream of the *mutator* complex. Interestingly, SIMR-1 also forms germline foci, but these foci are discrete from P granules, Z granules, and *Mutator* foci (Figures 1G and 2A; Manage *et al.* 2020). Rather, SIMR-1 colocalizes with RSD-2, a factor required for exogenous RNAi introduced at low doses and production of 22G-RNAs at ERGO-1 target mRNAs (Tijsterman *et al.* 2004; Han *et al.* 2008; Zhang *et al.* 2012; Sakaguchi *et al.* 2014) and that has no known association with the piRNA pathway. Thus, the current hypothesis is that the SIMR-1 foci function to promote interactions between primary and secondary small RNA pathways, RSD-2 for exogenous RNAi and ERGO-1 target genes and SIMR-1 for piRNA target genes. Also, likely co-localizing with SIMR-1 and RSD-2 is the SIMR-1 paralog, HPO-40. While no function has yet been attributed to HPO-40, it has a similar localization pattern as SIMR-1 and fails to colocalize completely with MUT-16 (Manage *et al.* 2020). Since no other components of the SIMR foci are currently known, further study will be necessary to reveal the molecular details of this compartment.

## Interactions between germ-granule compartments

The current model of germ granule organization in the *C. elegans* germline proposes sequential stacking of P granules, Z granules, and *Mutator* foci at the nuclear periphery (Figure 2A; Wan *et al.* 2018). SIMR foci are also found in tripartite structures, adjacent to Z granules and opposite P granules (Manage *et al.* 2020), however the orientation of all four condensates relative to one another is still undetermined. Nonetheless, the consistency of which these granules are found in this stacked organization suggests that the interaction between condensates may promote efficient small RNA-based silencing and inheritance. In the germline progenitor cells of early embryos, Z-granule proteins,

ZNFX-1 and WAGO-4, co-localize to P granules rather than forming discrete structures; however, after the 100-cell stage of embryonic development, the Z granules demix into discrete condensates (Figure 1C; Wan *et al.* 2018). Similarly, the *Mutator* foci and SIMR foci are first observed as robust granules around the 100-cell stage of embryonic development in the Z2/Z3 progenitor germ cells, but unlike Z granules, the *Mutator* foci and SIMR foci appear to nucleate *de novo* from cytoplasmically localized proteins rather than through demixing of proteins from the P granule (Figure 1D; Uebel *et al.* 2018, 2021; Wan *et al.* 2018). These events roughly coincide with initiation of transcription from embryonic germ cells, suggesting that the presence of newly synthesized mRNAs traversing the nuclear pores and their recognition by various small RNA pathway proteins may drive changes in germline condensate morphology (Seydoux and Dunn 1997; Wan *et al.* 2018).

Based on their protein composition, *Mutator* foci are considered hubs of WAGO 22G-RNA biogenesis, positioned near P granules and nuclear pores to capture recently-transcribed target mRNAs for small RNA amplification. Simultaneous RNAi knockdown of four core components of the P granule is sufficient to disrupt *Mutator* foci formation, while *Mutator* foci are not required for P-granule assembly (Phillips *et al.* 2012; Singh *et al.* 2021). SIMR foci also do not require *Mutator* foci for formation and, reciprocally, *Mutator* foci do not require SIMR foci; however, interactions between SIMR foci and other germ-granule compartments have not been thoroughly tested (Manage *et al.* 2020). Similar to *Mutator* foci, ZNFX-1 appears to require P granules for localization, as *csr-1* and *glh-1* mutants, which disrupt P granules, also disrupt ZNFX-1 localization (Ishidate *et al.* 2018). Interestingly, loss of the P-granule protein DEPS-1, alters P-granule, Z-granule and *Mutator* foci morphology, while loss of *mutator* complex proteins disrupt DEPS-1 localization, suggesting there may be a more complex interplay between condensates (Wan *et al.* 2018; Suen *et al.* 2020).

Similar to P granules, Z granules and *Mutator* foci have properties associated with phase-separated condensates, canonically characterized by rapid internal recovery after photobleaching (Brangwynne *et al.* 2009; Uebel *et al.* 2018; Wan *et al.* 2018). Furthermore, *Mutator* foci assemble after reaching a critical concentration threshold, which may explain their presence in germ cells but not somatic cells. They are disrupted by elevated temperature and, like P granules, they dissolve in low concentrations of an aliphatic alcohol that is thought to only affect weak hydrophobic interactions (Updike *et al.* 2011; Uebel *et al.* 2018). These liquid-like properties may allow a continuation of RNA exchange through distinct granule compartments, coordinating RNA silencing between granules.

## Proteins and RNAs not yet assigned to specific germ-granule compartments

While the above-described sub-granules constitute the most well-characterized of the germ-granule compartments, several other proteins have been associated with germ granules but whether they form distinct compartments or overlap with a known compartment is unclear. For example, the Tudor domain protein RSD-6 localizes to foci near P granules referred to as R2 bodies (Yang *et al.* 2014). RSD-6, along with the DEAD box helicase RDE-12, associate with mRNAs targeted by RNAi and are required for production of secondary siRNAs at ERGO-1 and exogenous RNAi targets (Zhang *et al.* 2012; Shirayama *et al.* 2014; Yang *et al.* 2014). Interestingly RDE-12 localizes to both the R2 bodies and to P granules, possibly shuttling between the two; it has been proposed that RDE-12 may carry primary siRNA-targeted mRNAs from P granules to R2 bodies for *mutator*-dependent siRNA amplification (Yang *et al.* 2014). Given the overlap in phenotypes and likely function between *rsd-2*, in SIMR foci, with *rsd-6* and *rde-12*, in R2 bodies, a likely conclusion is that SIMR foci and R2 bodies are one in the same; however, this inference has yet to be proven.

Another example of a germ granule-associated protein which has not been precisely localized to one of the known germ-granule compartments is FBF-1. FBF-1, and its paralog FBF-2, are PUF-family RNA binding proteins that are required in germline stem cells to silence the expression of mRNAs required for meiosis (Voronina *et al.* 2012). While FBF-2 seems to associate with P granules, FBF-1 forms both cytoplasmic and perinuclear foci, the majority of which do not overlap with P granules though many are immediately adjacent (Voronina *et al.* 2012). Two additional PUF-family proteins, PUF-3 and PUF-11, in combination with FBF-1 and FBF-2, are required for germline proliferation and have been localized to unidentified cytoplasmic and perinuclear foci germline foci (Haupt *et al.* 2020).

Similarly, the KH protein MINA-1, identified in a screen for apoptosis regulators, binds the 3′UTRs of genes associated with germ cell development and localizes to a germ-granule compartment adjacent to P granules (Sendoel *et al.* 2019). Curiously, MINA-1 down-regulates the Z-granule Argonaute protein WAGO-4, and loss of *mina-1* leads to enhanced exogenous RNAi (Sendoel *et al.* 2019). Further work will be necessary to uncover the precise role of MINA-1 in small RNA and gene regulatory pathways.

Finally, in addition to proteins that have yet to be localized to specific germ granules, mRNAs have also been localized to the perinuclear germ-granule regions. Specifically, mRNAs are differentially regulated by miRNAs in germ cells compared to somatic cells, where germline miRNA-targeted mRNAs are stabilized while somatic miRNA-targeted mRNAs are destabilized (Dallaire *et al.* 2018). These miRNA-targeted mRNAs localize adjacent to P granules dependent on GLH-1, but not to any specific, known compartment (Dallaire *et al.* 2018). Thus, the localization of RSD-6, FBF-1, PUF-3, PUF-11, MINA-1, and miRNA-targeted mRNAs to undetermined perinuclear germ-granule compartments will necessitate further co-localization studies to determine whether these proteins are components of known germ-granule compartments or whether they are the defining members of a newly identified compartments.

