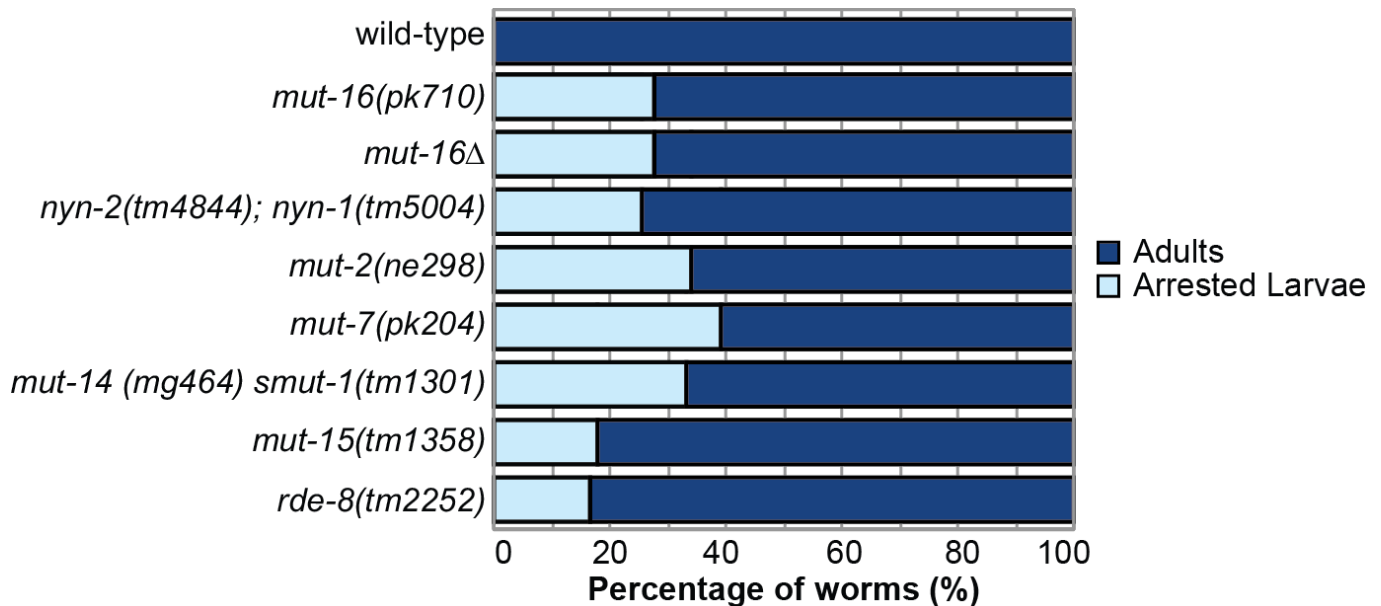# Disruption of the *mutator* complex triggers a low penetrance larval arrest phenotype

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Figure 1: Larval arrest is a shared phenotype of *mutator* mutants. Bar graph shows percentage of synchronized L1 larvae for each *mutator* mutant strain that reach adulthood (dark blue) or arrest at any larval stage (light blue) when cultured at 20°C. For each strain, n=1,000 animals.

Description

Mutator foci are perinuclear granules in the germline of *Caenorhabditis elegans* that are required for the amplification of 22G-small interfering RNAs (siRNAs) (Phillips *et al.,* 2012). These *mutator*-dependent siRNAs act downstream of primary endogenous and exogenous siRNA pathways and are necessary for robust and heritable silencing (Pak *et al.,* 2007; Sijen *et al.,* 2007; Gu *et al.,* 2009; Gent *et al.,* 2010; Vasale *et al.,* 2010; Phillips *et al.,* 2012). There are numerous factors that have been identified that localize to *Mutator* foci and are required for *mutator*-dependent siRNA biogenesis. These *mutator*-class proteins include the core component of *Mutator* foci MUT-16, the nucleotidyl transferase MUT-2, the 3'-5' exonuclease MUT-7, the DEAD-box RNA helicases MUT-14 and SMUT-1, the Zc3h12a-like ribonucleases RDE-8, NYN-1, and NYN-2, and two proteins of unknown function, MUT-15 and RDE-2 (Ketting *et al.,* 1999; Tijsterman *et al.,* 2002; Vastenhouw *et al.,* 2003; Chen *et al.,* 2005; Tops *et al.,* 2005; Phillips *et al.,* 2012; Phillips *et al.,* 2014; Tsai *et al.,* 2015). Additionally, the RNA-dependent RNA polymerase RRF-1 localizes to *Mutator* foci but is redundant with EGO-1 for *mutator*-dependent siRNA biogenesis (Phillips *et al.,* 2012; Gu *et al.,* 2009). It was previously shown that mutations in *mutator*-class genes are sterile at elevated temperature (Ketting *et al.,* 1999; Zhang *et al.,* 2011; Rogers and Phillips, 2020). Recently, we performed a brood size assay using wild-type and *mut-16* hermaphrodites cultured at 20°C. We found that compared to wild-type animals, *mut-16* mutant animals lay fewer eggs (56% fewer eggs laid compared to wild-type animals), and of those eggs, fewer *mut-16* mutant eggs hatch (81% of *mut-16* mutant eggs hatch compared to wild-type, where 100% of the eggs hatch) (Rogers and Phillips, 2020). Furthermore, 100% of wild-type larvae successfully mature to adulthood, whereas only 85% of *mut-16* mutant larvae mature to adulthood (Rogers and Phillips, 2020). The reduced hatching rates and larval arrest of *mut-16* mutant animals had not been previously reported.

