microPublication BIOLOGY

12/9/2022 - *Open Access*

Functional divergence of orthologous temperature-sensitive mutations in *C. elegans* and *C. briggsae*

Satheeja Santhi Velayudhan[1], Ronald E Ellis[1§]

[1]Rowan University SOM

[§]To whom correspondence should be addressed: ellisre@rowan.edu

Abstract

To learn if orthologous mutations are temperature-sensitive in related species, we studied four *C. briggsae* mutations orthologous to alleles of important *C. elegans* genes. Both *Cel-glp-4(bn2)* and *Cbr-glp-4(v473)* are temperature-sensitive, causing sterility at 25°C. By contrast, *Cel-fog-1*(*q253)* is strongly *ts*, but its ortholog *Cbr-fog-1(v442)* causes a loss-of-function at all temperatures. Finally, the *C. elegans glp-1* alleles *bn18* and *e2141* are *ts* sterile. However, their *C. briggsae* orthologs, *Cbr-glp-1(v429)* and *Cbr-glp-1(v438)* respectively, are wild-type at all temperatures. Thus, a *ts* mutation in one species provides clues about how to design *ts* alleles in another, but all theoretical outcomes are possible.

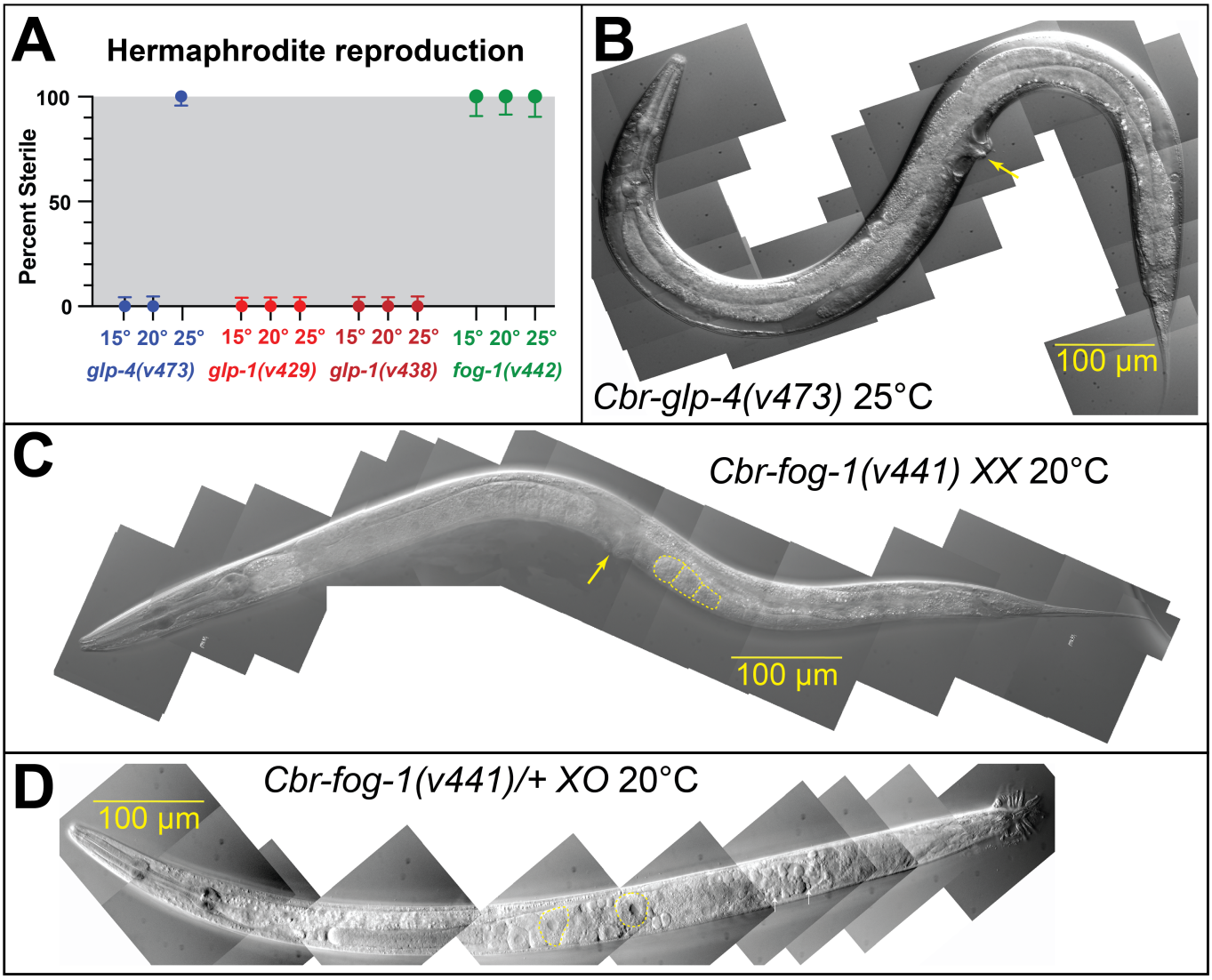

Figure 1. Analysis of temperature-sensitivity in orthologous mutations

(A) Graph showing sterility of *C. briggsae* mutant hermaphrodites at three temperatures. Circles represent the percent displaying the trait, and lines represent 95% confidence limits, calculated using the Wilson/Brown method, implemented by

GraphPad Prism. (B) Differential Interference Contrast (DIC) image of a *glp-4(v473) XX* animal at 25° C. The yellow arrow marks the vulva. There is no visible germ line. Anterior is left and ventral is down. (C) DIC image of a *fog-1(v441) XX* animal at 20°C. The yellow arrow marks the vulva. The uterus is empty and only oocytes are differentiating in the germ line. Three of them are outlined in yellow. (D) DIC image of a heterozygous *fog-1 XO* male, showing oocytes outlined in yellow, as well as sperm (white arrows).

Description