## Germ granules likely collaborate in heritable gene silencing

### Overview of transgenerational inheritance

In many eukaryotes, including *C. elegans*, endogenous siRNAs can transmit epigenetic information, including responses to environmental stress, from parents to offspring (Rechavi and Lev 2017). When siRNAs carried by either egg or sperm are deposited into the embryo, they are amplified through the activity of RdRPs. mRNA targets of the deposited siRNAs become RdRP templates, leading to further production of abundant secondary siRNAs and enhanced silencing. Loss of many RNAi pathway and germ-granule components lead to a germline mortal (Mrt) phenotype, where the animals become progressively less fertile over generations (Buckley *et al.* 2012; Simon *et al.* 2014; Sakaguchi *et al.* 2014; Spracklin *et al.* 2017; Ishidate *et al.* 2018; Wan *et al.* 2018, 2021; Manage *et al.* 2020; Placentino *et al.* 2021). This phenotype hints at the possibility that sterility arises from changes in germ cell gene

expression that become exacerbated by the transgenerational small RNA amplification cycle.

### Phenotypic variation can depend on parental or ancestral genotype

It has been observed that mutations in multiple germ-granule components, including *pgl-1*, *glh-1*, and *meg-3; meg-4*, are either RNAi defective or RNAi inheritance defective (Robert *et al.* 2005; Spike *et al.* 2008b; Wang *et al.* 2014; Spracklin *et al.* 2017; Ouyang *et al.* 2019; Dodson and Kennedy 2019). The implication being that germ granules are essential for RNAi. However, this RNAi-defective phenotype can be uncoupled, or "transgenerationally disconnected," from the germ granule-defective genotype. For example, wild-type animals *with meg-3; meg-4* mutant ancestors can exhibit RNAi defects and *meg-3; meg-4* mutants from wild-type ancestors can exhibit a wild-type RNAi response (Lev *et al.* 2019; Ouyang *et al.* 2019; Dodson and Kennedy 2019). This phenotypic disconnect can last >8 generations, indicating that loss of germ granules can have transgenerational repercussions. It is worth noting, however, that the expected coupling of phenotype and genotype ultimately returns, suggesting the information needed to coordinate small RNA gene silencing is genome-encoded (Dodson and Kennedy 2019).

### P granules and piRNAs coordinate siRNA biogenesis and transgenerational inheritance

If embryonic P granules are not required, per se, for germline RNAi, what role do they play in coordinating siRNA production and RNA silencing (Dodson and Kennedy 2019)? P granules house multiple RNAi pathway proteins, including Argonaute proteins and RdRPs, and in their absence, aberrant siRNAs are generated at some genes while siRNAs are lost at others. Changes in small RNA poly-uridylation have also been observed, which could affect small RNA stability or sorting into distinct Argonaute proteins, ultimately altering RNA silencing efficacy (van Wolfswinkel *et al.* 2009; Xu *et al.* 2018; Lev *et al.* 2019). Some of the aberrantly targeted genes include genes required for RNAi. For example, *rde-4* mRNA expression is reduced when P granules are dispersed in *deps-1* mutants (Spike *et al.* 2008a). In addition, *sid-1* and *rde-11* have increased small RNAs and reduced mRNA expression when embryonic P granules are dispersed in *meg-3; meg-4* double mutants, presumably reducing RNAi's efficacy in this mutant (Ouyang *et al.* 2019; Dodson and Kennedy 2019). Curiously, the misexpressed small RNAs tend to be very consistent between replicates and experiments, leading to the question of why specific genes are mistargeted and if certain sequences or features drive aberrant small RNA targeting following the loss of germ granules. One clue comes from the fact that mutants in *prg-1*, as well as transgenerational RNA silencing mutants *hrde-1* and *znfx-1*, can restore RNAi competence to *meg-3; meg-4* mutants (Ouyang *et al.* 2019). Both *sid-1* and *rde-11* appear to be direct targets of PRG-1, as their siRNA levels are reduced and their mRNA expression increased in a *prg-1* mutant (McMurchy *et al.* 2017; Shen *et al.* 2018; Ouyang *et al.* 2019). This comes as a surprise, as these genes are expressed in wild-type animals and required for an effective response to exogenous RNAi; however, the findings invoke a "safe harbor" model where P granules can protect some transcripts from transgenerational piRNA-mediated silencing (Ouyang *et al.* 2019).

While it is generally understood that piRNAs promote siRNA-mediated gene silencing, it may not be that simple. For example, PRG-1 is required maternally to coordinate siRNA biogenesis and prevent siRNA-mediated silencing of essential genes (de Albuquerque *et al.* 2015; Phillips *et al.* 2015). In fact, following the loss of PRG-1 and piRNAs, siRNA-mediated silencing of the rDNA locus directly contributes to transgenerational sterility (Wahba *et al.* 2021), while siRNA-mediated silencing at histone genes can become permanent (Barucci *et al.* 2020; Reed *et al.* 2020; Shukla *et al.* 2021). While the signal that predisposes some genes to silencing in *prg-1* mutants is unknown, it has been hypothesized that the lack of a poly(A) tail common in histone transcripts and rRNAs (Montgomery *et al.* 2020; Reed *et al.* 2020), or the susceptibility of transcripts to perpetual poly-UG (pUG) RNA/siRNA cycling (Shukla *et al.* 2020, 2021; Wahba *et al.* 2021), could make specific genes more permissive for siRNA biogenesis. Like the "safe harbor" model above, PRG-1 targeted transcripts may be protected from permanent RDE-3-mediated siRNA silencing of *Mutator* foci if they are sequestered within P granules and away from RDE-3 silencing in *Mutator* foci (Shukla *et al.* 2021). As these details are worked out, the roles of germ-granule demixing are sure to become more apparent.

## Outlook and outstanding questions

The occurrence of germ-granule demixing into distinct sub-granules leaves a number of questions unanswered. To start with, what are the events in germline blastomeres that trigger demixing? Is demixing a passive phenomenon caused by the localized synthesis of particular small RNAs, the clustering of similar RNA modifications and their binding proteins, or the condensation of phases similar to those that partition nucleolar sub-compartments (Feric *et al.* 2016; Lafontaine *et al.* 2021)? Instead, is demixing an active process that requires energy metabolism and the cytoskeleton? Is there directionality or progression from the emergence of one sub-granule to the next? Then once formed, how is the separation of these compartments maintained?

Determining what constitutes a P granule, Z granule, *Mutator* focus, or SIMR focus, their upper and lower size limits, and how each are defined is left to an investigator's discretion. This requires us to ask whether distinct compositions of germ-granule compartments exist and whether this indicates functional specialization. At any given time are the RNA and protein constituents of the individual sub-granules the same, and if not, does the association, loss, or exchange of RNAs and proteins constitute a new compartment with distinct functions? If the answer is yes, it will be important to understand the extent that proteins are shared across sub-granules, and if proteins moving between compartments are trafficking RNAs or other substrates from one location to the next. If so, what then is the trajectory of RNAs between sub-granules? Does this trajectory reflect changing functions for these RNAs, or an assembly line for their progressive modifications? And then, finally, how are these activities coordinated to ensure germline integrity? The answer to these and other questions should be attainable with further study and the increasing availability of new tools and improved imaging resolution.

## Data availability

The authors state that all data necessary for confirming the conclusions presented in the manuscript are represented fully within the manuscript.

## Conflicts of interest

The authors declare that there is no conflict of interest. Conclusions, implications and opinions expressed within the content are solely the authors.

## Literature cited


Al-Amin M, Min H, Shim Y-H, Kawasaki I. 2016. Somatically expressed germ-granule components, PGL-1 and PGL-3, repress programmed cell death in *C. elegans*. Sci Rep. 6:33884.doi:10.1038/srep33884.

Amiri A, Keiper BD, Kawasaki I, Fan Y, Kohara Y, *et al.* 2001. An isoform of eIF4E is a component of germ granules and is required for spermatogenesis in *C. elegans*. Development. 128:3899–3912.

Andralojc KM, Campbell AC, Kelly AL, Terrey M, Tanner PC, *et al.* 2017. ELLI-1, a novel germline protein, modulates RNAi activity and P-granule accumulation in *Caenorhabditis elegans*. PLoS Genet. 13:e1006611.doi:10.1371/journal.pgen.1006611.

