**om92, a glp-1 enhancer mutation, is an allele of ekl-1**

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**Abstract**

Germline stem cell proliferation in *C. elegans* requires activation of the GLP-1/Notch receptor, which is located on the germline plasma membrane and encoded by the *glp-1* gene. We previously identified several genes whose products directly or indirectly promote activity of the GLP-1 signaling pathway by finding mutations that enhance the germline phenotype of a *glp-1(ts)* allele, *glp-1(bn18)*. Here, we report phenotypic and molecular analysis of a new *ekl-1* allele, *ekl-1(om92)*, that enhances the *glp-1(bn18)* phenotype. *ekl-1(om92)* is a 244 bp deletion predicted to generate a frameshift and premature termination codon, yielding a severely truncated protein, suggesting it is a null allele.
Figure 1. *glp-1*(*bn18*) enhancer allele om92 disrupts germline function and is likely a null allele of the *ekl-1* gene:
Asterisks indicate the distal tip cell. Arrows indicate location of sperm. Arrowheads indicate location of oocytes. (B) Fluorescence images of representative DAPI-stained germlines from young adult hermaphrodites of the indicated strains raised at 20 °C. Marking symbols are the same as in (A). (C) Screenshot of ekl-1 gene location on LG I and gene organization from Wormbase (WS285, https://wormbase.org) indicating location and length of the ekl-1(om92) deletion. (D) Partial sequence alignment of ekl-1(om92) and ekl-1(+) genomic sequence indicating precise extent and location of the ekl-1(om92) deletion. Exon sequences are uppercase while intron sequences are lowercase. WT, wildtype. (E) Schematic diagram of predicted proteins encoded by ekl-1(om92) (above) and ekl-1(+) (below). Red boxes in the predicted wildtype EKL-1 protein indicate locations of Tudor domains according to UniProt (Release 2022_03, https://www.uniprot.org/). The black box in the predicted mutant EKL-1 protein indicates the extent of the amino acid sequence changes caused by the ekl-1(om92) deletion and resulting frameshift. The C-terminal amino acid sequence resulting from the frameshift (after residue T81) is shown. The predicted length of the mutant EKL-1 protein is 100 amino acids, and the wildtype protein is 606 amino acids.

**Description**

We have reported the identification of numerous genes that promote GLP-1/Notch signaling in the *C. elegans* germline through genetic analysis of EMS- and UV/TMP-induced mutations in a sensitized genetic background. Specifically, we have found a series of *ego* (enhancer of *glp*-gene) alleles that genetically enhance the reduced germline proliferation observed in a temperature-sensitive allele of *glp-1*, *glp-1(bn18)* (Qiao, et al., 1995; Smardon, et al., 2000; Liu and Maine, 2007; She, et al., 2009; Lissemore, et al., 2018). Molecular characterization of these *ego* alleles revealed genes that directly or indirectly promote GLP-1/Notch signaling, including the transcriptional regulator *lag-1*, cell cycle regulatory genes *cye-1* and *cdk-2*, and several genes encoding small RNA machinery components (*drh-3, ekl-1, ego-1*), among others (Qiao, et al., 1995; Smardon, et al., 2000; She, et al., 2009; Fox, et al., 2011). We present here molecular and phenotypic analysis of another allele from our previously reported UV/TMP screen for *ego* alleles, *om92*.

Wildtype young adult hermaphrodites have active germline proliferation (i.e., a progenitor zone) in the distal gonad arm (Figure 1A and 1B). As germline cells move proximally, they enter meiosis and in adults undergo oogenesis; oocytes mature in the proximal region prior to fertilization in the spermatheca. *unc-32(e189) glp-1 (bn18)* young adult hermaphrodites at 20 °C have reduced GLP-1 signaling, leading to a reduced progenitor zone compared to wildtype (Figure 1A and 1B). *unc-32(e189)* is tightly linked to *glp-1* and is used here as a marker for the presence of *glp-1(bn18)*. The overall organization of the *glp-1(bn18)* germline at 20 °C is the same as in wildtype although the size is somewhat smaller. Young adult hermaphrodites homozygous for *om92*, a UV/TMP-induced mutation, are sterile but have a substantial germline (Figure 1A and 1B). The distal region contains a progenitor zone, and more proximal germ cells undergo meiosis, forming sperm and sometimes oocytes; when present, oocytes are small and morphologically abnormal, as in the examples shown here. In addition, some oocytes appear to be aneuploid or to have a chromosome-pairing defect (i.e., > 6 DAPI-bright foci at diakinesis). Sperm appear normal and are located in the spermatheca. *om92; unc-32(e189) glp-1(bn18)* young adult hermaphrodites have abnormal germlines without distal mitotic cells or meiotic cells, and the size of the germline is correspondingly much smaller than wildtype (Figure 1A and 1B). Oocytes are absent, and sperm are visible scattered throughout the gonad arm. Based on this phenotype, it appears the germline progenitor zone was not maintained during larval development; instead, all germ cells entered meiosis and underwent spermatogenesis prior to young adult stage. Because *om92; unc-32(e189) glp-1(bn18)* germlines do not retain germline stem cells whereas *om92* and *glp-1(bn18)* single mutant homozygotes do, *om92* meets the criterion for an *ego* mutation.