Because one phenotype of mutants of the *mutator*-class genes is hopping of transposable elements, and thus they can exhibit spontaneous mutations (Ketting *et al.,* 1999), in this work we first sought to test whether the larval arrest phenotype is found in other *mut-16* mutant alleles and not due to a background mutation in the *mut-16(pk710)* strain. We performed a larval arrest assay in which we counted the total number of individuals that mature to adulthood or arrest as larvae for wild-type (N2) and *mut-16* mutants. We used two *mut-16* alleles, *mut-16(pk710)*, the same allele as the original assay which carries an early stop

codon, and *mut-16(cmp185)* (referred to here as *mut-16Δ*), an in-frame 560 amino acid deletion (Uebel *et al.,* 2018). One thousand L1 stage animals of each strain were plated at 20°C. After seventy-two hours, the developmental stage of the individuals was assessed. We found that 28% of both *mut-16Δ* and *mut-16(pk710)* mutant individuals arrested as larvae compared to 0% of wild-type individuals (Figure 1).

To determine whether larval arrest is a common phenotype amongst other *mutator* mutants, we counted the larval arrest rate for six additional *mutator*-class mutants. We observed that a portion of the L1 stage animals from each *mutator*-class mutant arrested at either the L1 or L2 stage – *nyn-1(tm5004); nyn-2(tm4844)* mutants (26% arrest), *mut-2(ne298)* mutants (34% arrest), *mut-7(pk204)* mutants (39% arrest), *mut-14(mg464) smut-1(tm1301)* mutants (33% arrest), *mut-15(tm1358)* mutants (18% arrest), and *rde-8(tm2252)* mutants (16% arrest) (Figure 1). These data indicate that larval arrest is a low penetrance phenotype found in between 16% and 39% of mutant individuals, and that *mutator*-class proteins, such as MUT-16, play an important role in ensuring the development of *C. elegans*.

While elevated temperature triggers sterility in *mutator*-class mutants, here we show that *mutator*-class mutants also exhibit a larval arrest phenotype at permissive temperature. Larval arrest can occur in *C. elegans* for many reasons, including but not limited to, stressful conditions such as starvation – which could occur due to a lack of food (Johnson *et al.,* 1984), the inability to consume food or perform pharyngeal pumping (Fay *et al.,* 2003; Furuya *et al.,* 2005; Mango, 2007), or the inability to absorb nutrients in the gut (Thieringer *et al.,* 2003) – or mis-regulation of cell cycle components (Boxem *et al.,* 1999; van den Heuvel, 2005), proteasome components (Takahashi *et al.,* 2002), or other pathways that affect development. The individuals assayed in our experiments were not grown under stressful conditions or on densely populated plates. Thus, the underlying cause of larval arrest in *mutator*-class mutants could be due to the inability of the animals to consume food, absorb nutrients, or due to mis-regulation of factors necessary for proper development of *C. elegans*. Previously, we showed that *mut-16* has a maternal and paternal effect on sterility when animals are raised at elevated temperature (25°C) (Rogers and Phillips, 2020). Thus, the low penetrance larval arrest phenotype of *mut-16* mutants could arise from maternal effects, paternal effects, zygotic effects, or a combination. Further experiments will be needed to determine the underlying cause of larval arrest in *mutator*-class mutants. It is interesting to note that when *mut-16* mutant larvae are synchronized by starvation 28% arrest as larvae, whereas when we previously performed a brood size assay, where no L1 starvation occurred, 15% of *mut-16* mutant larvae arrest (Rogers and Phillips, 2020). This difference suggests that, while the larval arrest phenotype of *mutator* mutants can occur either when larvae hatch from eggs in the presence of food or when starved as L1s, starvation may exacerbate the arrest phenotype. Taken together with the reduced egg laying of *mut-16* mutants (Rogers and Phillips, 2020), these findings suggest that MUT-16, and other *mutator*-class proteins, play key roles in both maintaining fertility and promoting development in *C. elegans*.