Aoki ST, Kershner AM, Bingman CA, Wickens M, Kimble J. 2016. PGL germ granule assembly protein is a base-specific, single-stranded RNase. Proc Natl Acad Sci U S A. 113:1279–1284. doi:10.1073/pnas.1524400113.

Aoki ST, Lynch TR, Crittenden SL, Bingman CA, Wickens M, *et al.* 2021. *C. elegans* germ granules require both assembly and localized regulators for mRNA repression. Nat Commun. 12:996. doi:10.1038/s41467-021–21278-1.

Ariz M, Mainpal R, Subramaniam K. 2009. *C. elegans* RNA-binding proteins PUF-8 and MEX-3 function redundantly to promote germline stem cell mitosis. Dev Biol. 326:295–304. doi:10.1016/j.ydbio.2008.11.024.

Ashe A, Sapetschnig A, Weick E-M, Mitchell J, Bagijn MP, *et al.* 2012. piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. Cell. 150:88–99. doi:10.1016/j.cell.2012.06.018.

Audhya A, Hyndman F, McLeod IX, Maddox AS, Yates JR, *et al.* 2005. A complex containing the Sm protein CAR-1 and the RNA helicase CGH-1 is required for embryonic cytokinesis in *Caenorhabditis elegans*. J Cell Biol. 171:267–279. doi:10.1083/jcb.200506124.

Bagijn MP, Goldstein LD, Sapetschnig A, Weick E-M, Bouasker S, *et al.* 2012. Function, targets, and evolution of *Caenorhabditis elegans* piRNAs. Science. 337:574–578. doi:10.1126/science.1220952.

Barbee SA, Lublin AL, Evans TC. 2002. A novel function for the Sm proteins in germ granule localization during *C. elegans* embryogenesis. Curr Biol. 12:1502–1506. doi:10.1016/S0960-9822(02)01111-9.[pii]

Barucci G, Cornes E, Singh M, Li B, Ugolini M, *et al.* 2020. Small-RNA-mediated transgenerational silencing of histone genes impairs fertility in piRNA mutants. Nat Cell Biol. 22:235–245. doi:10.1038/s41556-020–0462-7.

Batista PJ, Ruby JG, Claycomb JM, Chiang R, Fahlgren N, *et al.* 2008. PRG-1 and 21U-RNAs interact to form the piRNA complex required for fertility in *C. elegans*. Mol Cell. 31:67–78. doi:10.1016/j.molcel.2008.06.002.

Beshore EL, McEwen TJ, Jud MC, Marshall JK, Schisa JA, *et al.* 2011. *C. elegans* Dicer interacts with the P-granule component GLH-1 and both regulate germline RNPs. Dev Biol. 350:370–381. doi:10.1016/j.ydbio.2010.12.005.

Billi AC, Alessi AF, Khivansara V, Han T, Freeberg M, *et al.* 2012. The *Caenorhabditis elegans* HEN1 ortholog, HENN-1, methylates and stabilizes select subclasses of germline small RNAs. PLoS Genet. 8:e1002617.doi:10.1371/journal.pgen.1002617.

Boag PR, Nakamura A, Blackwell TK. 2005. A conserved RNA-protein complex component involved in physiological germline apoptosis regulation in *C. elegans*. Development. 132:4975–4986. doi:10.1242/dev.02060.

Brangwynne CP, Eckmann CR, Courson DS, Rybarska A, Hoege C, *et al.* 2009. Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science. 324:1729–1732. doi:10.1126/science.1172046.

Brown KC, Svendsen JM, Tucci RM, Montgomery BE, Montgomery TA. 2017. ALG-5 is a miRNA-associated Argonaute required for proper developmental timing in the *Caenorhabditis elegans* germline. Nucleic Acids Res. 45:9093–9107. doi:10.1093/nar/gkx536.

Buckley BA, Burkhart KB, Gu SG, Spracklin G, Kershner A, *et al.* 2012. A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality. Nature. 489:447–451. doi:10.1038/nature11352.

Campbell AC, Updike DL. 2015. CSR-1 and P granules suppress sperm-specific transcription in the *C. elegans* germline. Development. 142:1745–1755. doi:10.1242/dev.121434.

Cecere G, Hoersch S, O'Keeffe S, Sachidanandam R, Grishok A. 2014. Global effects of the CSR-1 RNA interference pathway on the transcriptional landscape. NatStruct Mol Biol. 21:358–65. 10.1038/nsmb.2801. 24681887.

Charlesworth AG, Seroussi U, Lehrbach NJ, Renaud MS, Sundby AE, et al. 2021. Two isoforms of the essential C. elegans Argonaute CSR-1 differentially regulate sperm and oocyte fertility. Nucleic Acids Res. 49:8836–8865. 10.1093/nar/gkab619. 34329465.

Chen C-CG, Simard MJ, Tabara H, Brownell DR, McCollough JA, *et al.* 2005. A member of the polymerase beta nucleotidyltransferase superfamily is required for RNA interference in *C. elegans*. Curr Biol. 15:378–383. doi:10.1016/j.cub.2005.01.009.

Chen J-X, Cipriani PG, Mecenas D, Polanowska J, Piano F, *et al.* 2016. In vivo interaction proteomics in *Caenorhabditis elegans* embryos provides new insights into P granule dynamics. Mol Cell Proteom. 15:1642–1657. doi:10.1074/mcp.M115.053975.

Chen W, Hu Y, Lang CF, Brown JS, Schwabach S, *et al.* 2020. The dynamics of P granule liquid droplets are regulated by the *Caenorhabditis elegans* germline RNA helicase GLH-1 via its ATP hydrolysis cycle. Genetics. 215:421–434. doi:10.1534/genetics.120.303052.

Ciosk R, DePalma M, Priess JR. 2006. Translational regulators maintain totipotency in the *Caenorhabditis elegans* germline. Science. 311:851–853. doi:10.1126/science.1122491.

Cipriani PG, Bay O, Zinno J, Gutwein M, Gan HH, *et al.* 2021. Novel LOTUS-domain proteins are organizational hubs that recruit *C. elegans* Vasa to germ granules. Elife. 10:e60833. doi:10.7554/eLife.60833.

Claycomb JM, Batista PJ, Pang KM, Gu W, Vasale JJ, *et al.* 2009. The Argonaute CSR-1 and its 22G-RNA cofactors are required for holocentric chromosome segregation. Cell. 139:123–134. doi:10.1016/j.cell.2009.09.014.

Collins J, Saari B, Anderson P. 1987. Activation of a transposable element in the germ line but not the soma of *Caenorhabditis elegans*. Nature. 328:726–728. doi:10.1038/328726a0.

Conine CC, Batista PJ, Gu W, Claycomb JM, Chaves DA, *et al.* 2010. Argonautes ALG-3 and ALG-4 are required for spermatogenesis-specific 26G-RNAs and thermotolerant sperm in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A. 107:3588–3593. doi:10.1073/pnas.0911685107.

Conine CC, Moresco JJ, Gu W, Shirayama M, Conte D, *et al.* 2013. Argonautes promote male fertility and provide a paternal memory of germline gene expression in *C. elegans*. Cell. 155:1532–1544. doi:10.1016/j.cell.2013.11.032.

Cordeiro Rodrigues RJ, de Jesus Domingues AM, Hellmann S, Dietz S, de Albuquerque BFM, *et al.* 2019. PETISCO is a novel protein

complex required for 21U RNA biogenesis and embryonic viability. Genes Dev. 33:857–870. doi:10.1101/gad.322446.118.

Dallaire A, Frédérick P-M, Simard MJ. 2018. Somatic and germline microRNAs form distinct silencing complexes to regulate their target MRNAs differently. Dev Cell. 47:239–247.e4. doi: 10.1016/j.devcel.2018.08.022.

de Albuquerque BFM, Placentino M, Ketting RF. 2015. Maternal piRNAs are essential for germline development following de novo establishment of endo-siRNAs in *Caenorhabditis elegans*. Dev Cell. 1–9. doi:10.1016/j.devcel.2015.07.010.

