**Introduction**

During animal development, somatic cells display ever more restricted developmental potential as they terminally differentiate into mature tissues, whereas germ cells must retain the ability to produce generation after generation of an organism and all of the cell types of each generation. Loss of these attributes of immortality and totipotency in the germ line results in sterility; conversely, acquisition of these attributes by somatic cells are hallmarks of both cancer and regenerative stem cells. A key to understanding how germ cells are regulated resides in the germ plasm, which contains germline determinants (Illmensee and Mahowald, 1974; Ikenishi et al., 1986). Ribonucleoprotein (RNP) aggregates called germ granules are found in the germ plasm of many species (Eddy, 1975; Extavour and Akam, 2003). Although depletion of many germ-granule components results in infertility (Chuma et al., 2009), the importance of the assembly of those components into granules within germ plasm has yet to be determined.

*Caenorhabditis elegans* germ granules (P granules) are dynamic, moving within the germline cytoplasm much like liquid droplets (Brangwynne et al., 2009). In somatic cells, P granules dissolve in part through protein degradation and autophagy (DeRenzo et al., 2003; Zhang et al., 2009). It was recently proposed that the primary dissolution of P granules that remain in the somatic blastomere after the first embryonic cell division is the result of under-saturation of P-granule components. In contrast, in the germ line, the concentration at which soluble P-granule components are saturated decreases, likely through nucleating factors specific to the germ line, resulting in super-saturation and aggregation of P-granule components (Brangwynne et al., 2009). In *Drosophila*, the fly-specific protein Oskar nucleates Vasa recruitment to germ granules (Ephrussi and Lehmann, 1992). In zebrafish, germ granules are nucleated by the vertebrate-specific Bucky Ball protein (Bontems et al., 2009). This theme is continued in *C. elegans*, where we show that the worm-specific protein PGL-1 nucleates worm Vasa homologues to form granules.

The DEAD-box helicase Vasa is a germ-granule component that is conserved across phyla. In *C. elegans*, the Vasa-related proteins GLH-1, GLH-2, and GLH-4 contain a phenylalanine-glycine (FG) repeat domain. Multiple FG residues separated by 10–15 amino acids are a hallmark of many nuclear pore complex (NPC) proteins or Nups. These FG-rich domains form a cohesive meshwork of filaments through hydrophobic interactions involving the phenylalanines in FG motifs (Ribbeck and Görlich, 2002; Patel et al., 2007). Within NPCs, these interactions create a size-exclusion barrier that...
Results and discussion

P granules create a size-exclusion barrier
Hydrophobic FG-repeat domains within the NPC act both to prevent passive diffusion of proteins >45 kD into the nucleus and to facilitate transport of karyopherins and their cargo. Fluorescently labeled dextrans of varying sizes are commonly used to determine size-exclusion barriers in living cells (Peters, 1986). In *C. elegans*, fluorescent dextrans can be injected into the gonad syncytium and labeled dextrans of varying sizes are commonly used to determine size-exclusion barriers in living cells (Peters, 1986). In *C. elegans*, fluorescent dextrans can be injected into the gonad syncytium and imaged in developing embryos 4–5 h later (Galy et al., 2003). At the end of each cell cycle, the nuclear envelope reforms around chromatin and excludes large dextrans (70 and 155 kD).

Because the constitutive P-granule components GLH-1, GLH-2, and GLH-4 contain Nup-like FG domains, we tested if P granules could also establish a size-exclusion barrier (Fig. 1 A). TRITC-labeled dextrans were injected into worms, and dextrans and GFP-tagged P granules (GFP::PGL-1) were simultaneously imaged in early embryos. As reported previously (Galy et al., 2003), we observed that dextrans of 70 and 155 kD are excluded from nuclei but dextrans of 10 and 40 kD are not (Fig. 1 B). We found that P granules also exclude 70- and 155-kD dextrans, but not 10- and 40-kD dextrans. These results demonstrate that, like NPCs, P granules also establish a size-exclusion barrier somewhere between 40 and 70 kD, a barrier that may be somewhat less stringent than NPCs, as 40-kD dextran is at least partially excluded from the nucleus but not from P granules. The size-exclusion barrier created by P granules may be the result of interactions between the FG repeats of GLH-1, GLH-2, and GLH-4 and other P-granule proteins.