To determine the molecular identity of *om92*, we used the one-step whole genome sequencing and SNP mapping method (Doitsidou, et al., 2010) to delimit the mutation to the region between 6.5-7.5 Mbp on chromosome I. Variant calling (Smith, et al., 2016) revealed a single novel candidate mutation within that interval, a 244 bp deletion in the *ekl-1* gene, that was confirmed by Sanger sequencing of PCR amplicons from *om92* homozygotes (Figure 1C and 1D). *ekl-1* encodes a Tudor domain protein with multiple known germline functions, including chromosome segregation and small RNA biogenesis and stability (Rocheleau, et al., 2008; Claycomb, et al., 2009; Thivierge, et al., 2011). She, et al. (2009) previously showed, using RNAi and mutational analysis, that loss of *ekl-1* function enhances *glp-1(bn18)*. The *om92* deletion removes parts of exons 2 and 3 along with all of intron 2 and is predicted to generate a frameshift downstream of codon 81 (Figure 1E, black box in upper figure). Full-length EKL-1 is predicted to include 606 amino acids. In contrast, the truncated EKL-1 protein is predicted...
to encode only 100 amino acids and lack both Tudor domains, which have been shown in other organisms to bind to methylated amino acid residues in histones and other proteins (Lorton and Shechter, 2019), hence it is very likely to be non-functional. Thus, ekl-1(om92) should be considered a null mutation.

The phenotype of ekl-1(om92) homozygotes generally resembles the phenotypes reported for ekl-1(om56) and ekl-1(om83) (She, et al., 2009). Specifically, we note the presence of small, abnormal oocytes in a subset of mutants. In addition, She, et al., 2009 noted incompletely penetrant chromosomal pairing defects in the oocytes of ekl-1(om53) and ekl-1(om86), similar to what we observe here for ekl-1(om92). (These similarities were not noted in the preliminary phenotypic analysis conducted at the time ekl-1(om92) was isolated, and therefore She et al. did not analyze ekl-1(om92).) We do not, however, observe variable sperm morphology in ekl-1(om92) as She, et al., 2009, reported for ekl-1(om56) and ekl-1(om83). We do not have a definitive explanation for the observed phenotypic differences, but they might be attributed to the substantial differences in the predicted protein products from the different alleles. The ekl-1(om92) predicted protein product contains only 100 amino acids and lacks both Tudor domains. In contrast, ekl-1(om83) is a deletion mutation resulting in a predicted protein product containing 314 amino acids while ekl-1(om56) is a nonsense mutation resulting in a predicted protein product with 318 amino acids. Both proteins would contain the first Tudor domain which might result in these proteins having some residual function that the protein encoded by ekl-1(om92) lacks.

Methods

Nematode strains and worm maintenance:
Worms were maintained using standard methods.

Microscopy:
DIC and DAPI microscopy were carried out essentially as described in Lissemore, et al., 2018.

SNP mapping/whole genome sequencing (WGS):
Combined SNP mapping and WGS were carried out essentially as described (Smith, et al., 2016) using genomic DNA isolated from om92; unc-32(e189) glp-1(bn18) sterile homozygous worms.

PCR and Sanger DNA sequencing:
Single worm PCR was carried out on ekl-1(om92) homozygous sterile worms and N2 worms using standard methods. Two different pairs of primers flanking the putative deletion were tested, eklA/eklB and eklC/eklD, and each pair gave the same results. Sanger DNA sequencing was carried out by Eton Bioscience, Inc. (San Diego, CA); primers used for PCR were also used for sequencing amplicons.