Temperature-sensitive mutations have a wild-type phenotype at the normal (or permissive) temperature and a mutant phenotype at the restrictive temperature, and are versatile tools for studying gene function. The temperature-sensitive proteins may exhibit altered stability, failure to fold or aggregate, resistance to proteolysis, or be cleared more quickly because of partial unfolding, since any of these factors might reduce function at unfavorable temperatures (Poultney et al., 2011). *C. elegans* has several *ts* alleles of critical genes that are helpful for genetic and developmental studies. To determine whether the temperature-sensitive behavior of mutations in *C. elegans* and *C. briggsae* is evolutionarily conserved, we constructed orthologs of four *C. elegans* germline *ts* mutations in *C. briggsae* using CRISPR-Cas9 (Farbound and Meyer, 2015) or TALEN (Wood et al., 2011, Wei et al., 2014) mediated gene editing.

The *glp-4* gene encodes a Valyl Aminoacyl tRNA Synthetase, essential for populating the germline with sufficient numbers of cells for gametogenesis (Rastogi et al. 2015). At the restrictive temperature of 25°C, *C. elegans glp-4(bn2ts)* mutants are sterile, because they produce very few germ cells, all of which arrest at meiotic prophase (Beanan and Strome 1992). By contrast, they are self-fertile hermaphrodites at the permissive temperature of 15°C. There is 87% sequence identity between Cel-GLP-4 and Cbr-GLP-4, and even higher amino acid sequence conservation (96%) within 50 amino acids of the *bn2* allele. This allele alters the Gly 296 residue to aspartic acid, and the orthologous CRISPR mutation in *C. briggsae* changes Gly 293 to aspartic acid. This ortholog, *C. briggsae glp-4(v473) I*, is also temperature-sensitive (Fig.1A); much like its *C. elegans* ortholog, it results in sterility and germ cells that fail to differentiate into gametes only when grown at 25°C (Fig. 1B). Thus, *Cbr-glp-4(v473ts)* provides a valuable new germline-proliferation-defective *ts* allele for *C. briggsae*.