Hydrophobic interactions are required for P-granule integrity
Treatment of cells with low concentrations of aliphatic alcohols such as 1,6-hexanediol (HD) disrupts hydrophobic interactions between FG residues and compromises the nucleocytoplasmic size-exclusion barrier; less hydrophobic alcohols such as 1,2,3-hexanetriol (HT) are less effective (Ribbeck and Görlich, 2002; Shulga and Goldfarb, 2003). To determine if hydrophobic interactions are important for P-granule structure, we exposed dissected gonads from a GLH-1::GFP—expressing strain to a 5% final volume of HD or HT in buffer, or buffer alone (control) (Fig. 2, A and B; Video 1). We observed rapid dispersal of GLH-1::GFP granules when exposed to HD compared with the control (50% of granules had dispersed by 18 s after the addition of HD, P < 0.0005 compared with control). We observed less rapid dispersal of GLH-1::GFP granules when exposed to HT (50% of granules had dispersed by 34 s after HT, P = 0.046 compared with control). Three observations make it unlikely that the dispersal of GLH-1::GFP granules resulted from the effect of HD on NPCs. First, both cytoplasmic and perinuclear GLH-1::GFP granules dispersed. Although cytoplasmic P granules in maturing oocytes are associated with NPC components, cytoplasmic P granules in the rachis are not; both classes of cytoplasmic P granules dissolved. Second, perinuclear GLH-1::GFP granules began to disperse before the influx of GFP from the cytoplasm into the nucleus (Video 2). Third, P granules dissolved after exposure to HD instead of detaching from the nuclear periphery as they do after Nup RNAi (Updike and Strome, 2009). Our results suggest that the integrity of P granules depends on hydrophobic interactions.

The above results could be explained by alcohol disruption of GLH-1 interactions within granules and dispersal specifically of GLH-1, or by disruption of hydrophobic interactions that hold P granules together. To distinguish between these possibilities, we examined a different P-granule protein, PGL-1 is a constitutive P-granule protein that lacks an FG domain. We exposed dissected gonads from a GFP::PGL-1—expressing strain to low concentrations of HD and HT (Fig. 2 C). Both HD and HT dispersed PGL-1 granules (P < 0.0005 compared with control). Our results suggest that hydrophobic interactions within P granules contribute to the integrity of P granules as a whole.

To investigate the specificity of granule disruption by aliphatic alcohols, we also exposed dissected gonads from GFP::SPD-2 worms to HD and HT (Fig. 2 D; Video 3). SPD-2 is a component of pericentriolar material and is also found in cytoplasmic granules in the germ line. GFP::SPD-2 granules were only subtly affected by HD or HT, suggesting that the much more substantial P-granule disruption by HD and HT is a consequence of disrupting hydrophobic interactions between P-granule components.

GLH proteins must be nucleated to form granules
Our results indicate that P granules, like NPCs, establish a size-exclusion barrier and depend on hydrophobic interactions for their structural integrity. We hypothesize that these granule properties are attributable to interactions between the FG domains of P-granule proteins. There are two nonexclusive models for the role of FG repeats within NPCs: (1) noncohesive FG domains create hydrophobic, entropic barriers at pores, and/or (2) cohesive FG domains interact to form a size-selective hydrogel (Weis, 2007).
To test the sufficiency of P-granule proteins and domains to form granules in vivo but outside the context of the germ line and therefore independently of other germline-specific factors, we drove ectopic expression of those proteins and domains in...
concentration can also be accomplished by multimerizing FG domains. Indeed, intestinal expression of three fused NPP-4 FG domains caused GFP granules to form more readily than a single FG domain in both the nucleus and the cytoplasm (Fig. 3 F).

To determine if the FG domain of GLH-1 is sufficient for granule formation, we tagged the domain with GFP. The GLH-1 FG domain was dispersed throughout the nucleus and cytoplasm (Fig. 3 G). However, like the multimerized NPP-4 FG intestinal cells of C. elegans embryos (Fig. 3). For comparison of GLH-1 to a bona fide NPC component, we tested NPP-4. After expression of the NPP-4 FG domain (Fig. 3 B) behind the intestine-specific elt-2 promoter, most NPP-4(FG)::GFP localized to the nucleus, but we also observed NPP-4(FG)::GFP in the cytoplasm, some of it in granular form (Fig. 3 E). Weak FG interactions are facilitated by anchoring domains within the NPC, which increases the local FG concentration. An increase in local FG concentration can also be accomplished by multimerizing FG domains. Indeed, intestinal expression of three fused NPP-4 FG domains caused GFP granules to form more readily than a single FG domain in both the nucleus and the cytoplasm (Fig. 3 F).