Methods
Request a detailed protocol

***C. elegans* strains.** All animals were grown at 20°C according to standard conditions (Brenner 1974). All strains are in the wild-type (N2) background and have been outcrossed at least four times.

Larval arrest assay. Worms were synchronized by bleaching and were then plated on NGM plates, 20 L1 stage animals per plate and one thousand individuals per genotype. After 72 hours at 20°C, the number of individuals that reached adulthood or arrested as L1-L2s were counted.

Reagents
N2 – wild-type.

NL1810 – *mut-16(pk710) I.*

GR1747 – *mut-15(tm1358) V.*

GR1948 – *mut-14(mg464) smut-1(tm1301) V.*

WM30 – *mut-2(ne298) I.*

NL1820 – *mut-7(pk204) III.*

FX2252 – *rde-8(tm2252) IV.*

USC880 – *nyn-2(tm4844) I; nyn-1(tm5004) IV.*

USC1148 – *mut-2(cmp42[(mut-2::gfp::3xFLAG)]) mut-16(cmp185[mut-16ΔE-K::mCherry::2xHA]) I.*

We used USC1148 (referred to here as *mut-16Δ*) for the *mut-16* deletion allele. It should be noted that USC1148 contains MUT-2::GFP::3xFLAG, which does not affect the function of MUT-2.

References

Brenner, S. (1974). The Genetics of *Caenorhabditis elegans*. Genetics 77(1), 71–94. PMID: 4366476.

Boxem M, Srinivasan DG, van den Heuvel S. (1999). The *Caenorhabditis elegans* gene ncc-1 encodes a cdc2-related kinase required for M phase in meiotic and mitotic cell divisions, but not for S phase. Development 126(10), 2227-2239. PMID: 10207147.

Chen CC, Simard MJ, Tabara H, Brownell DR, McCollough JA, Mello CC. (2005). A member of the polymerase beta nucleotidyltransferase superfamily is required for RNA interference in *C. elegans*. Curr Bio 15(4), 378-383. doi: 10/1016/j.cub.2005.01.009. PMID: 15723801.

Fay DS, Large E, Han M, Darland M. (2003). lin-35/Rb and ubc-18, an E2 ubiquitin-conjugating enzyme, function redundantly to control pharyngeal morphogenesis in *C. elegans*. Development 130(14), 3319-3330. doi: 10.1242/dev.00561. PMID: 12783801.

Furuya M, Qadota H, Chisholm AD, Sugimoto A. (2005). The *C. elegans* eyes absent ortholog EYA-1 is required for tissue differentiation and plays partially redundant roles with PAX-6. Dev Bio 286(2), 452-463. doi: 10.1016/j.ydbio,2005.08.011. PMID: 16154558.

Gent JI, Lamm AT, Pavelec DM, Maniar JM, Parameswaran P, Tao L, Kennedy S, Fire AZ. (2010). Distinct phases of siRNA synthesis in an endogenous RNAi pathway in *C. elegans* soma. Mol Cell 37(5), 679-689. doi: 10.1016.j.molcel.2010.01.012. PMID: 20116306.

Gu W, Shirayama M, Conte D Jr, Vasale J, Batista PJ, Claycomb JM, Moresco JJ, Youngman EM, Keys J, Stoltz MJ, Chen CC, Chaves DA, Duan S, Kasschau KD, Fahlgren N, Yates JR 3rd, Mitani S, Carrington JC, Mello CC. (2009). Distinct argonaute-mediated 22G-RNA pathways direct genome surveillance in the *C. elegans* germline. Mol Cell 36(2), 231-244. doi: 10.1016/j.molcel.2009.09.020. PMID: 19800275.

Johnson TE, Mitchell DH, Kline S, Kemal R, Foy J. (1984). Arresting development arrests aging in the nematode *Caenorhabditis elegans*. Mech Ageing Dev 28(1), 23-40. doi: 10.1016/0047-6374(84)90150-7. PMID: 6542614.

Ketting RF, Haverkamp TH, van Luenen HG, 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(2), 133-141. doi: 10.1016/s0092-8674(00)81645-1. PMID: 10535732.

Mango SE. (2007). The *C. elegans* pharynx: a model for organogenesis. *WormBook* ed. The *C. elegans* Research Community, WormBook. doi/10.1895/wormbook.1.129.1. http://www.wormbook.org DOI: 10.1895/wormbook.1.129.1

Pak J, Fire A. (2007). Distinct populations of primary and secondary effectors during RNAi in *C. elegans*. Science 315(5809), 241-244. doi: 10.1126/science.1132839. PMID: 17124291.

