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Molecules and Cells

Two Mutations in *pab-1* Encoding Poly(A)-Binding Protein Show Similar Defects in Germline Stem Cell Proliferation but Different Longevity in *C. elegans*

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Four new alleles, *bn116*, *bn117*, *bn118*, and *bn119,* on LG I were isolated in *C. elegans* with defects in germline stem cell proliferation. Using genetic mapping and snip-SNP mapping, *bn116*, *bn117, bn118,* and *bn119* were located 5.0 cM, 1.3 cM, 2.3 cM, and 5.0 cM, respectively, to the right of *dpy-5* on LG I. Further, *bn116* and *bn119* were grouped into the same complementation group by a complementation test. They are loss-of-function recessive alleles that produce homozygous sterile worms whose germ cells do not proliferate during larval development. However, the worms contained normal somatic gonadal structures including distal tip cells and gonadal sheath cells, suggesting that the defect in germline proliferation was not caused by the absence of somatic signaling. Although DAF-16 was localized to the nucleus in all four mutants, the life span was extended only in the three mutants except *bn116*. These results suggest that the defect in germline stem cell proliferation, the presence of normal somatic gonadal tissues, and DAF-16 nuclear translocation were sufficient for extending the lifespan of the *bn117, bn118,* and *bn119* mutants, but not the *bn116* mutant. Intriguingly, *bn116* and *bn119* were identified as two different mutations on the same gene, *pab-1,* which encodes a poly(A)-binding protein. Therefore, although the *bn116* and *bn119* mutations cause similar defects in germ cell proliferation, their effects on life span are different.

INTRODUCTION

The transmission of genetic information across generations is an essential process for the organisms, and the germline is responsible for this process. If there are defects in the germline that cause sterility, offspring will not be produced. Therefore, elucidating the molecular basis of germline development, including mitotic germ cell division, meiosis, and gametogenesis is important.

C. elegans is an excellent model system for studying germline development because it simultaneously displays mitotically proliferating germ cells, early meiotic germ cells, as well as differentiating oocytes and sperm within a transparent gonad tube (Hirsh et al., 1976). Furthermore, a transparent body allows the observation of the inside of the gonad and the identification of phenotypic alterations in the germ line. There are two somatic gonad precursor cells, Z1 and Z4, and two primordial germ cells, Z2 and Z3, in the newly hatched first (L1) stage larva. The number of germ cells is gradually increased during larval development, and the adult hermaphrodite contains more than 1,000 germ cells (Riddle et al., 1997; William et al., 1988). During this process, germline stem cells are continuously proliferated mitotically to provide germ cells that are differentiated into oocytes or sperm (Hirsh, 1976).

Two major factors that regulate germline stem cell proliferation are the GLP-1 receptor expressed in germline stem cells and the LAG-2 ligand expressed in somatic gonadal distal tip cells (DTCs). They promote mitotic proliferation of nearby germ cells (reviewed in Byre and Kimble, 2009). Other germline proliferation (*glp*)-defective mutants, *glp-2* (unpublished), *glp-3* (Kadyk et al., 1997), and *glp-4* (Beanan and Strome, 1992), also affect mitotic germ cell proliferation. Cell cycle regulators are also important for the regulation of germline stem cell proliferation. For example, a loss-of-function mutant of *cdc-25.1*, which encodes a phosphatase that dephosphorylates the inhibitory phosphate on CDK-1, shows severe defects in germline stem cell proliferation (Kim et al., 2009). A screen using a combination of cDNA subtraction and RNAi testing identified 15 germline-specific genes that are essential for *C. elegans* germline development (Hanazawa et al., 2001). In the screen three genes were categorized as *glp* genes. We assume more genes are involved in this process and are yet to be identified.

In this study, we performed forward genetic screening to isolate more *glp* mutants. Forward genetics is a slow process and requires much effort. Reverse genetics with massive RNAi treatment is a more rapid process for screening genes of interest. However, accumulated data obtained from massive RNAi treatment experiments are sometimes not consistent, and moreover, penetrance and expressivity of RNAi phenotypes fluctuate (Fraser et al., 2000; Gonczy et al., 2000; Hanazawa et al., 2001; Maeda et al., 2001). Since the *glp* phenotype has an

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obvious morphology for screening, and due to the increasing availability of powerful genetic tools for fine mapping, such as snip-SNP mapping, we performed a forward genetic screening for new *glp* mutants, and successfully isolated four such alleles, *bn116*, *bn117*, *bn118*, and *bn119* on LG I. In this study, we describe the initial characterization of these mutants.