Dodson AE, Kennedy S. 2019. Germ granules coordinate RNA-based epigenetic inheritance pathways. Dev Cell. 50:704–715.e4. doi: 10.1016/j.devcel.2019.07.025.

Draper BW, Mello CC, Bowerman B, Hardin J, Priess JR. 1996. MEX-3 is a KH domain protein that regulates blastomere identity in early *C. elegans* embryos. Cell. 87:205–216. doi: 10.1016/S0092-8674(00)81339-2.[pii]

Eckmann CR, Kraemer B, Wickens M, Kimble J. 2002. GLD-3, a bicaudal-C homolog that inhibits FBF to control germline sex determination in *C. elegans*. Dev Cell. 3:697–710. doi:S1534580702003222.[pii]

Eddy EM. 1975. Germ plasm and the differentiation of the germ cell line. Int Rev Cytol. 43:229–280. doi:10.1016/s0074-7696(08)60070-4.

Eide D, Anderson P. 1985. Transposition of Tc1 in the nematode *Caenorhabditis elegans*. Proc Natl Acad Sci U S A. 82:1756–1760. doi: 10.1073/pnas.82.6.1756.

Feric M, Vaidya N, Harmon TS, Mitrea DM, Zhu L, *et al.* 2016. Coexisting liquid phases underlie nucleolar subcompartments. Cell. 165:1686–1697. doi:10.1016/j.cell.2016.04.047.

Folkmann A, Putnam A, Lee CF, Seydoux G. 2021. Pickering stabilization of a dynamic intracellular emulsion. bioRxiv. doi: 10.1101/2021.06.22.449249.

Friday AJ, Henderson MA, Morrison JK, Hoffman JL, Keiper BD. 2015. Spatial and temporal translational control of germ cell mRNAs mediated by the eIF4E isoform IFE-1. J Cell Sci. 128:4487–4498. doi:10.1242/jcs.172684.

Gallo CM, Munro E, Rasoloson D, Merritt C, Seydoux G. 2008. Processing bodies and germ granules are distinct RNA granules that interact in *C. elegans* embryos. Dev Biol. 323:76–87. doi: 10.1016/j.ydbio.2008.07.008.

Gao G, Deeb F, Mercurio JM, Parfenova A, Smith PA, *et al.* 2012. PAN-1, a P-granule component important for *C. elegans* fertility, has dual roles in the germline and soma. Dev Biol. 364:202–213. doi:10.1016/j.ydbio.2012.02.006.

Gartner A, Boag PR, Blackwell TK. 2008. Germline survival and apoptosis. WormBook. Sep 4;1–20. doi:10.1895/wormbook.1.145.1.

Gent JI, Lamm AT, Pavelec DM, Maniar JM, Parameswaran P, *et al.* 2010. Distinct phases of siRNA synthesis in an endogenous RNAi pathway in *C. elegans* soma. Mol Cell. 37:679–689. doi: 10.1016/j.molcel.2010.01.012.

Grishok A, Sinskey JL, Sharp PA. 2005. Transcriptional silencing of a transgene by RNAi in the soma of *C. elegans*. Genes Dev. 19:683–696. doi:10.1101/gad.1247705.

Gruidl ME, Smith P. A, Kuznicki K. A, McCrone JS, Kirchner J, *et al.* 1996. Multiple potential germ-line helicases are components of the germ-line-specific P granules of *Caenorhabditis elegans*. Proc Natl Acad Sci U S A. 93:13837–13842.

Gu W, Shirayama M, Conte D, Vasale J, Batista PJ, *et al.* 2009. Distinct argonaute-mediated 22G-RNA pathways direct genome surveillance in the *C. elegans* germline. Mol Cell. 36:231–244. doi: 10.1016/j.molcel.2009.09.020.

Gu W, Lee H-C, Chaves D, Youngman EM, Pazour GJ, *et al.* 2012. CapSeq and CIP-TAP identify Pol II start sites and reveal capped small RNAs as *C. elegans* piRNA precursors. Cell. 151:1488–1500. doi:10.1016/j.cell.2012.11.023.

Guedes S, Priess JR. 1997. The *C. elegans* MEX-1 protein is present in germline blastomeres and is a P granule component. Development. 124:731–739.

Han W, Sundaram P, Kenjale H, Grantham J, Timmons L. 2008. The *Caenorhabditis elegans* rsd-2 and rsd-6 genes are required for chromosome functions during exposure to unfavorable environments. Genetics. 178:1875–1893. doi:10.1534/genetics.107.085472.

Hanazawa M, Yonetani M, Sugimoto A. 2011. PGL proteins self associate and bind RNPs to mediate germ granule assembly in *C. elegans*. J Cell Biol. 192:929–937. doi:10.1083/jcb.201010106.

Haupt KA, Law KT, Enright AL, Kanzler CR, Shin H, *et al.* 2020. A PUF hub drives self-renewal in *Caenorhabditis elegans* germline stem cells. Genetics. 214:147–161. doi:10.1534/genetics.119.302772.

Hegner RW. 1911. The germ cell determinants in the eggs of chrysomelid beetles. Science. 33:71–72. doi:10.1126/science.33.837.71.

Henderson MA, Cronland E, Dunkelbarger S, Contreras V, Strome S, *et al.* 2009. A germline-specific isoform of eIF4E (IFE-1) is required for efficient translation of stored mRNAs and maturation of both oocytes and sperm. J Cell Sci. 122:1529–1539. doi:10.1242/jcs.046771.

Hubbard EJA, Greenstein D. 2005. Introduction to the germ line. WormBook. Sep 1;1–4. doi:10.1895/wormbook.1.18.1.

Hubert A, Anderson P. 2009. The C. elegans sex determination gene laf-1 encodes a putative DEAD-box RNA helicase. Dev Biol. 330:358–67. 10.1016/j.ydbio.2009.04.003. 19361491.

Huggins HP, Subash JS, Stoffel H, Henderson MA, Hoffman JL, *et al.* 2020. Distinct roles of two eIF4E isoforms in the germline of *Caenorhabditis elegans*. J Cell Sci. 133:jcs237990. doi:10.1242/jcs.237990.

Ishidate T, Ozturk AR, Durning DJ, Sharma R, Zhi Shen E, *et al.* 2018. ZNFX-1 functions within perinuclear nuage to balance epigenetic signals. Mol Cell. 70:639–649.e6. doi:10.1016/j.molcel.2018.04.009.

Jedrusik-Bode M, Studencka M, Smolka C, Baumann T, Schmidt H, *et al.* 2013. The sirtuin SIRT6 regulates stress granule formation in *C. elegans* and mammals. J Cell Sci. 126:5166–5177. doi: 10.1242/jcs.130708.

Jones AR, Francis R, Schedl T. 1996. GLD-1, a cytoplasmic protein essential for oocyte differentiation, shows stage- and sex-specific expression during *Caenorhabditis elegans* germline development. Dev Biol. 180:165–183. doi:10.1006/dbio.1996.0293

Kamminga LM, van Wolfswinkel JC, Luteijn MJ, Kaaij LJT, Bagijn MP, *et al.* 2012. Differential impact of the HEN1 homolog HENN-1 on 21U and 26G RNAs in the germline of *Caenorhabditis elegans*. PLoS Genet. 8:e1002702.doi:10.1371/journal.pgen.1002702.

Kawasaki I, Shim YH, Kirchner J, Kaminker J, Wood WB, *et al.* 1998. PGL-1, a predicted RNA-binding component of germ granules, is essential for fertility in *C. elegans*. Cell. 94:635–645. doi: S0092-8674(00)81605-0.[pii]

Kawasaki I, Amiri A, Fan Y, Meyer N, Dunkelbarger S, *et al.* 2004. The PGL family proteins associate with germ granules and function redundantly in *Caenorhabditis elegans* germline development. Genetics. 167:645–661. doi:10.1534/genetics.103.023093.

Kemphues KJ, Priess JR, Morton DG, Cheng NS. 1988. Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. Cell. 52:311–320. doi:S0092-8674(88)80024-2.[pii]

Ketting RF, Haverkamp TH, van Luenen HGA, Plasterk RH. 1999. Mut-7 of *C. elegans*, required for transposon silencing and RNA

interference, is a homolog of Werner syndrome helicase and RNaseD. Cell. 99:133–141. doi:10.1016/s0092-8674(00)81645-1.