Phillips CM, Montgomery TA, 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(13), 1433-1444. doi: 10.1101/gad.193904.112. PMID: 22713602.

Phillips CM, Montgomery BE, Breen PC, Roovers EF, Rim YS, Ohsumi TK, Newman MA, van Wolfswinkel JC, Ketting RF, Ruvkun G, Montgomery TA. (2014). MUT-14 and SMUT-1 DEAD box RNA helicases have overlapping roles in germline RNAi and endogenous siRNA formation. Curr Bio 24(8), 839-844. doi: 10.1016/j.cub.2014.02.060. PMID: 24684932.

Rogers AK, Phillips CM. (2020). RNAi pathways repress reprogramming of *C. elegans* germ cells during heat stress. Nucleic Acids Res pii: gkaa174. doi: 10.1093/nar/gkaa174. PMID: 32187370.

Sijen T, Steiner FA, Thijssen KL, Plasterk RH. (2007). Secondary siRNAs result from unprimed RNA synthesis and form a distinct class. Science 315(5809), 244-247. doi: 10.1126/science. 1136699. PMID: 17158288.

Takahasi M, Iwasaki H, Inoue H, Takahashi K. (2002). Reverse genetic analysis of the *Caenorhabditis elegans* 26S proteasome subunits by RNA interference. Biol Chem 383(7-8), 1263-1266. doi: 10.1515/BC.2002.140. PMID: 12437114.

Thieringer H, Mollers B, Dodt G, Kunau WH, Driscoll M. (2003). Modeling human peroxisome biogenesis disorders in the nematode *Caenorhabditis elegans*. J Cell Sci 116(Pt 9), 1797-1804. doi: 10.1242/jcs. 00380. PMID: 12665560.

Tijsterman M, Ketting RF, Okihara KL, Sijen T, Plasterk RH. (2002). RNA helicase MUT-14-dependent gene silencing triggered in *C. elegans* by short antisense RNAs. Science 295(5555), 694-697. doi: 10.1126/science/1067534. PMID: 11809977.

Tops BB, Tabara H, Sijen T, Simmer F, Mello CC, Plasterk RH, Ketting RF. (2005). RDE-2 interacts with MUT-7 to mediate RNA interference in *Caenorhabditis elegans.* Nucleic Acids Res 33(1), 347-355. doi: 10.1093/nar/gki183. PMID: 15653635.

Tsai HY, Chen CC, Conte D Jr, Moresco JJ, Chaves DA, Mitani S, Yates JR 3rd, Tsai MD, Mello CC. (2015). A ribonuclease coordinates siRNA amplification and mRNA cleavage during RNAi. Cell 160(3), 407-419. doi: 10.1016/j.cell.2015.01.010. PMID: 25635455.

Uebel CJ, Anderson DC, Mandarino LM, Manage KI, Aynaszyan S, Phillips CM. (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 genetics 14(7), e1007542. doi: 10.1371/journal.pgen.1007542. PMID: 30036386.

van den Heuvel S. (2005). Cell-cycle regulation. *WormBook*, ed. The *C. elegans* Research Community, WormBook. doi/10.1895/wormbook.1.28.1. http://www.wormbook.org DOI: 10.1895/wormbook.1.28.1

Vasale JJ, Gu W, Thiverge C, Batista PJ, Claycomb JM, Youngman EM, Duchaine TF, Mello CC, Conte D Jr. (2010). Sequential rounds of RNA-dependent RNA transcription drive endogenous small-RNA biogenesis in the ERGO-1/Argonaute pathway. PNAS 107(8), 3582-3587. doi: 10.1073/pnas.0911908107. PMID: 20133583.

Vastenhouw NL, Fischer SE, Robert VJ, Thijssen KL, Fraser AG, Kamath RS, Ahringer J, Plasterk RH. (2003). A genome-wide screen identifies 27 genes involved in transposon silencing in *C. elegans*. Curr Bio 13(15), 1331-1336. doi: 10.1016/s0960-9822(03)00539-6. PMID: 12906791.

Zhang C, Montgomery TA, Gabel HW, Fischer SE, Phillips CM, Fahlgren N, Sullivan CM, Carrington JC, Ruvkun G. (2011). *mut-16* and other *mutator* class genes modulate 22G and 26G siRNA pathways in *Caenorhabditis elegans*. PNAS 108(4), 1201-1208. doi: 10.1073/pnas.1018695108. PMID: 21245313.

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