MATERIALS AND METHODS

Worm culture and strains

Caenorhabditis elegans strains were cultured and manipulated as described (Brenner, 1974). Bristol N2 strain was used as wild type in all experiments. All strains were maintained at 20°C on Nematode Growth Medium (NGM) agar plates containing *Escherichia coli* strain OP50. The following strains were used: SS674: *pab-1(bn116)/+ I,* SS676: *bn117/+ I; him-3(e1147) IV,* SS680: *bn118/+ I; him-3(e1147) IV,* SS681: *pab-1(bn119)/+ I; him-3(e1147) IV,* SS104: *glp-4(bn2) I,* CB61: *dpy-5(e61) I,* CB1072: *unc-29(e1072) I,* TJ1052: *age-1(hx546) II,* CF1038: *daf-16(m86) I*. Before phenotype analysis, SS674, SS676, SS680, and SS681 were outcrossed four times against N2 to get rid of additional mutations. Deficiency strains used were: JK1726: *qDf16/dpy-5(e61) unc-15(e1402) I,* JK1534: *ces-1 (n703) qDf5/unc-29(e193) mec-8(e398) dpy-24(s71) I,* JK1545: *ces-1(n703) qDf8/unc-13(e1091) lin-11(n566) I,* JK1573: *ces-1(n703) qDf6/dpy-14(e188) unc-13(e51) I,* MT2181: *nDf24/unc-13(e1091) lin-11(n566) I.* To examine somatic gonad development of *bn*-sterile mutants, L4 hermaphrodites of strain XA6226: *mrg-1(qa6200)/qC1 dpy-19(e1259) glp-1(q339) [qIs 26] III* or strain DG1575: *tnIs6 [lim-7::GFP + rol-6(su1006)]* were mated with *bn-sterile mutation/hT2[qIs48:myo-2::GFP] (I;III)* males. Worms expressing the LAG-2::GFP or LIM-7::GFP transgene were then observed at the adult stage using a fluorescence microscope (Axioplan 2 or Axioskop 2 MOT, ZEISS). To construct a strain carrying a *bn-sterile mutation* with GFP::DAF-16, *bn-sterile mutation/hT2[qIs48:myo-2::GFP] (I;III)* males were mated with L4 hermaphrodites of strain GR1352: *daf-16(mgDf47) I; xrIs87[daf-16alpha::GFP::DAF-16B+rol-6(su1006)].* The intestinal nuclear localization of GFP::DAF-16 fusion protein was observed at the adult stage using a fluorescence microscope.

Sterile mutant screening and maintenance

Worms were mutagenized with 40 mM ethyl methanesulfonate (EMS) as described previously (Brenner, 1974), followed by screening for recessive sterile mutants as follows: After mutagenizing P0 worms, healthy-looking, late L4-stage F1 worms were transferred to individual plates and allowed to produce self-progeny. Then, F1 clones that produced about one fourth of the F2 sterile progeny were maintained by transferring F2 fertile heterozygous siblings to new plates. Heterozygous mutants were eventually balanced by mating with *mes-3/hT2 [qIs48:myo-2::GFP] (I;III)* males to produce *sterile mutation/hT2 [qIs48:myo-2::GFP] (I;III)* strains. *hT2[qIs48:myo-2:: GFP]* is a GFP-marked variant of *hT2*, a well-characterized effective balancer for the left portion of chromosome I (LG I) and right portion of chromosome III (LG III). Since we only used this balancer to isolate and maintain new sterile mutants, our screen was restricted to sterile mutations located on LG I and III. Homozygous non-GFP sterile worms were easily distinguished from their heterozygous GFP-marked fertile siblings using a fluorescence dissecting microscope (SMZ1500, Nikon).