Kim JK, Gabel HW, Kamath RS, Tewari M, Pasquinelli A, *et al.* 2005. Functional genomic analysis of RNA interference in *C. elegans*. Science. 308:1164–1167. doi:10.1126/science.1109267.

Kim KW, Nykamp K, Suh N, Bachorik JL, Wang L, *et al.* 2009. Antagonism between GLD-2 binding partners controls gamete sex. Dev Cell. 16:723–733. doi:10.1016/j.devcel.2009.04.002.

Knutson AK, Egelhofer T, Rechtsteiner A, Strome S. 2017. Germ granules prevent accumulation of somatic transcripts in the adult *Caenorhabditis elegans* Germline. Genetics. 206:163–178. doi: 10.1534/genetics.116.198549.

Kulkarni A, Extavour CG. 2017. Convergent evolution of germ granule nucleators: a hypothesis. Stem Cell Res. 24:188–194. doi: 10.1016/j.scr.2017.07.018.

Kuznicki KA, Smith PA, Leung-Chiu WM, Estevez AO, Scott HC, *et al.* 2000. Combinatorial RNA interference indicates GLH-4 can compensate for GLH-1; these two P granule components are critical for fertility in *C. elegans*. Development. 127:2907–2916.

Lafontaine DLJ, Riback JA, Bascetin R, Brangwynne CP. 2021. The nucleolus as a multiphase liquid condensate. Nat Rev Mol Cell Biol. 22:165–182. doi:10.1038/s41580-020–0272-6.

Lall S, Piano F, Davis RE. 2005. *Caenorhabditis elegans* decapping proteins: localization and functional analysis of Dcp1, Dcp2, and DcpS during embryogenesis. Mol Biol Cell. 16:5880–5890. doi: 10.1091/mbc.E05-07-0622.[pii]10.1091/mbc.E05-07–0622

Leacock SW, Reinke V. 2008. MEG-1 and MEG-2 are embryo-specific P-granule components required for germline development in *Caenorhabditis elegans*. Genetics. 178:295–306. doi:10.1534/genetics.107.080218.[pii]10.1534/genetics.107.080218

Lee H-C, Gu W, Shirayama M, Youngman E, Conte D, *et al.* 2012. *C. elegans* piRNAs mediate the genome-wide surveillance of germline transcripts. Cell. 150:78–87. doi:10.1016/j.cell.2012.06.016.

Lee C-YS, Putnam A, Lu T, He S, Ouyang JPT, *et al.* 2020. Recruitment of mRNAs to P granules by condensation with intrinsically-disordered proteins. Elife. 9:e52896. doi: 10.7554/eLife.52896.

Lev I, Toker IA, Mor Y, Nitzan A, Weintraub G, *et al.* 2019. Germ granules govern small RNA inheritance. Curr Biol. 29:2880–2891.e4. doi:10.1016/j.cub.2019.07.054.

Lewis A, Berkyurek AC, Greiner A, Sawh AN, Vashisht A, *et al.* 2020. A family of argonaute-interacting proteins gates nuclear RNAi. Mol Cell. 78:862–875.e8. doi:10.1016/j.molcel.2020.04.007.

Li W, DeBella LR, Guven-Ozkan T, Lin R, Rose LS. 2009. An eIF4E-binding protein regulates katanin protein levels in *C. elegans* embryos. J Cell Biol. 187:33–42. doi:10.1089/jcb.200903003.[pii]10.1083/jcb.200903003

Luteijn MJ, van Bergeijk P, Kaaij LJT, Almeida MV, Roovers EF, *et al.* 2012. Extremely stable Piwi-induced gene silencing in *Caenorhabditis elegans*. Embo J. 31:3422–3430. doi:10.1038/emboj.2012.213.

Mahowald AP, Illmensee K, Turner FR. 1976. Interspecific transplantation of polar plasm between *Drosophila* embryos. J Cell Biol. 70: 358–373. doi:10.1083/jcb.70.2.358.

Manage KI, Rogers AK, Wallis DC, Uebel CJ, Anderson DC, *et al.* 2020. A tudor domain protein, SIMR-1, promotes siRNA production at piRNA-targeted mRNAs in *C. elegans*. Elife. 9:e56731. doi:10.7554/eLife.56731.

Marnik EA, Fuqua JH, Sharp CS, Rochester JD, Xu EL, *et al.* 2019. Germline maintenance through the multifaceted activities of GLH/Vasa in *Caenorhabditis elegans* P granules. Genetics. 213: 923–939. doi:10.1534/genetics.119.302670.

Marnik EA, Almeida MV, Cipriani PG, Chung G, Caspani E, *et al.* 2021. The *Caenorhabditis elegans* TDRD5/7-like protein. LOTR-1, interacts with the helicase ZNFX-1 to balance epigenetic signals in the germline. bioRxiv. doi:10.1101/2021.06.18.448978.

McMurchy AN, Stempor P, Gaarenstroom T, Wysolmerski B, Dong Y, *et al.* 2017. A team of heterochromatin factors collaborates with small RNA pathways to combat repetitive elements and germline stress. Elife. 6:e21666. doi:10.7554/eLife.21666.

Mello CC, Schubert C, Draper B, Zhang W, Lobel R, *et al.* 1996. The PIE-1 protein and germline specification in *C. elegans* embryos. Nature. 382:710–712. doi:10.1038/382710a0.

Min H, Shim Y, Kawasaki I. 2016. Loss of PGL-1 and PGL-3, members of a family of constitutive germ-granule components, promotes germline apoptosis in *C. elegans*. J Cell Sci. 129:341–353. doi: 10.1242/jcs.174201.

Min H, Lee Y-U, Shim Y-H, Kawasaki I. 2019. Autophagy of germ-granule components, PGL-1 and PGL-3, contributes to DNA damage-induced germ cell apoptosis in *C. elegans*, (M. P. Colaiácovo, Ed. ). PLoS Genet. 15:e1008150.doi:10.1371/journal.pgen.1008150.

Montgomery TA, Rim Y-S, Zhang C, Dowen RH, Phillips CM, *et al.* 2012. PIWI associated siRNAs and piRNAs specifically require the *Caenorhabditis elegans* HEN1 ortholog henn-1. PLoS Genet. 8: e1002616.doi:10.1371/journal.pgen.1002616.

Montgomery BE, Vijayasarathy T, Marks TN, Reed KJ, Montgomery TA. 2020. piRNAs prevent runaway amplification of siRNAs from ribosomal RNAs and histone mRNAs. bioRxiv. doi: 10.1101/2020.06.15.153023.

Navarro RE, Shim EY, Kohara Y, Singson A, Blackwell TK. 2001. cgh-1, a conserved predicted RNA helicase required for gametogenesis and protection from physiological germline apoptosis in *C. elegans*. Development. 128:3221–3232.

Nguyen DAH, Phillips CM. 2021. Arginine methylation promotes siRNA-binding specificity for a spermatogenesis-specific isoform of the Argonaute protein CSR-1. Nat Commun. 12:4212.doi: 10.1038/s41467-021–24526-6.

Ogura K, Kishimoto N, Mitani S, Gengyo-Ando K, Kohara Y. 2003. Translational control of maternal glp-1 mRNA by POS-1 and its interacting protein SPN-4 in *Caenorhabditis elegans*. Development. 130:2495–2503.

Ouyang JPT, Folkmann A, Bernard L, Lee C-Y, Seroussi U, *et al.* 2019. P granules protect RNA interference genes from silencing by piRNAs. Dev Cell. 50:716–728.e6. doi:10.1016/j.devcel.2019.07.026.

Parker DM, Winkenbach LP, Boyson S, Saxton MN, Daidone C, *et al.* 2020. mRNA localization is linked to translation regulation in the *Caenorhabditis elegans* germ lineage. Development. 147:dev186817. doi:10.1242/dev.186817.