FOG-1 is a Cytoplasmic Polyadenylation Element Binding (CPEB) protein that controls the sperm fate in *C. elegans* (Barton and Kimble, 1990, Luitjens et al., 2000, Jin et al., 2001a). In *fog-1* mutants, germ cells that would normally develop into sperm instead become oocytes. Furthermore, *fog-1* is required for spermatogenesis in both *XO* males and *XX*hermaphrodites. The *C. elegans fog-1* allele *q253ts* is a replacement of Thr 366 by Ile (Jin et al., 2001). It is strongly temperature sensitive, causing *XX* animals to become self-sterile at 25°C but not at the permissive temperature of 15°(Barton and Kimble, 1990). Although Cel-FOG-1 and Cbr-FOG-1 share only 54% sequence identity, they have higher conservation near the site of *q253*. The *Cbr-fog-1(v442) I* allele has an orthologous change (Thr 391 to Ile) that we made using TALEN gene editing. In contrast to *C. elegans q253ts*, the *Cbr-fog-1(v442)* allele causes a loss of function at all temperatures (Fig. 1A). The *XX* hermaphrodites made only oocytes (Fig. 1C) and the *XO* males produced oocytes at both permissive and non-permissive temperatures. As in *C. elegans*, this mutation is semidominant in males (Fig. 1D), which implies conservation of how *fog-1* is regulated in this sex. Since the homozygotes are self-sterile, we balanced *Cbr-fog-1(v442)* with *unc-40(v270)*.

GLP-1 is a notch receptor protein that regulates the mitotic proliferation of germ cells (reviewed by Kimble and Crittenden, 2007). The *C. elegans glp-1* alleles *bn18* and *e2141* are strongly temperature-sensitive, blocking germline proliferation at restrictive temperatures and causing sterility (Kodoyianni et al. 1992, Mello et al., 1994,). *C. elegans glp-1(e2141ts*) is a missense allele that changes arginine 974 to cysteine, and is orthologous to *Cbr-glp-1(v438)*, which we made using CRISPR/Cas9. Although the *C. briggsae* allele changes arginine 955 to cysteine, it is not *ts*, since the mutants appear wild type at both permissive and restrictive temperatures (Fig. 1A).

We also compared *Cel-glp-1*(*bn18*ts) with *C. briggsae*. The *bn18ts* allele replaces alanine 1034 with threonine, and is orthologous to *Cbr-glp-1(v429)*, an alanine 1020 to threonine substitution that we made using CRISPR-Cas9. Unlike *bn18ts*, *Cbr-glp-1(v429)* is not sterile at either the restrictive or permissive temperatures, but instead develops like the wild type.

Although *C. elegans glp-1(ts)* alleles are sterile at the non-permissive temperature, neither of the *C. briggsae* orthologues showed sterility or defective germline development. However, a loss of function allele located nearby in the transcript does exhibit a Glp phenotype. *Cbr-glp-1(v439)* is a frameshift mutation caused by the deletion of nucleotide 2867 T in the coding sequence, near the site of *v438*. These mutant worms are sterile and display the Glp-1 defective phenotype at all temperatures (Rudel and Kimble, 2001). Thus, the *glp-1* loss-of-function phenotype is conserved between *C. briggsae* and *C. elegans* but not the temperature-sensitivity of key alleles.

Taken together, our findings show that one of the four *C. elegans ts* alleles we studied had similar behavior in *C. briggsae*. However, the other alleles were only *ts* in one species. Thus, mutations orthologous to known *ts* alleles can be temperature-sensitive in other species, and provide a promising guide for generating such alleles, but their behavior often differs. Although

high levels of structural conservation might suggest that mutations will behave similarly in both species, we found it hard to predict which alleles will be *ts* without experimental tests. We suspect that *C. elegans* and *C. briggsae* sometimes differ because some genetic backgrounds are more sensitive to perturbation than others.

Reagents

Reagents

Strain	Genotype	Phenotype	Availability
CB4037	*glp-1(e2141) III*		CGC
DG2389	*glp-1(bn18) II.*		CGC
SS104	*glp-4(bn2) I*		CGC
JK560	*fog-1(q253) I*		CGC
RE1206	*Cbr-glp-1(v438) III*	wildtype	Ellis Lab
RE1208	*Cbr-glp-1(v439)/ Cbr-lin-39(bh20) III*	Glp	Ellis Lab
RE1186	*Cbr-glp-1(v429) III*	wildtype	Ellis Lab
RE1274	*Cbr-glp-4(v473ts) I*	TS Glp	Ellis Lab
RE1229	*Cbr-fog-1(v442)/Cbr-unc-40(v270) I*	Fog	Ellis Lab

Acknowledgements: We thank the Caenorhabditis Genetics Center for C. elegans strains, Jon Harbin and Yongquan Shen for advice about microinjections and Dr. Bhagwati Gupta for Cbr-lin-39(bh20) III. We also thank NIH for the funding.