Genetic mapping, complementation test, and sequencing analyses

Linkage group analysis was performed using triply marked strains, MT465: *dpy-5(e61) I; bli-2(e768) II; unc-32(e189) III* and MT464: *unc-5(e53) IV; dpy-11(e224) V; lon-2(e678) X*. Genetic mapping on LG I was performed by two-factor analysis using *dpy-5(e61)* and *unc-29(e1072).* Deficiency mapping was performed using *qDf5, qDf6, qDf8, qDf16,* and *nDf24,* all of which are located to the right of *dpy-5* on LG I. A complementation test between *bn*-sterile alleles and several other sterile mutant alleles, which were already mapped by others to the right of *dpy-5* on LG I, was also performed. Sequence analysis was performed to confirm the presence of mutations on the *pab-1* locus of the genomic DNA prepared from *bn116* and *bn119* homozygous animals. The DNA was prepared by digesting 50 worms in 50 μl of lysis buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Tween-20, 0.45% NP-40, and 0.01% gelatin) containing 100 μg/ml of proteinase K at 60°C for 1 h and at 95°C for 15 min. The *pab-1* locus was PCR-amplified using the following primer pairs: 5′-CAC GAT GAA ACG AAG TGT AGC-3′ and 5′-CGG GAC ACA AGG ACG CAG G-3′; 5′-CTT CGT CGA CCT TCG AGC G-3′ and 5′-AGC CAT GTA CAA CCC AAC ACA-3′; 5′-GAA CCA TCT GTT GTC CAC CG-3′ and 5′-GCT GCG AAG TCA TGA CTG TC-3′; 5′-GAA GTT TCA AGT CAG TTC CCT-3′ and 5′-CAT TGG GCT ATG CCT ACG TC-3′; 5′-GCC GTG AAG AGC CTC GAA G-3′ and 5′-CGA ACC GTT ACT TGT TTC CGT-3′ (GenBank accession No. NM_001026538). The PCR products were sequenced using AB1310 DNA sequencer (PE Biosystem).

SNP mapping

SNP mapping was conducted using the strain CB4856, a Hawaiian wild isolate with high density of polymorphisms against N2 (Wicks et al., 2001). CB4856 males were mated to *dpy-5*-marked heterozygous *bn-sterile* mutant hermaphrodites, *dpy-5(e61) bn-sterile/dpy-5(e61) +*. The F1 hybrid progeny, *dpy-5(e61) bn-sterile/CB4856,* were allowed to self-fertilize, after which their dumpy non-sterile F2 congenic progeny that caused recombination between *dpy-5* and *bn-sterile* loci were screened. The congenic recombinant worms were then tested for their patterns (either N2 or Hawaiian pattern) of snip-SNP markers. To detect the snip-SNP marker on F21C3, the following primer pairs were used: 5′-AGA TTG AGG CTG AAA TAT GGT G-3′ and 5′-GTC GAG CAG CAC CAG TTA TTG-3′ (GenBank accession No. Z71261).

Immunostaining and microscopy

Immunostaining was performed as previously described (Kawasaki et al., 1998; Strome and Wood, 1983) with minor adaptations. Synchronized animals were placed in 10 μl of M9 buffer on a 0.01% polylysine-coated slide glass, covered with a cover slip, and quickly frozen in liquid nitrogen. Worms in the adult stage were dissected with a 26 1/2-gauge needle to extrude their gonads. The slide glass was recovered from liquid nitrogen, the cover slip quickly removed, and the sample fixed in methanol for 10 min followed by post-fixing in acetone for 10 min at 4°C. Dried specimens were blocked with 30 μl of 3% BSA (bovine serum albumin) in PBS (phosphate-buffered saline) for 1 h at RT in a humidity chamber. After wicking off the BSA, 30 μl of rabbit polyclonal anti-PGL-1 (diluted 1:5,000 in PBS; Kawasaki et al., 1998) was applied. The specimens were incubated in a humidity chamber for 16 h at 4°C, washed three times in PBS for 20 min at 16°C, and treated with 30 μl of 3% BSA for 15 min at 4°C for the second blocking. After wicking off the BSA, goat anti-rabbit IgG-FITC (Santa Cruz Biotechnology, diluted 1:200 in PBS) was applied and incubated for 4 h at 4°C in a humidity chamber in the dark. The specimens were washed 3 times in PBS for 10 min. At the second PBS wash,