Penners A. 1922. Die Furchung von Tubifex rivulorum Lam. Verh Deutsch Zool Ges. 29:69–73.

Perez-Borrajero C, Podvalnaya N, Holleis K, Lichtenberger R, Karaulanov E, *et al.* 2021. Structural basis of PETISCO complex assembly during piRNA biogenesis in *C. elegans*. Genes Dev. 35: 1304–1323., doi:10.1101/gad.348648.121.

Phillips CM, Montgomery T. A, Breen PC, Ruvkun G. 2012. MUT-16 promotes formation of perinuclear Mutator foci required for RNA silencing in the *C. elegans* germline. Genes Dev. 26:1433–1444. doi: 10.1101/gad.193904.112.

Phillips CM, Montgomery BE, Breen PC, Roovers EF, Rim Y-S, *et al.* 2014. MUT-14 and SMUT-1 DEAD box RNA helicases have overlapping roles in germline RNAi and endogenous siRNA formation. Curr Biol. 24:839–844. doi:10.1016/j.cub.2014.02.060.

Phillips CM, Brown KC, Montgomery BE, Ruvkun G, Montgomery TA. 2015. piRNAs and piRNA-dependent siRNAs protect conserved and essential *C. elegans* genes from misrouting into the RNAi pathway. Dev Cell. 34:457–465. doi:10.1016/j.devcel.2015.07.009.

Pitt JN, Schisa JA, Priess JR. 2000. P granules in the germ cells of *Caenorhabditis elegans* adults are associated with clusters of nuclear pores and contain RNA. Dev Biol. 219:315–333. doi:10.1006/dbio.2000.9607.

Placentino M, de Jesus Domingues AM, Schreier J, Dietz S, Hellmann S, *et al.* 2021. Intrinsically disordered protein PID-2 modulates Z granules and is required for heritable piRNA-induced silencing in the *Caenorhabditis elegans* embryo. Embo J. 40:e105280.doi:10.15252/embj.2020105280.

Price IF, Hertz HL, Pastore B, Wagner J, Tang W. 2021. Proximity labeling identifies LOTUS domain proteins that promote the formation of perinuclear germ granules in *C. elegans*. Elife. 10. doi:10.7554/eLife.72276.

Putnam A, Cassani M, Smith J, Seydoux G. 2019. A gel phase promotes condensation of liquid P granules in *Caenorhabditis elegans* embryos. Nat Struct Mol Biol. 26:220–226. doi:10.1038/s41594-019–0193-2.

Quarato P, Singh M, Cornes E, Li B, Bourdon L, *et al.* 2021. Germline inherited small RNAs facilitate the clearance of untranslated maternal mRNAs in *C. elegans* embryos. Nat Commun. 12:1441.doi:10.1038/s41467-021–21691-6.

Raiders SA, Eastwood MD, Bacher M, Priess JR. 2018. Binucleate germ cells in *Caenorhabditis elegans* are removed by physiological apoptosis. PLoS Genet. 14:e1007417.doi:10.1371/journal.pgen.1007417.

Rechavi O, Lev I. 2017. Principles of transgenerational small RNA inheritance in *Caenorhabditis elegans*. Curr Biol. 27: R720–R730. doi:10.1016/j.cub.2017.05.043.

Reed KJ, Svendsen JM, Brown KC, Montgomery BE, Marks TN, *et al.* 2020. Widespread roles for piRNAs and WAGO-class siRNAs in shaping the germline transcriptome of *Caenorhabditis elegans*. Nucleic Acids Res. 48:1811–1827. doi:10.1093/nar/gkz1178.

Ritter R. 1890. Die Entwicklung der Geschlechtsorgane und des Darmes bei Chiromomus. Zeit Furr Wiss Zool Bd. 50:408–427.

Robert VJP, Sijen T, van Wolfswinkel J, Plasterk RHA. 2005. Chromatin and RNAi factors protect the *C. elegans* germline against repetitive sequences. Genes Dev. 19:782–787. doi:10.1101/gad.332305.

Rogers AK, Phillips CM. 2020a. RNAi pathways repress reprogramming of *C. elegans* germ cells during heat stress. Nucleic Acids Res. 48:4256–4273. doi:10.1093/nar/gkaa174.

Rogers AK, Phillips CM. 2020b. A small-RNA-mediated feedback loop maintains proper levels of 22G-RNAs in *C. elegans*. Cell Rep. 33: 108279.doi:10.1016/j.celrep.2020.108279.

Rose L, Gönczy P. 2014. Polarity establishment, asymmetric division and segregation of fate determinants in early *C. elegans* embryos. WormBook. Dec 30;1–43. doi:10.1895/wormbook.1.30.2.

Ruby JG, Jan C, Player C, Axtell MJ, Lee W, *et al.* 2006. Large-scale sequencing reveals 21U-RNAs and additional microRNAs and endogenous siRNAs in *C. elegans*. Cell. 127:1193–1207. doi:10.1016/j.cell.2006.10.040.

Rybarska A, Harterink M, Jedamzik B, Kupinski AP, Schmid M, *et al.* 2009. GLS-1, a novel P granule component, modulates a network of conserved RNA regulators to influence germ cell fate decisions. PLoS Genet. 5:e1000494.doi:10.1371/journal.pgen.1000494.

Saha S, Weber CA, Nousch M, Adame-Arana O, Hoege C, *et al.* 2016. Polar positioning of phase-separated liquid compartments in cells regulated by an mRNA competition mechanism. Cell. 166: 1572–1584.e16. doi:10.1016/j.cell.2016.08.006.

Sakaguchi A, Sarkies P, Simon M, Doebley A-L, Goldstein LD, *et al.* 2014. *Caenorhabditis elegans* RSD-2 and RSD-6 promote germ cell immortality by maintaining small interfering RNA populations. Proc Natl Acad Sci U S A. 111: E4323–E4331. doi:10.1073/pnas.1406131111.

Salinas LS, Maldonado E, Macias-Silva M, Blackwell TK, Navarro RE. 2007. The DEAD box RNA helicase VBH-1 is required for germ cell function in *C. elegans*. Genesis. 45:533–546. doi:10.1002/dvg.20323.

Schisa JA, Pitt JN, Priess JR. 2001. Analysis of RNA associated with P granules in germ cells of *C. elegans* adults. Development. 128: 1287–1298.

Schmid M, Kuchler B, Eckmann CR. 2009. Two conserved regulatory cytoplasmic poly(A) polymerases, GLD-4 and GLD-2, regulate meiotic progression in *C. elegans*. Genes Dev. 23:824–836. doi:10.1101/gad.494009. [pii]10.1101/gad.494009

Schmidt HB, Görlich D. 2016. Transport selectivity of nuclear pores, phase separation, and membraneless organelles. Trends Biochem Sci. 41:46–61. doi:10.1016/j.tibs.2015.11.001.

Schmidt H, Putnam A, Rasoloson D, Seydoux G. 2021. Protein-based condensation mechanisms drive the assembly of RNA-rich P granules. Elife. 10:e63698. doi:10.7554/eLife.63698.

Schreier J, Dietz S, Jesus Domingues A. D, Seistrup A-S, Nguyen DAH, *et al.* 2021. A membrane-associated condensate drives paternal epigenetic inheritance in *C. elegans*. bioRxiv. doi:10.1101/2020.12.10.417311v1.

Sendoel A, Subasic D, Ducoli L, Keller M, Michel E, *et al.* 2019. MINA-1 and WAGO-4 are part of regulatory network coordinating germ cell death and RNAi in *C. elegans*. Cell Death Differ. 26:2157–2178. doi:10.1038/s41418-019–0291-z.

Sengupta MS, Low WY, Patterson JR, Kim H-M, Traven A, *et al.* 2013. ifet-1 is a broad-scale translational repressor required for normal P granule formation in *C. elegans*. J Cell Sci. 126:850–859. doi:10.1242/jcs.119834.

Seth M, Shirayama M, Gu W, Ishidate T, Conte D, *et al.* 2013. The *C. elegans* CSR-1 argonaute pathway counteracts epigenetic silencing to promote germline gene expression. Dev Cell. 27:656–663. doi:10.1016/j.devcel.2013.11.014.