To determine if the FG domain of GLH-1 is sufficient for granule formation, we tagged the domain with GFP. The GLH-1 FG domain was dispersed throughout the nucleus and cytoplasm (Fig. 3 G). However, like the multimerized NPP-4 FG
domain, three multimerized GLH-1 FG domains readily formed granules in the intestine (Fig. 3 H). We also tested the granule-forming capacity of the FG domain in the context of full-length GLH-1 and tested full-length GLH-3, which naturally lacks an FG domain (Fig. 3, B, I, and K–N). These full-length proteins remained dispersed throughout the cytoplasm and did not form granules. However, by inserting two additional FG domains in the N terminus of full-length GLH-1, granules readily formed (Fig. 3 J). These results show that GLH-1 on its own does not form granules outside of the germ line. The FG repeats must reach a critical concentration threshold before granules can form (Pappu et al., 2008). This suggests a requirement for GLH nucleation factors in the germ line.

PGL-1 promotes granule formation and nucleates the formation of GLH-1 and GLH-3 granules

Because the PGL proteins have been shown to interact with each other by yeast two-hybrid and in vitro pull-down assays (Kawasaki et al., 2004), we tested if PGL-1 can self-aggregate into granules when expressed outside of the germ line. PGL-1::GFP and untagged PGL-1 were ectopically expressed in the intestine (Fig. 4 A). Both untagged and GFP-tagged PGL-1 formed granules. Because PGL-1 is sufficient for granule formation outside of the germ line, we reasoned that PGL-1 may act as a nucleating factor for the aggregation of other P-granule components like GLH-1. To test this, lines expressing PGL-1::GFP in the intestine were crossed with lines expressing GLH-1::GFP in the intestine, implicating PGL-1 as a P-granule–nucleating component (Fig. 4 B). Untagged PGL-1 also recruited GLH-1::GFP to granules (Fig. 4 C; 21/22 worms expressing PGL-1::GFP had granular GLH-1::GFP, 3/24 worms lacking expression of PGL-1 had granular GLH-1::GFP). To determine if the recruitment of GLH protein to PGL-1 granules requires an FG domain, lines expressing PGL-1::GFP in the intestine were crossed with lines expressing GLH-3::mCherry in the intestine. PGL-1::GFP recruited GLH-1::mCherry to granules in the intestine, implicating PGL-1 as a P-granule–nucleating component (Fig. 4 B). Untagged PGL-1 also recruited GLH-1::GFP to granules (Fig. 4 C; 21/22 worms expressing PGL-1::GFP had granular GLH-1::GFP, 3/24 worms lacking expression of PGL-1 had granular GLH-1::GFP). To determine if the recruitment of GLH protein to PGL-1 granules requires an FG domain, lines expressing PGL-1::GFP in the intestine were crossed with lines expressing GLH-3::mCherry in the intestine. Despite the absence of an FG domain in GLH-3 (Fig. 3 B), GLH-3 was also recruited to PGL-1::GFP granules (Fig. 4 D), revealing that PGL-1 recruitment does not require an FG domain. Consistent with recruitment of GLH-1 to PGL-1 granules independently of GLH-1’s FG domain, untagged PGL-1 did not recruit the 1XFG domain from GLH-1 to granules (Fig. 4 E, n = 30).
GLH-1 is necessary but not sufficient for the perinuclear association of PGL granules in intestinal cells

We noticed that PGL-1 granules were not concentrated around the nuclear periphery in the intestine (Fig. 5 A), whereas granules of GLH-1 with 3X multimerized FG domains were often perinuclear (Fig. 5 B). To test if GLH-1 is sufficient to recruit PGL-1 granules to a perinuclear location, we generated worms that coexpress PGL-1 and GLH-1, or coexpress PGL-1 and 3XFG-GLH-1, or express PGL-1 fused to the 3XFG domain of GLH-1 in their intestines (Fig. 5 D). In all of the lines examined, fewer than 10% of PGL-1 granules were in contact with the nuclear envelope (n = 10 embryos per line). Thus, association or attachment of a single or multimerized FG domain was not sufficient to drive PGL-1 granules to perinuclear locations.

hpl-2 mutants offer another avenue to investigate P granules in intestinal cells. In hpl-2 mutants, germline genes are ectopically expressed in the intestine and aggregates of P-granule components like PGL-1, PGL-3, and GLH-1 form around intestinal nuclei (Fig. 5 E, middle; Wang et al., 2005; Petrella et al., 2011).