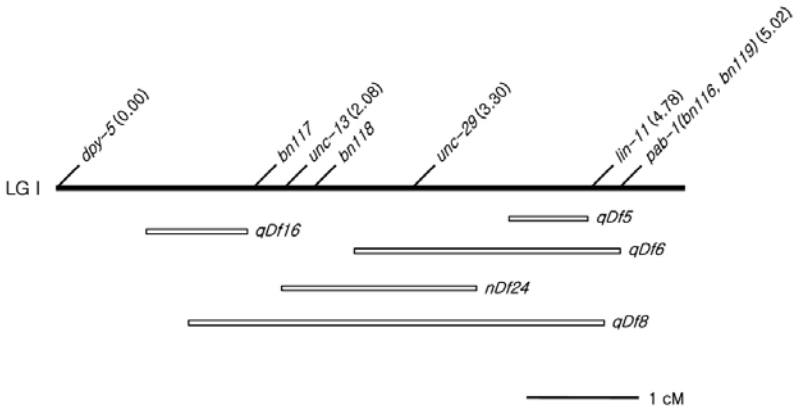


Fig. 1. Genetic mapping of *bn116, bn117, bn118,* and *bn119* on LG I. The four sterile mutations were mapped by two-factor analysis with *dpy-5* or *unc-29*, by deficiency mapping with *qDf5, qDf6, qDf8, qDf16,* or *nDf24,* and by complementation tests against several sterile mutations that have been mapped to the right of *dpy-5* including *pab-1(ar232),* as described in "Materials and Methods". Deduced mapping positions of the four mutations are drawn along with those of the genes and the deficiencies used for the mapping. Numbers in parentheses indicate map positions of the genes in cM (centi-Morgan).

0.5 μg/ml of Hoechst 33342 dye was included for DNA staining. The specimens were rinsed with distilled water then mounted in Sigma Mounting Medium (M1289) beneath a cover slip and sealed with nail polish. The mounted specimens were observed under a fluorescence microscope (Axioplan 2 or Axioskop 2 MOT, ZEISS). Images were acquired using an Orca ERG digital camera (Hamamatsu) and processed with Openlab software (Improvision).

Life span analysis

Life span was analyzed at 20°C. Synchronized embryos were obtained by letting gravid adult worms lay eggs on NGM plates for 3 h, after which the laid embryos were allowed to develop on the plates for 3 days. Then, grown-up, semi-synchronized worms were cloned to individual plates when at the L4 larval stage, transferred to new plates every other day, and scored for viability (sterile mutants were not transferred to new plates). Worms were judged as dead when they did not respond to touch. Statistical analysis was performed using Student's *t*-test and values of $p < 0.05$ were considered as significant.

RESULTS

Isolation of new sterile mutations on LG I

New mutant alleles that show defects in germline proliferation (*glp* mutants) were isolated after EMS mutagenesis as described in "Materials and Methods". By linkage group analysis, four of these alleles, *bn116, bn117, bn118,* and *bn119,* were linked to LG I. They were further mapped on LG I by two-factor mapping with *dpy-5* and *unc-29* (Fig. 1). *bn116, bn117,* and *bn118* were mapped ~5.0 cM, ~1.3 cM, and ~2.3 cM to the right of *dpy-5* (0.0 cM), respectively. *bn119* was mapped ~1.67 cM to the right of *unc-29* (3.30 cM) and was estimated to be at position ~5.0 cM (Fig. 1).

Deficiency mapping was also performed using *qDf5, qDf6, qDf8, qDf16,* and *nDf24,* all of which are located to the right of *dpy-5* on LG I (Fig. 1). *bn117* was complemented by *qDf16* (0.96-1.81 cM), *qDf8* (1.14-4.67 cM), and *nDf24* (1.92-3.72 cM), suggesting that *bn117* is not located within these deficiency loci. However, since this result does not match that of two-factor mapping, and since these deficiencies are not intact and some portions within the 'deleted' regions are still remaining, there is still the possibility that *bn117* is located within these deficiencies. On the other hand, *bn118* was not complemented by *nDf24*, indicating that *bn118* is located within the deficiency locus of *nDf24*. In addition, *bn116* and *bn119* were complemented by *qDf8* and *qDf5* (4.02-4.60 cM) but not by *qDf6* (2.69-5.08 cM), indicating that both *bn116* and *bn119* are located between 4.67 cM and 5.08 cM (Fig. 1).