References

Barton MK, Kimble J. 1990. *fog-1*, a regulatory gene required for specification of spermatogenesis in the germ line of *Caenorhabditis elegans.* Genetics 125: 29-39. PubMed ID: 2341035

Beanan MJ, Strome S. 1992. Characterization of a germ-line proliferation mutation in *C. elegans*. Development 116: 755-66. PubMed ID: 1289064

Farboud B, Meyer BJ. 2015. Dramatic enhancement of genome editing by CRISPR/Cas9 through improved guide RNA design. Genetics 199: 959-71. PubMed ID: 25695951

Jin SW, Kimble J, Ellis RE. 2001a. Regulation of cell fate in *Caenorhabditis elegans* by a novel cytoplasmic polyadenylation element binding protein. Dev Biol 229: 537-53. PubMed ID: 11150246

Jin SW, Arno N, Cohen A, Shah A, Xu Q, Chen N, Ellis RE. 2001b. In *Caenorhabditis elegans*, the RNA-binding domains of the cytoplasmic polyadenylation element binding protein FOG-1 are needed to regulate germ cell fates. Genetics 159: 1617-30. PubMed ID: 11779801

Kimble J, Crittenden SL. 2007. Controls of germline stem cells, entry into meiosis, and the sperm/oocyte decision in *Caenorhabditis elegans*. Annu Rev Cell Dev Biol 23: 405-33. PubMed ID: 17506698

Kodoyianni V, Maine EM, Kimble J. 1992. Molecular basis of loss-of-function mutations in the *glp-1* gene of *Caenorhabditis elegans*. Mol Biol Cell 3: 1199-213. PubMed ID: 1457827

Luitjens C, Gallegos M, Kraemer B, Kimble J, Wickens M. 2000. CPEB proteins control two key steps in spermatogenesis in *C. elegans*. Genes Dev 14: 2596-609. PubMed ID: 11040214

Mello CC, Draper BW, Priess JR. 1994. The maternal genes *apx-1* and *glp-1* and establishment of dorsal-ventral polarity in the early *C. elegans* embryo. Cell 77: 95-106. PubMed ID: 8156602

Poultney CS, Butterfoss GL, Gutwein MR, Drew K, Gresham D, Gunsalus KC, Shasha DE, Bonneau R. 2011. Rational design of temperature-sensitive alleles using computational structure prediction. PLoS One 6: e23947. PubMed ID: 21912654

Rastogi S, Borgo B, Pazdernik N, Fox P, Mardis ER, Kohara Y, Havranek J, Schedl T. 2015. Caenorhabditis elegans *glp-4* Encodes a Valyl Aminoacyl tRNA Synthetase. G3 (Bethesda) 5: 2719-28. PubMed ID: 26464357

Rudel D, Kimble J. 2001. Conservation of *glp-1* regulation and function in nematodes. Genetics 157: 639-54. PubMed ID: 11156985

Wei Q, Shen Y, Chen X, Shifman Y, Ellis RE. 2014. Rapid creation of forward-genetics tools for *C. briggsae* using TALENs: lessons for nonmodel organisms. Mol Biol Evol 31: 468-73. PubMed ID: 24194560

Wood AJ, Lo TW, Zeitler B, Pickle CS, Ralston EJ, Lee AH, et al., Meyer BJ. 2011. Targeted genome editing across species using ZFNs and TALENs. Science 333: 307. PubMed ID: 21700836

Funding: NIH R01 GM121688

Author Contributions: Satheeja Santhi Velayudhan: writing - original draft, investigation. Ronald E Ellis: supervision, writing - review editing.

Reviewed By: Anonymous

History: Received October 26, 2022 **Accepted** December 9, 2022 **Published Online** December 9, 2022 **Indexed** December 23, 2022

Citation: Velayudhan, SS; Ellis, RE (2022). Functional divergence of orthologous temperature-sensitive mutations in *C. elegans* and *C. briggsae*. microPublication Biology. 10.17912/micropub.biology.000705