Figure 4. PGL-1 nucleates granule formation. (A) GFP-tagged and untagged PGL-1 expressed in the intestine. (B and D) GLH-1::mCherry (B) or GLH-3::mCherry (D) coexpressed with PGL-1::GFP in intestinal cells. (C and E) Young larvae expressing GLH-1::GFP (C) or the FG domain of GLH-1 tagged with GFP (E) in intestinal cells. The larvae in the right panels additionally express untagged PGL-1 in intestinal cells (transgenic PGL-1-expressing worms identified by myo-3::mCherry expression in body muscle).
Likewise, full-length GLH-1::GFP assembles into cytoplasmic and perinuclear granules in hpl-2 mutant embryos (Fig. 5 C, 44/50 embryos with perinuclear GLH-1 granules). When GLH-1 was depleted from hpl-2 mutants by RNAi, PGL-3-containing granules were no longer concentrated around the nuclear periphery but instead were found throughout the intestinal cytoplasm (Fig. 5 E, bottom; dispersal of PGL-3 granules in 39/52 hpl-2; glh-1 (RNAi) worms). Thus, GLH-1 is required for the perinuclear localization of PGL granules. These findings in the intestine are consistent with findings in the germ line: loss of GLH-1 by RNAi or mutation causes PGL granules to lose their nuclear association. Although long-term loss results in variable degrees of dispersal of PGL proteins from granules (Kawasaki et al., 1998; Spike et al., 2008), a more immediate effect of glh-1 RNAi is the presence of small PGL-1 granules in the intestine. Ectopic PGL-1 granules in the intestine (arrowhead), endogenous PGL-1 granules in the germ cells (arrow). (B) Embryo expressing 3X(FG)-GLH-1 in the intestine. Cytoplasmic granules (arrowhead), perinuclear granules (arrow). (C) hpl-2(tm1489) embryo expressing GLH-1 in the intestine. Cytoplasmic granules (arrowhead), perinuclear granules (arrow). (D) GLH-1 (left) and 3X(FG)-GLH-1 (middle) co-expressed with PGL-1 in intestinal cells. 3XF from GLH-1 fused to PGL-1 (right). Ectopic PGL-1 granules in the intestine (arrowhead), endogenous PGL-1 granules in the germ cells (arrow). (E) Wild-type and hpl-2(tm1489) L1s grown at 26°C on control bacteria and glh-1(RNAi) bacteria and immunostained for GLH-1 and PGL-3. Germ cells (arrowheads). (F) Germ lines from wild-type worms grown from L1 to adulthood at 26°C on control bacteria and glh-1(RNAi) bacteria.

Concluding remarks
The P-granule assembly pathway, worked out by molecular epistasis tests over the past decade, placed GLH-1 upstream of PGL proteins: in the absence of GLH-1, small PGL granules disperse throughout the cytoplasm, while the combined absence of the PGL proteins has no obvious effect on the perinuclear location.
of GLH-1 granules in the adult germ line (Kawasaki et al., 1998, 2004; Spike et al., 2008). Our studies of the properties of individual P-granule proteins expressed in the intestine, as well as the studies of Hanazawa et al. (2011), refine this pathway by showing that PGL-1 and PGL-3 are capable of self assembling into granules in the absence of other germine-specific proteins and that GLH-1 requires nucleating factors, like PGL-1, for de novo granule formation. These insights required examining the roles of P-granule proteins under conditions of de novo granule assembly (in early embryos and in the intestine) instead of under conditions of granule maintenance (the adult germ line). One role of GLH-1 is to retain PGL aggregates in large assemblies at the nuclear periphery; however, GLH-1 cannot do this on its own. We speculate that in the germ line, GLH-1 aggregation seeded by the PGL proteins facilitates interaction with other FG-containing proteins to retain P granules at the nuclear periphery, potentially explaining why P granules detach from the periphery when certain Nups are depleted (Updike and Strome, 2009). It was recently reported that in mouse testes, Vasa immunoprecipitates with the peripheral FG-rich Nup98, but this interaction was not observed in tissue culture cells (Voronina and Seydoux, 2010), suggesting that Vasa, like GLH-1, requires nucleation by additional germine components to localize to the nuclear periphery.