To further narrow down the genetic loci, snip-SNP mapping was performed against *bn117* and *bn118* using congenic recombinants between Hawaiian strain CB4856 and *bn*-sterile worms marked with *dpy-5(e61)*, as described in "Materials and Methods". In the case of *bn117*, all dumpy non-sterile recombinants showed a Hawaiian pattern for the snip-SNP marker F21C3 (1.87 cM), suggesting that *bn117* is located close to 1.87 cM. In the case of *bn118*, snip-SNP mapping failed to define the location. In the case of *bn116* and *bn119*, all dumpy non-sterile recombinants showed a Hawaiian pattern for the snip-SNP marker F59C6 (5.05 cM), suggesting that both *bn116* and *bn119* are located close to 5.05 cM.

Moreover, a complementation test between *bn*-sterile alleles and several other sterile mutant alleles, which have already been mapped by others to the right of *dpy-5* on LG I, was also performed. We found that *bn116* and *bn119* failed to complement each other. Further, they failed to complement *pab-1(ar232)* (Maciejowski et al., 2005), indicating that *bn116* and *bn119* are two new alleles of *pab-1* (see below). On the other hand, *bn117* and *bn118* complemented all sterile mutants tested so far, including *let-545, let-395, let-378, let-513, let-602, let-611, let-604, let-390, let-544, let-605, let-394,* and *let-610* (McDowall and Rose, 1997a; 1997b).

***bn*-sterile mutants are defective in germline stem cell proliferation**

To characterize the mutant phenotype, the four *bn*-sterile mutants were observed through postembryonic development by Hoechst 33342 nuclear staining and anti-PGL-1 immunostaining (Fig. 2). PGL-1 is one of the constitutive protein components of germ granules in *C. elegans* and is present in all germ cells except mature sperm (Kawasaki et al., 1998). All four mutants possessed normal-looking primordial germ cells, Z2 and Z3, at the L1 larval stage as in wild-type N2 (Fig. 2, A2-E2, F). In N2, germ cells proliferated extensively during the larval stages, reaching over 100 per gonad arm at the L4 larval stage (Fig. 2, A4, F) and even more at the adult stage (Fig. 2, A6). However, in the four *bn*-sterile mutants, the germ cells stopped proliferation during the L2-L3 larval stages, with only a few germ cells present at the L4 stage (Fig. 2, B4-E4, F) and the adult stage (Fig. 2, B6-E6, F).

DTCs and gonadal sheath cells are intact in *bn*-sterile mutants

Germ cell proliferation is controlled by somatic gonadal structures including DTCs (Distal Tip Cells) and gonadal sheath cells. DTCs positively regulate germ cell mitotic proliferation by pre-

Table 1. Lifespan analysis of *bn*-sterile mutants

Strain	Life span (days)	n	*p* value
N2	19.37 ± 0.4	210	
age-1(hx546)	32.98 ± 0.4	211	0.00172
cdc-25.1(bn115)	29.12 ± 1.7	100	0.00551
bn116	18.10 ± 1.8	137	0.42469
bn117	25.42 ± 0.2	122	0.00031
bn118	23.95 ± 1.0	132	0.02012
bn119	25.30 ± 0.1	101	0.00024

Life span was analyzed at 20°C and presented as means ± SEM (days). n, the number of worms examined. *p* values of mean life span compared to that of wild-type N2 are shown.