Seydoux G, Fire A. 1994. Soma-germline asymmetry in the distributions of embryonic RNAs in *Caenorhabditis elegans*. Development. 120:2823–2834.

Seydoux G, Dunn MA. 1997. Transcriptionally repressed germ cells lack a subpopulation of phosphorylated RNA polymerase II in early embryos of *Caenorhabditis elegans* and Drosophila melanogaster. Development. 124:2191–2201.

Seydoux G. 2018. The P granules of *C. elegans*: a genetic model for the study of RNA–protein condensates. J Mol Biol. 430:4702–4710. doi:10.1016/j.jmb.2018.08.007.

Shen E, Chen H, Ozturk AR, Tu S, Shirayama M, *et al.* 2018. Identification of piRNA binding sites reveals the argonaute regulatory landscape of the *C. elegans* germline. Cell. 172:937–951.e18. doi:10.1016/j.cell.2018.02.002.

Sheth U, Pitt J, Dennis S, Priess JR. 2010. Perinuclear P granules are the principal sites of mRNA export in adult *C. elegans* germ cells. Development. 137:1305–1314. doi:10.1242/dev.044255.

Shimada M, Kawahara H, Doi H. 2002. Novel family of CCCH-type zinc-finger proteins, MOE-1, -2 and -3, participates in *C. elegans* oocyte maturation. Genes Cells. 7:933–947. doi:10.1046/j.1365–2443.2002.00570.x.[pii]

Shirayama M, Seth M, Lee H-C, Gu W, Ishidate T, *et al.* 2012. PiRNAs initiate an epigenetic memory of nonself RNA in the *C. elegans* germline. Cell. 150:65–77. doi:10.1016/j.cell.2012.06.015.

Shirayama M, Stanney W, Gu W, Seth M, Mello CC. 2014. The Vasa Homolog RDE-12 engages target mRNA and multiple argonaute

proteins to promote RNAi in *C. elegans*. Curr Biol. 24:845–851. doi: 10.1016/j.cub.2014.03.008.

Shukla A, Yan J, Pagano DJ, Dodson AE, Fei Y, *et al.* 2020. poly(UG)-tailed RNAs in genome protection and epigenetic inheritance. Nature. 582:283–288. doi:10.1038/s41586-020-2323-8.

Shukla A, Perales R, Kennedy S. 2021. piRNAs coordinate poly(UG) tailing to prevent aberrant and perpetual gene silencing. Curr Biol. CB. 31:4473–4485.e3. 10.1016/j.cub.2021.07.076. 34428467.

Simon M, Sarkies P, Ikegami K, Doebley A-L, Goldstein LD, *et al.* 2014. Reduced insulin/IGF-1 signaling restores germ cell immortality to *Caenorhabditis elegans* Piwi mutants. Cell Rep. 7:762–773. doi: 10.1016/j.celrep.2014.03.056.

Singh M, Cornes E, Li B, Quarato P, Bourdon L, *et al.* 2021. Translation and codon usage regulate Argonaute slicer activity to trigger small RNA biogenesis. Nat Commun. 12:3492.doi: 10.1038/s41467-021-23615-w.

Smith LD. 1966. The role of a "germinal plasm" in the formation of primordial germ cells in Rana pipiens. Dev Biol. 14:330–347. doi: 10.1016/0012–1606(66)90019-4.

Smith J, Calidas D, Schmidt H, Lu T, Rasoloson D, *et al.* 2016. Spatial patterning of P granules by RNA-induced phase separation of the intrinsically-disordered protein MEG-3. Elife. 5:e21337.doi: 10.7554/eLife.21337.

Spichal M, Heestand B, Billmyre KK, Frenk S, Mello CC, *et al.* 2021. Germ granule dysfunction is a hallmark and mirror of Piwi mutant sterility. Nat Commun. 12:1420.doi:10.1038/s41467-021-21635-0.

Spike CA, Bader J, Reinke V, Strome S. 2008a. DEPS-1 promotes P-granule assembly and RNA interference in *C. elegans* germ cells. Development. 135:983–993. doi:10.1242/dev.015552.

Spike C, Meyer N, Racen E, Orsborn A, Kirchner J, *et al.* 2008b. Genetic analysis of the *Caenorhabditis elegans* GLH family of P-granule proteins. Genetics. 178:1973–1987. doi:10.1534/genetics.107.083469.

Spracklin G, Fields B, Wan G, Becker D, Wallig A, *et al.* 2017. The RNAi inheritance machinery of *Caenorhabditis elegans*. Genetics. 206: 1403–1416. doi:10.1534/genetics.116.198812.

Squirrell JM, Eggers ZT, Luedke N, Saari B, Grimson A, *et al.* 2006. CAR-1, a protein that localizes with the mRNA decapping component DCAP-1, is required for cytokinesis and ER organization in *Caenorhabditis elegans* embryos. Mol Biol Cell. 17:336–344. doi: 10.1091/mbc.e05-09–0874.

Stitzel ML, Cheng KC-C, Seydoux G. 2007. Regulation of MBK-2/Dyrk kinase by dynamic cortical anchoring during the oocyte-to-zygote transition. Curr Biol. 17:1545–1554. doi: 10.1016/j.cub.2007.08.049.

Strome S, Wood WB. 1982. Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*. Proc Natl Acad Sci U S A. 79: 1558–1562.

Strome S. 2005. Specification of the germ line. WormBook. Jul 28: 1–10. doi:10.1895/wormbook.1.9.1.

Subramaniam K, Seydoux G. 1999. nos-1 and nos-2, two genes related to *Drosophila nanos*, regulate primordial germ cell development and survival in *Caenorhabditis elegans*. Development. 126: 4861–4871.

Suen KM, Braukmann F, Butler R, Bensaddek D, Akay A, *et al.* 2020. DEPS-1 is required for piRNA-dependent silencing and PIWI condensate organisation in *Caenorhabditis elegans*. Nat Commun. 11: 4242.doi:10.1038/s41467-020-18089-1.

Tabara H, Hill RJ, Mello CC, Priess JR, Kohara Y. 1999a. pos-1 Encodes a cytoplasmic zinc-finger protein essential for germline specification in *C. elegans*. Development. 126:1–11.

Tabara H, Sarkissian M, Kelly WG, Fleenor J, Grishok A, *et al.* 1999b. The rde-1 gene, RNA interference, and transposon silencing in *C. elegans*. Cell. 99:123–132. doi:10.1016/s0092-8674(00)81644-x.

Tang W, Tu S, Lee H-C, Weng Z, Mello CC. 2016. The RNase PARN-1 trims piRNA 3′ ends to promote transcriptome surveillance in *C. elegans*. Cell. 164:974–984. doi:10.1016/j.cell.2016.02.008.

Thivierge C, Makil N, Flamand M, Vasale JJ, Mello CC, *et al.* 2011. Tudor domain ERI-5 tethers an RNA-dependent RNA polymerase to DCR-1 to potentiate endo-RNAi. Nat Struct Mol Biol. 19:90–97. doi:10.1038/nsmb.2186.

Tijsterman M, Ketting RF, Okihara KL, Sijen T, Plasterk RHA. 2002. RNA helicase MUT-14-dependent gene silencing triggered in *C. elegans* by short antisense RNAs. Science. 295:694–697. doi: 10.1126/science.1067534.

Tijsterman M, May RC, Simmer F, Okihara KL, Plasterk RHA. 2004. Genes required for systemic RNA interference in *Caenorhabditis elegans*. Curr Biol. 14:111–116. doi:10.1016/j.cub.2003.12.029.

Tops BBJ, Tabara H, Sijen T, Simmer F, Mello CC, *et al.* 2005. RDE-2 interacts with MUT-7 to mediate RNA interference in *Caenorhabditis elegans*. Nucleic Acids Res. 33:347–355. doi: 10.1093/nar/gki183.

Tsai H-Y, Chen C-CG, Conte D, Moresco JJ, Chaves DA, *et al.* 2015. A ribonuclease coordinates siRNA amplification and mRNA cleavage during RNAi. Cell. 160:407–419. doi:10.1016/j.cell.2015.01.010.