Reagents

Primers

| PRIMER NAME | SEQUENCE (5’--->3’) |
|-------------|---------------------|
| eklA        | ATGATTGCAGTGAGATCCCG |
| eklB        | TGGTCACAGATAATGGGGA |
| eklC        | CCGAATCCGGAAGAACCCT |
| eklD        | GGAGAGAAACAAGTCCACAC |

Strains
| STRAIN | GENOTYPE | AVAILABLE FROM |
|--------|----------|---------------|
| N2     | *Caenorhabditis elegans* | CGC           |
| JL50   | ekl-1(om92)/+ | Lissemore Lab |
| JL51   | ekl-1(om92)/+; unc-32(e189) glp-1( bn18) | Lissemore Lab |
| JL52   | ekl-1(om92) I/hT2[ bli-4(e937) let-?( q782) qIs48]; unc-32(e189) glp-1( bn18)/ hT2[ bli-4(e937) let-?(q782) qIs48] | Lissemore Lab |

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References

Claycomb JM, Batista PJ, Pang KM, Gu W, Vasale JJ, van Wolfswinkel JC, et al., Mello CC. 2009. The Argonaute CSR-1 and its 22G-RNA cofactors are required for holocentric chromosome segregation. Cell 139: 123-34. PubMed ID: [19804758](#)

Doitsidou M, Poole RJ, Sarin S, Bigelow H, Hobert O. 2010. *C. elegans* mutant identification with a one-step whole-genome-sequencing and SNP mapping strategy. PLoS One 5: e15435. PubMed ID: [21079745](#)

Fox PM, Vought VE, Hanazawa M, Lee MH, Maine EM, Schedl T. 2011. Cyclin E and CDK-2 regulate proliferative cell fate and cell cycle progression in the *C. elegans* germline. Development 138: 2223-34. PubMed ID: [21558371](#)

Lissemore JL, Connors E, Liu Y, Qiao L, Yang B, Edgley ML, et al., Maine EM. 2018. The Molecular Chaperone HSP90 Promotes Notch Signaling in the Germline of *Caenorhabditis elegans*. G3 (Bethesda) 8: 1535-1544. PubMed ID: [29507057](#)

Liu Y, Maine EM. 2007. The Bro1-domain protein, EGO-2, promotes Notch signaling in *Caenorhabditis elegans*. Genetics 176: 2265-77. PubMed ID: [17603118](#)

Lorton BM, Shechter D. 2019. Cellular consequences of arginine methylation. Cell Mol Life Sci 76: 2933-2956. PubMed ID: [31101937](#)

Qiao L, Lissemore JL, Shu P, Smardon A, Gelber MB, Maine EM. 1995. Enhancers of glp-1, a gene required for cell-signaling in *Caenorhabditis elegans*, define a set of genes required for germline development. Genetics 141: 551-69. PubMed ID: [8647392](#)

Rocheleau CE, Cullison K, Huang K, Bernstein Y, Spilker AC, Sundaram MV. 2008. The *Caenorhabditis elegans ekl* (enhancer of ksr-1 lethality) genes include putative components of a germline small RNA pathway. Genetics 178: 1431-43. PubMed ID: [18245826](#)

She X, Xu X, Fedotov A, Kelly WG, Maine EM. 2009. Regulation of heterochromatin assembly on unpaired chromosomes during *Caenorhabditis elegans* meiosis by components of a small RNA-mediated pathway. PLoS Genet 5: e1000624. PubMed ID: [19714217](#)

Smardon A, Spoerke JM, Stacey SC, Klein ME, Mackin N, Maine EM. 2000. EGO-1 is related to RNA-directed RNA polymerase and functions in germ-line development and RNA interference in *C. elegans*. Curr Biol 10: 169-78. PubMed ID: [10704412](#)

Smith HE, Fabritius AS, Jaramillo-Lambert A, Golden A. 2016. Mapping Challenging Mutations by Whole-Genome Sequencing. G3 (Bethesda) 6: 1297-304. PubMed ID: [26945029](#)
Thivierge C, Makil N, Flamand M, Vasale JJ, Mello CC, Wohlschlegel J, Conte D Jr, Duchaine TF. 2011. Tudor domain ERI-5 tethers an RNA-dependent RNA polymerase to DCR-1 to potentiate endo-RNAi. Nat Struct Mol Biol 19: 90-7. PubMed ID: 22179787

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