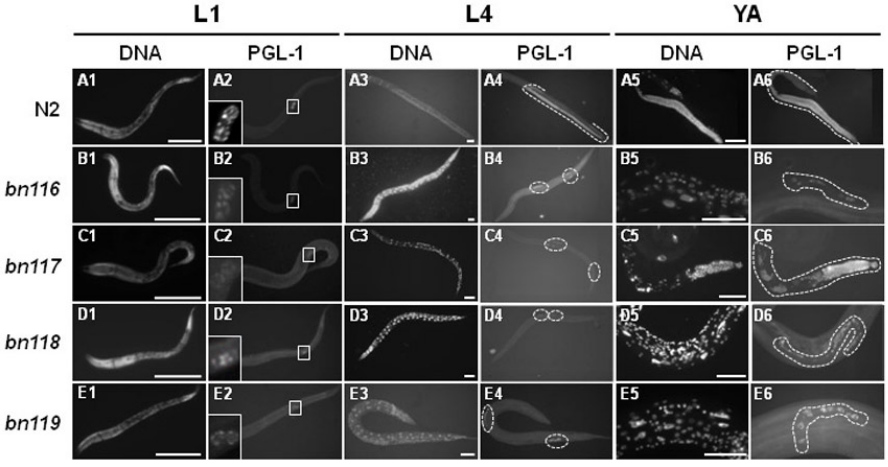
senting the LAG-2 ligand to nearby germ cells expressing the GLP-1 receptor (Henderson et al., 1997). LAG-2-bound GLP-1 is proteolyzed and internalized into germ nuclei for the signal transduction of mitotic proliferation. Gonadal sheath cells are necessary for germline meiotic progression and for ovulation of mature oocytes into spermatheca (McCarter et al., 1997). Therefore, the presence of intact DTCs and gonadal sheath cells is essential for normal postembryonic germline development. To examine whether DTCs and gonadal sheath cells are affected in *bn116*, *bn117*, *bn118*, and *bn119* mutants, the four *bn*-sterile mutant worms containing a transgene, *lag-2::gfp* or *lim-7::gfp*, which are specifically expressed in DTCs or gonadal sheath cells, respectively, were examined with a fluorescence microscope. We found that all the four *bn*-sterile mutants contained two DTCs as well as normal numbers of gonadal sheath cells (Fig. 3). These results indicate that the defect in germline proliferation of the four *bn*-sterile mutants was not caused by a defect in DTCs or gonadal sheath cells.

Life span of *bn*-sterile mutants

The life span of worms is influenced by the proliferating germline stem cells (Arantes-Oliveira et al., 2002). Therefore we examined whether the four *bn*-sterile mutants, which are defective in germline stem cell proliferation, show extended life spans (Table 1). We found that *bn117*, *bn118,* and *bn119* indeed showed extended life spans compared to N2, similar to the *age-1* mutant. The *cdc-25.1(bn115)* mutant, which is defective in germline stem cell proliferation (Kim et al., 2009), also showed an extended life span. Interestingly, we found that the *bn116* mutant did not show an extended life span (Table 1), although it was also defective in germline stem cell proliferation (Fig. 2). Longevity regulated by germline stem cells is DAF-16 activity-dependent (Hsin and Kenyon, 1999). Likewise, we confirmed that the life span extension observed in *bn*-sterile mutants was also DAF-16 dependent, since *daf-16* RNAi treatment of *bn*-sterile mutants suppressed the life span extension (data not shown). DAF-16 is activated by translocation from the cytoplasm to the nucleus upon reception of physiological signals (Henderson and Johnson, 2001). To examine whether DAF-16 is activated in the *bn116* mutant as in other *bn*-sterile mutants, we observed GFP::DAF-16 nuclear localization (Fig. 4). We found that GFP::DAF-16 was localized to the nucleus in all four *bn*-sterile mutants including *bn116* at the adult stage, but not in wild type (Fig. 4, only *bn116* and *bn119* are shown). These results indicate that failure of life span extension in the *bn116* mutant was not due to failure of DAF-16 activation.

bn116* and *bn119* are two new alleles of *pab-1

As described above, *bn116* and *bn119* failed to complement each other. Furthermore, they failed to complement *ar232,* a



F

	Number of germ cells		
	L1	L4	YA
N2	2	134.3 ± 9.2	N. D.
bn116	2	8.0 ± 1.0	7.7 ± 0.5
bn117	2	24.0 ± 14.0	35.6 ± 10.0
bn118	2	14.2 ± 1.6	13.7 ± 4.9
bn119	2	9.4 ± 1.3	6.7 ± 1.2