Tu S, Wu MZ, Wang J, Cutter AD, Weng Z, *et al.* 2014. Comparative functional characterization of the CSR-1 22G-RNA pathway in *Caenorhabditis* nematodes. Nucleic Acids Res. 1–17. doi: 10.1093/nar/gku1308.

Uebel CJ, Anderson DC, Mandarino LM, Manage KI, Aynaszyan S, *et al.* 2018. Distinct regions of the intrinsically disordered protein MUT-16 mediate assembly of a small RNA amplification complex and promote phase separation of Mutator foci. PLoS Genet. 14: e1007542.doi:10.1371/journal.pgen.1007542.

Uebel CJ, Agbede D, Wallis DC, Phillips CM. 2020. Mutator foci are regulated by developmental stage, RNA, and the germline cell cycle in *Caenorhabditis elegans*. G3 (Bethesda). 10:3719–3728. doi: 10.1534/g3.120.401514.

Uebel CJ, Manage KI, Phillips CM. 2021. SIMR foci are found in the progenitor germ cells of *C. elegans* embryos. microPubl Biol. Feb 22;2021. doi:10.17912/micropub.biology.000374.

Updike DL, Strome S. 2009. A genomewide RNAi screen for genes that affect the stability, distribution and function of P granules in *Caenorhabditis elegans*. Genetics. 183:1397–1419. doi:10.1534/genetics.109.110171.

Updike D, Strome S. 2010. P granule assembly and function in *Caenorhabditis elegans* germ cells. J Androl. 31:53–60. doi: 10.2164/jandrol.109.008292.

Updike DL, Hachey SJ, Kreher J, Strome S. 2011. P granules extend the nuclear pore complex environment in the *C. elegans* germ line. J Cell Biol. 192:939–948. doi:10.1083/jcb.201010104.

Updike DL, Knutson AK, Egelhofer TA, Campbell AC, Strome S. 2014. Germ-granule components prevent somatic development in the *C. elegans* germline. Curr Biol. 24:970–975. doi:10.1016/j.cub.2014.03.015.

van Wolfswinkel JC, Claycomb JM, Batista PJ, Mello CC, Berezikov E, *et al.* 2009. CDE-1 affects chromosome segregation through uridylation of CSR-1-bound siRNAs. Cell. 139:135–148. doi: 10.1016/j.cell.2009.09.012.

Vastenhouw NL, Fischer SEJ, Robert VJP, Thijssen KL, Fraser AG, *et al.* 2003. A genome-wide screen identifies 27 genes involved in transposon silencing in *C. elegans*. Curr Biol. 13:1311–1316.

Voronina E, Paix A, Seydoux G. 2012. The P granule component PGL-1 promotes the localization and silencing activity of the PUF

protein FBF-2 in germline stem cells. Development. 139: 3732–3740. doi:10.1242/dev.083980.

Voronina E, Seydoux G. 2010. The *C. elegans* homolog of nucleoporin Nup98 is required for the integrity and function of germline P granules. Development. 137:1441–1450. doi:10.1242/dev.047654.

Vought VE, Ohmachi M, Lee M-H, Maine EM. 2005. EGO-1, a putative RNA-directed RNA polymerase, promotes germline proliferation in parallel with GLP-1/notch signaling and regulates the spatial organization of nuclear pore complexes and germline P granules in *Caenorhabditis elegans*. Genetics. 170:1121–1132. doi:10.1534/genetics.105.042135.

Wahba L, Hansen L, Fire AZ. 2021. An essential role for the piRNA pathway in regulating the ribosomal RNA pool in *C. elegans*. Dev Cell. 56:2295–2312.e6. doi:10.1016/j.devcel.2021.07.014.

Wallis DC, Nguyen DAH, Uebel CJ, Phillips CM. 2019. Visualization and quantification of transposon activity in *Caenorhabditis elegans* RNAi pathway mutants. G3 (Bethesda). 9:3825–3832. doi: 10.1534/g3.119.400639.

Wan G, Fields BD, Spracklin G, Shukla A, Phillips CM, *et al.* 2018. Spatiotemporal regulation of liquid-like condensates in epigenetic inheritance. Nature. 557:679–683. doi:10.1038/s41586-018–0132-0.

Wan G, Bajaj L, Fields B, Dodson AE, Pagano D, *et al.* 2021. ZSP-1 is a Z granule surface protein required for Z granule fluidity and germline immortality in *Caenorhabditis elegans*. Embo J. 40:e105612.doi: 10.15252/embj.2020105612.

Wang L, Eckmann CR, Kadyk LC, Wickens M, Kimble J. 2002. A regulatory cytoplasmic poly(A) polymerase in *Caenorhabditis elegans*. Nature. 419:312–316. doi:10.1038/nature01039nature01039.[pii]

Wang G, Reinke V. 2008. A *C. elegans* Piwi, PRG-1, regulates 21U-RNAs during spermatogenesis. Curr Biol. 18:861–867. doi: 10.1016/j.cub.2008.05.009.

Wang JT, Smith J, Chen B-C, Schmidt H, Rasoloson D, *et al.* 2014. Regulation of RNA granule dynamics by phosphorylation of serine-rich, intrinsically-disordered proteins in *C. elegans*. Elife. 3: e04591. doi:10.7554/eLife.04591.

Wedeles CJ, Wu MZ, Claycomb JM. 2013. Protection of germline gene expression by the *C. elegans* Argonaute CSR-1. Dev Cell. 27: 664–671. doi:10.1016/j.devcel.2013.11.016.

Witze ES, Field ED, Hunt DF, Rothman JH. 2009. *C. elegans* pur alpha, an activator of end-1, synergizes with the Wnt pathway to specify endoderm. Dev Biol. 327:12–23. doi: 10.1016/j.ydbio.2008.11.015.

Xu F, Feng X, Chen X, Weng C, Yan Q, *et al.* 2018. A Cytoplasmic Argonaute protein promotes the inheritance of RNAi. Cell Rep. 23:2482–2494. doi:10.1016/j.celrep.2018.04.072.

Yang H, Vallandingham J, Shiu P, Li H, Hunter CP, *et al.* 2014. The DEAD box helicase RDE-12 promotes amplification of RNAi in cytoplasmic foci in *C. elegans*. Curr Biol. 24:832–838. doi: 10.1016/j.cub.2014.01.008.

Zeng C, Weng C, Wang X, Yan Y-H, Li W-J, *et al.* 2019. Functional proteomics identifies a PICS complex required for piRNA maturation and chromosome segregation. Cell Rep. 27:3561–3572.e3. doi: 10.1016/j.celrep.2019.05.076.

Zhang Y, Yan L, Zhou Z, Yang P, Tian E, *et al.* 2009. SEPA-1 mediates the specific recognition and degradation of P granule components by autophagy in *C. elegans*. Cell. 136:308–321. doi: 10.1016/j.cell.2008.12.022.

Zhang C, Montgomery TA, Gabel HW, Fischer SEJ, Phillips CM, *et al.* 2011. Mut-16 and other mutator class genes modulate 22G and 26G siRNA pathways in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A. 108:1201–1208. doi:10.1073/pnas.1018695108.

Zhang C, Montgomery TA, Fischer SEJ, Garcia SMDA, Riedel CG, *et al.* 2012. The *Caenorhabditis elegans* RDE-10/RDE-11 complex regulates RNAi by promoting secondary siRNA amplification. Curr Biol. 22:881–890. doi:10.1016/j.cub.2012.04.011.

Zhang G, Wang Z, Du Z, Zhang H. 2018a. mTOR regulates phase separation of PGL granules to modulate their autophagic degradation. Cell. 174:1492–1506.e22. doi:10.1016/j.cell.2018.08.006.

Zhang D, Tu S, Stubna M, Wu W-S, Huang W-C, *et al.* 2018b. The piRNA targeting rules and the resistance to piRNA silencing in endogenous genes. Science. 359:587–592. doi:10.1126/science.aao2840.

*Communicating editor: J. Kim*