Similar to FG repeats, hydrophobic GW/GW repeats are found in the Argonaute-interacting protein GW182, a component of P bodies (Eulalio et al., 2009). P granules share many properties with P bodies and also contain Argonautes like PRG-1, WAGO-1, ALG-3, and CSR-1 (Batista et al., 2008; Claycomb et al., 2009; Gu et al., 2009; Conine et al., 2010). The Argonautes in P granules may interact with FG repeats like those found in GLH-1, GLH-2, and GLH-4. Another interesting protein that may share roles with the GLHs is the embryo implantation factor Trophinin, whose C terminus contains 45 FGs spaced every 10–15 aa. Trophinin is expressed in the cytoplasm of mammalian germ cells and localizes to the nuclear periphery (Aoyama et al., 2005).

More than 40 proteins associated with P granules either possess RNA-binding domains or are thought to regulate translation (Updike and Strome, 2010). We propose that the NPC-like properties of P granules provide a specialized hydrophobic microenvironment in germ cells that may facilitate post-transcriptional processing events while selectively excluding large protein complexes from gaining access to mRNAs and endogenous sRNAs. The aggregation tendencies of P granules may help stabilize or sequester regulatory proteins, such as translation initiation factors and Argonautes (Amiri et al., 2001; Updike and Strome, 2009), within the granules. Reconstituting germ granules in the intestine offers a way to further investigate germ granule assembly and function.

Materials and methods

Microscopy

A Velocity acquisition and spinning disk confocal system (PerkinElmer) fitted on an inverted microscope (Eclipse TE2000E; Nikon) with an EM-CCD camera (Hamamatsu Photonics) was used to acquire all images. A Nikon 60x oil objective (NA 1.4) was used to acquire Figs. 1, 3, 4 (A, B, and D), and 5 (A–D and F). A Nikon 40x oil objective (NA 1.3) was used to acquire Figs. 2, 4 (C and E), and 5 E, and Videos 1–3. All images were acquired at room temperature.

Injection of TRITC-labeled dextrans

A final concentration of 4 mg/ml of TRITC-labeled dextran (10, 40, 70, and 155 Kd; Sigma-Aldrich) in injection buffer (20 mM KPO4, pH 7.5, 3 mM K citrate, and 2% polyethylene glycol-6000) was injected into the syncytial gonad of worms as described previously (Galy et al., 2003). Injected bns-1[pie-1p::GFP::PGL-1] worms were incubated 4–4.5 h at 24°C, then dissected to harvest embryos. 28–50 cell stage embryos were mounted on an agar pad in egg buffer (25 mM Hepes, 120 mM NaCl2, 2 mM MgCl2, 2 mM CaCl2, and 48 mM KCI3) and single planes of green and red channels were acquired every 10 for 2 min.

Dispersal of GFP granules with aliphatic alcohols

Gonads expressing GLH-1::GFP, GFP::PGL-1, GFP::SPD-2, or both GFP::SPD-2 and PGL-1::RFP were dissected in 15 µl egg buffer on polylysine-coated coverslips and mounted drop up on an inverted microscope. Fluorescence images were acquired every 2 s for 1 min. At 20 s, an equal 1.5µl volume of egg buffer alone (control) or egg buffer containing 10% (final 5%) 1,6-hexanediol [Acros Organics] in egg buffer or 10% (final 5%) 1,2,3 hexanetriol [Acros Organics] was added. 20 dissected gonads were imaged for each transgene in the three solutions (coded A, B, or C to allow the experiment to be scored blind). Velocity was used to find and count GFP granules larger than 200 nm2 with an intensity that was at least 40% of saturation. Typically, the first time point contained ~100–200 granules defined by these parameters. P-granule quantity was then normalized using the average of the first nine time points. Image sequences were not corrected for photobleaching, accounting for the slopes before and after the addition of each solution at t = 20 s. An iterative permutation test was used to calculate the significance between the plotted lines.