Fig. 2. *bn116, bn117, bn118,* and *bn119* mutants are defective in germline stem cell proliferation. Postembryonic germline development of the four sterile mutants was observed by immunostaining with anti-PGL-1, a germline-specific marker, along with Hoechst 33342 nuclear staining at the L1 and L4 larval and the young adult (YA) stages. (A1-A6) Wild-type N2, (B1- B6) *bn116*, (C1-C6) *bn117*, (D1-D6) *bn118*, and (E1-E6) *bn119*. Insets in A2-E2 show enlarged images of the two primordial germ cells Z2 and Z3. In A4-E4 and A6-E6, positions of the germ cells are indicated by dotted lines. Scale bars, 50 μm. (F) Numbers of the germ cells counted in N2 and the four sterile mutants at each developmental stage. PGL-1-positive cells were counted as germ cells. Numbers of total germ cells per worm are shown for L1, and numbers of germ cells per gonad arm are shown as means ± SD for L4 and young adult (YA).

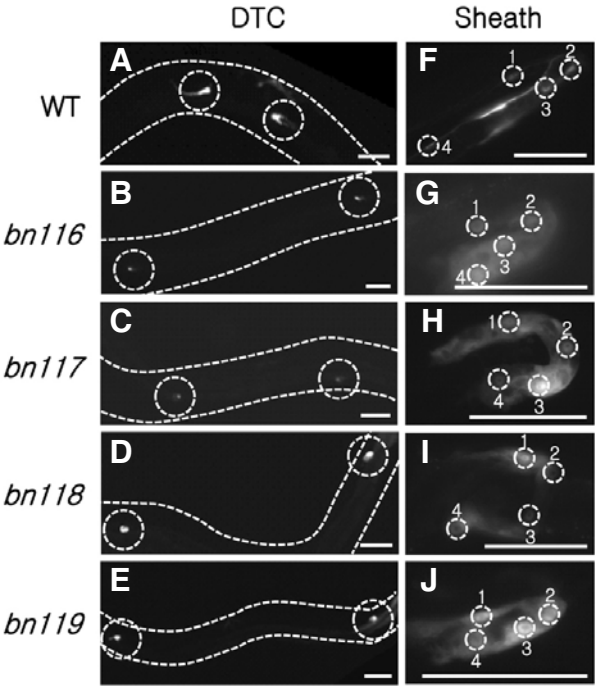


Fig. 3. Somatic gonad development in *bn116, bn117, bn118,* and *bn119* mutants. (A-E) LAG-2::GFP expression in distal tip cells (DTCs). Two DTCs (dotted circles) expressing LAG-2::GFP were observed in all strains. Dotted lines indicate outline of the worms. (F-J) LIM-7::GFP expression in gonadal sheath cells (Sheath). Four pairs of gonadal sheath cells (dotted circles) expressing LIM-7::GFP were observed in all strains. Numbers indicate order of the sheath cell pairs relative to the orientation of the gonad (most distal pair is numbered 1). Scale bars, 50 μm.

pab-1 mutant allele, indicating that *bn116* and *bn119* are two new alleles of *pab-1*. These results were confirmed by identifying mutations in the *pab-1* gene of the *bn116* and *bn119* mutants using DNA sequencing analysis. *pab-1* encodes three isoforms (a, b, and c) of poly(A)-binding protein due to alternative splicing (Fig. 5). Genomic sequencing revealed that *bn116* has a single base-pair substitution at position 1863, which should affect all three isoforms. *bn119* also has a single base-pair substitution at position 64, which should affect only isoforms a and c (Fig. 5).