Strain construction

C. elegans strains were maintained as described previously (Brenner, 1974). Unless otherwise stated, transgenic lines were created by injecting 20 ng/µl of the plasmid and generating simple extrachromosomal arrays. For each transgene, at least 10 transgenic lines were isolated and examined for expression consistency. The line carrying the most stable array was used for this study. These lines are listed in Table S1.

Intestinal expression experiments

For consistency in comparing transgenic expression at the same developmental time point, images were acquired in comma to early 1.5-fold embryos, except for the early larval stages shown in Figs. 4, C and E, and 5 E. 10–µm projections are shown in Figs. 3–5 with the exception of Fig. 5, A–D, which required single planes to show the subcellular location of granules.

Immunocytochemistry

Embryos and worms were fixed using methanol/acetone (Strome and Wood, 1983). Antibody dilutions were 1:30,000 rabbit anti–PGL-1 (Kawasaki et al., 1998), 1:5,000 mouse anti-NPC mAb414 (Covance; Blobel, 1985), 1:5,000 rat anti–PGL-3 (Kawasaki et al., 2004), 1:1,000 rabbit anti–GLH-1 (Gruisell et al., 1996; Kawasaki et al., 2004), and 1:400 Alexa Fluor 488 goat anti–rabbit IgG, Alexa Fluor 594 goat anti–mouse IgG, Alexa Fluor 488 goat anti–rat IgG, and Alexa Fluor 594 goat anti–rabbit IgG (Invitrogen). For this study. These lines are listed in Table S1.

gh-1 RNAi

gh-1 RNAi by feeding (Spike et al., 2008) was performed on wild-type and hpl-2(tm1489) worms grown at 26°C.

Online supplemental material

Video 1 is a time-lapse movie of Fig. 2 A showing GLH-1::GFP granules (green) in dissected C. elegans gonads exposed to HD, HT, and egg buffer alone. Video 2 is a time-lapse movie showing cytoplasmic and perinuclear GLH-1::GFP granules (green) in a dissected C. elegans gonad exposed to HD. Video 3 is a time-lapse movie showing GFP::SPD-2 (green) and GFP::RFP (red) granules in a dissected C. elegans gonad exposed to HD. Table S1 is a list of all C. elegans strains used in this study. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201010104/DC1.

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References


Shulg


Supplemental material

Updike et al., http://www.jcb.org/cgi/content/full/jcb.201010104/DC1

Video 1. **Time-lapse confocal microscopy of GLH-1::GFP granules (green) in dissected C. elegans gonads exposed to HD, HT, and egg buffer (EB) alone.** Images collected on a Volocity spinning disk confocal system (PerkinElmer) with an inverted microscope (Eclipse TE2000-E; Nikon) at 1 frame every 2 s for 1 min were compressed to 3 s.

Video 2. **Cytoplasmic and perinuclear GLH-1::GFP granules (green) in a C. elegans gonad disperse after exposure to HD.** Shown are images from time-lapse confocal microscopy collected on a Volocity spinning disk confocal system (PerkinElmer) with an inverted microscope (Eclipse TE2000-E; Nikon) at 1 frame every 2 s for 1 min and then compressed to 3 s. The movie zooms in to oocytes and replays.