DISCUSSION

In this study, four new alleles, *bn116, bn117, bn118*, and *bn119*, with defects in germline stem cell proliferation were isolated and characterized. By genetic mapping and a complementation test, we identified that *bn116* and *bn119* are two new alleles of *pab-1*, which encodes a poly(A)-binding protein. The genetic loci of *bn117* and *bn118* remain to be further identified. In the four *bn*-sterile mutants, the two primordial germ cells, Z2 and Z3, were present, but their postembryonic mitotic proliferation was prohibited during the L2-L3 stages. The presence of Z2 and Z3 in newly hatched *bn*-sterile mutant larvae suggests that either their gene products are not required for the formation of Z2 and Z3 or that their maternally-loaded gene products support the formation. The four *bn*-sterile mutants contained normal numbers of DTCs and gonadal sheath cells, suggesting that the defect in germline proliferation was not caused by a defect in somatic gonad cells. Excessive apoptotic germ cells were not observed in the four *bn*-sterile mutants when examined with Acridine-Orange (AO) staining (data not shown). AO is a fluorescent dye that has been used to specifically stain apoptotic cells in live animals (Abrams et al., 1993; White et al., 1994). Engulfed apoptotic germ cells are specifically stained in worms that ingest AO-labeled bacteria (Lettre et al., 2004). This result suggests that the low number of germ cells in *bn*-sterile mutants is not a consequence of enhanced apoptosis in germ cells.

It was reported that the reproductive system influences the aging process in *C. elegans* via DAF-16 activation (Arantes-Oliveira et al., 2002). When the germline precursor cells Z2 and Z3 were ablated, the life span of *C. elegans* was extended (Hsin and Kenyon, 1999).

In addition, several germline mutants including *mes-1(bn7)*, which lacks germline precursor cells (Capowski et al., 1991), and *glp-1(q158)*, whose germ cells are severely underproliferated (Austin and Kimble, 1987), showed extended life spans (Arantes-Oliveira et al., 2002). As we expected, three of the four *bn*-sterile mutants showed extended life spans that were dependent on DAF-16 activity. However, the *bn116* mutant showed a mean life span that was indistinguishable from that of wild-type N2 after scoring more than a hundred individuals (Table 1, $p = 0.42469$, n = 137). Since *bn116* and *bn119* are two mutations of the same gene, *pab-1*, and since the *bn119* mutant

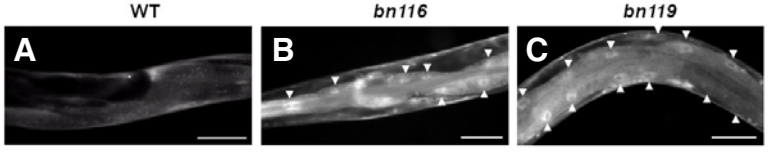


Fig. 4. Nuclear localization of GFP::DAF-16 in *bn116* and *bn119* mutants. *bn116* and *bn119* mutant worms carrying a *gfp::daf-16* transgene were grown to adults on normal NGM plates containing bacteria, and subcellular localization of the GFP::DAF-16 fusion protein in intestine and other cells was observed by fluorescence microscopy. (A) GFP::DAF-16 localization in wild type (WT). No specific localization was observed. (B) GFP::DAF-16 localization in *bn116* mutant. (C) GFP::DAF-16 localization in *bn119* mutant. GFP::DAF-16 was translocated to nuclei (white triangles) in *bn116* and *bn119* mutants. Scale bars, 50 μm.

Fig. 5. *bn116* and *bn119* are two mutant alleles of *pab-1*. *pab-1* encodes three isoforms (a, b, and c) of poly(A)-binding protein due to alternative splicing. *bn116* has a single base-pair substitution from G to A at position 1863, and *bn119* has a single base-pair substitution from C to T at position 64. Black boxes and carets indicate exons and introns, respectively.

showed an extended life span, the effect of *bn116* on life span must be allele-specific and not gene-specific. Genomic sequencing revealed that *bn116* has a single base-pair substitution at position 1863 on *pab-1* that changes TGG to a TGA stop codon, which should affect all three isoforms, resulting in the production of three relatively large truncated proteins. On the other hand, *bn119* has a single base-pair substitution at position 64 on *pab-1* that changes CAA to a TAA stop codon, which should affect only two of the three isoforms and resulting in the production of one intact protein and two small truncated proteins. Alterations in protein production may cause differences in life span between these two alleles. How two of the *pab-1* mutant alleles cause different longevity remains to be elucidated.

Germline stem cells are essential for providing germ cells that will differentiate into oocytes or sperm. For this purpose, germline stem cells continuously divide until the animals stop reproduction. Therefore, the molecular mechanism for controlling germline stem cell proliferation is essential to the organism, and thus, may be tightly linked to the mechanism that controls life span. Further analysis of *bn*-sterile mutants isolated in this study may provide clues about this issue.

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