Video 3. **Time-lapse confocal imaging of GFP::SPD-2 (green) and PGL-1::RFP (red) granules in a dissected C. elegans gonad exposed to HD.** Images collected on a Volocity spinning disk confocal system (PerkinElmer) with an inverted microscope (Eclipse TE2000-E; Nikon) at 1 frame every 2 s for 1 min were compressed to 3 s.
<table>
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<tr>
<th>Name</th>
<th>Genotype</th>
<th>Construction</th>
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<tr>
<td>SS947</td>
<td>bnEx35(glh-1p::GLH-1::GFP + myo-3p::dsRed2)</td>
<td>Microinjection of 0.5 ng/µl linear pDU12 (Fosmid WRM0640aH10 containing GLH-1 was recombineered [Sarov et al., 2006] to insert GFP between the GLH-1 stop and its 3’ UTR) and 0.5 ng/µl linear coinjection marker pER1 (myo-3p::dsRed2) from the David Miller laboratory and 40 ng/µl PvuII-digested N2 genomic filler DNA</td>
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<td>SS747</td>
<td>bns1(pie-1p::GFP::PLG-1)</td>
<td>Parental strains were OC95 containing GFP::SPD-2 and FT459 containing PGL-1::RFP</td>
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<td>bns2(pie-1p::GFP::SPD-2); {myo-2p::PLG-1::RFP}</td>
<td>Microinjection of pDU16 (5-kb el2 promoter from pOLB10 [Olaf Bossinger laboratory] cloned between PacI and XbaI of the GFP vector pD95.77 [Andy Fire laboratory]) and coinjection marker pRF4(dominant rol-6 marker)</td>
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<td>bns3(e2p::GFp + rol-6)</td>
<td>Microinjection of pDU23 (GFP from pDU16 cut out at AgeI and EcoRI sites and replaced with a worm mCherry amplicon from pCFJ104 [Erik Jorgensen laboratory] and coinjection marker pPD118.33(myo-2p::mCherry from the Andy Fire laboratory)</td>
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<td>bns4(e2p::mCherry + myo-2p::GFP)</td>
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<td>Microinjection of pDU27 (amplicon coding for the first 174 aa of NPP-4 inserted into the Xmal site of pDU16 replacing the 5’ Xmal with NgaMV to allow subsequent insertion of multimerized repeats) and coinjection marker pCFJ90(myo-2p::mCherry from the Erik Jorgensen laboratory)</td>
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<td>SS972</td>
<td>bns16(e2p::PGL-1::GFP + myo-2p::mCherry)X</td>
<td>Microinjection of pDU36 (GFP removed from pDU26 so that PGL-1 terminates followed by the unc-54 3’ UTR and coinjection marker pCFJ104</td>
</tr>
<tr>
<td>SS994</td>
<td>bns17(e2p::PGL-1::GFP + myo-2p::mCherry)</td>
<td>Microinjection of pDU26 with no coinjection marker</td>
</tr>
<tr>
<td>SS1015</td>
<td>bns18(e2p::PGL-1::GFP)</td>
<td>Microinjection of pDU26 with no coinjection marker</td>
</tr>
<tr>
<td>SS1016</td>
<td>bns19(e2p::PGL-1::GFP)</td>
<td>Microinjection of pDU26 with no coinjection marker</td>
</tr>
<tr>
<td>SS1017</td>
<td>bns20(e2p::PGL-1::GFP)</td>
<td>Microinjection of pDU25 with no coinjection marker</td>
</tr>
<tr>
<td>SS1021</td>
<td>bns21(e2p::PGL-1::GFP); bns22(e2p::PGL-1::mCherry)</td>
<td>Parental strains were SS973 and SS994</td>
</tr>
<tr>
<td>SS1022</td>
<td>bns23(e2p::PGL-1::GFP); bns24(e2p::PGL-1::mCherry)</td>
<td>Parental strains were SS973 and SS994</td>
</tr>
<tr>
<td>SS996</td>
<td>bns25(e2p::PGL-1::GFP + myo-2p::mCherry); bns26(e2p::PGL-1 + myo-3p::mCherry)</td>
<td>Parental strains were SS973 and SS994</td>
</tr>
<tr>
<td>SS1006</td>
<td>bns27(e2p::PGL-1::GFP + myo-2p::mCherry); bns28(e2p::PGL-1 + myo-3p::mCherry)</td>
<td>Parental strains were SS973 and SS994</td>
</tr>
<tr>
<td>SS1019</td>
<td>bns29(e2p::PGL-1::GFP)</td>
<td>Microinjection of pDU39 (full-length GLH-1 containing two additional FG repeats which were sequentially inserted into the Scal site of pDU22)</td>
</tr>
<tr>
<td>SS1030</td>
<td>bns30(e2p::PGL-1::GFP)</td>
<td>Microinjection of pDU42 (full-length PGL-1 inserted into the Xmal site of pDU32)</td>
</tr>
<tr>
<td>SS1031</td>
<td>bns31(e2p::PGL-1::GFP)</td>
<td>Microinjection of pDU43 (full-length PGL-1 inserted into the Xmal site of pDU34)</td>
</tr>
</tbody>
</table>
References

