COMMENTARY
Worm 4:3, e1046031; July/August/September 2015; Published with license by Taylor & Francis Group, LLC

20 Years of *unc-119* as a transgene marker

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This fall marks 20 years since the cloning of *unc-119* was reported. Despite having a strong phenotype that makes animals somewhat difficult to grow and handle, *unc-119* mutant rescue has become one of the most frequently-used markers for *C. elegans* transformation. In this Commentary, I describe the history of how *unc-119* rescue traveled through the worm community, contributing to the development of transgene methods in *C. elegans*.

Keywords: *C. elegans*, CRISPR/Cas9, miniMos, MosDEL, MosSCI, transformation, transgenesis, *unc-119*

Abbreviations: Unc, uncoordinated; CGC, *Caenorhabditis* Genetics Center; MRC, Medical Research Council; YAC, Yeast Artificial Chromosome; GFP, Green Fluorescent Protein; HRG4, Human Retinal Gene 4; NIH, National Institutes of Health; MosTIC, Mos1 excision-induced transgene-instructed gene conversion; MosSCI, Mos1-mediated single-copy insertion; MosDEL, Mos1-mediated deletion; CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR-associated protein 9; sgRNA, single-guide ribonucleic acid; DSLR, Digital Single Lens Reflex

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Submitted: 04/08/2015

Revised: 04/23/2015

Accepted: 04/23/2015

http://dx.doi.org/10.1080/21624054.2015.1046031

Introduction

Transgenes introduced into *C. elegans* can be followed by many methods.[1] A phenotypic way of distinguishing transformants from non-transformants makes the job much easier under the dissecting microscope. While there are many markers that work well, *unc-119* rescue has clearly become widespread in the field: A search of the *Caenorhabditis* Genetics Center (CGC) database (http://www.cgc.cbs.umn.edu) finds some 2000 strains that contain at least one transgene rescued by *unc-119*; a search of the Materials section of published *C. elegans* papers using Textpresso (http://www.textpresso.org/celegans/) finds approximately 1000 that contain "*unc-119*"; and the plasmid deposition site Addgene (http://www.addgene.org) contains some 50 plasmids that refer to *unc-119*. As described below, the popularity of *unc-119* rescue resulted from the connectivity of the *C. elegans* community, coupled with the suitability of this marker for a wide variety of applications.

Identification of *unc-119*

In the fall of 1991, my graduate research project was to work with a visible uncoordinated (Unc) mutant isolated by my thesis advisor, David Pilgrim (University of Alberta, Canada) when he was a postdoc with Jonathan Hodgkin (MRC Laboratory of Molecular Biology, Cambridge, UK). After performing crosses in which the transposable element Tc1 was free to mobilize, Pilgrim recovered a mutant, *e2498*, that defined a new locus, subsequently named *unc-119*. This was not the first time the gene had been mutated: Acetaldehyde mutagenesis had previously disrupted *unc-119* via a chromosomal break that created 2 independently segregating pieces of *LG III*, the free duplication *eDp6*, and the deficiency chromosome, *eDf2*.[2] The *eDf2; eDp6* strain has the same phenotype as *unc-119 (e2498)*, and the 2 fail to complement. Working with the small deficiency *tDf2*, I obtained 3 additional EMS-induced alleles of the locus in a classic F_1 noncomplementation screen: *ed3, ed4* and *ed9*. All failed to complement *e2498* and had identical phenotypes[3] (**Fig. 1**).

At first, molecular identification of *unc-119* turned out to be more challenging than it ought to have been. Although *e2498* was likely to be a Tc1 insertion, extra bands that might have contained the gene were not apparent in Southern blots probed with Tc1. Using strains containing *eDf2* or *eDp6* and cosmid clones on either side, I was able to localize *unc-119* to the overlap between 2 YACs, Y39A1 and Y60D9, by the summer of 1993. I subcloned the YACs by purifying them from pulsed-field gels and making *Sst*I fragment libraries in pBluescript. Fortuitously, one of the first clones picked, pDP#MM008, happened to contain most of the *unc-119* gene, though not enough to rescue. As I was about to clone a larger fragment from a genomic plasmid library, I read in the February, 1994 Worm Breeder's Gazette

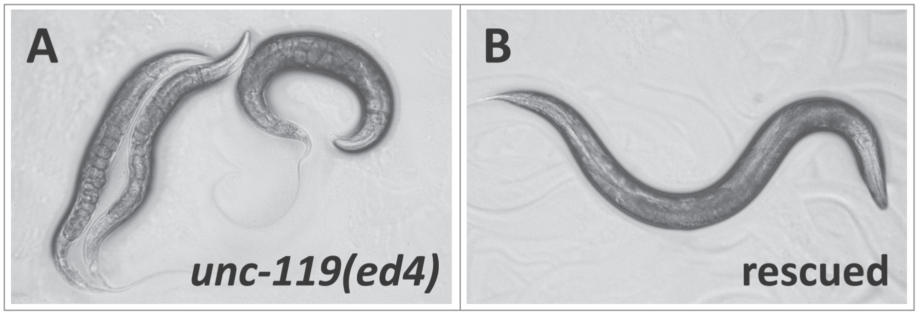

Figure 1. Appearance of adult *unc-119* mutant and wild-type (rescued) hermaphrodites on agar plates and summary of phenotypes. (**A**) *unc-119(ed4)* adults displaying short morphology and curled appearance. Mutants cannot form dauer larvae. (**B**) *unc-119* mutant rescued with an integrated *Cbr-unc-119(+)* single-copy transgene, displaying normal morphology and sinusoidal locomotion. Rescued animals can also form dauer larvae. Images were taken at the same scale. The rescued animal is approximately 1mm long. Phase-contrast images were taken with a Canon DSLR through a 10x objective on an upright Olympus BX-51 compound light microscope.

that Julie Ahringer (also at the MRC at the time, now at the University of Cambridge, UK) had cloned the nearby gene *vab-7*. She sent me a *vab-7*-rescuing cosmid, M142, with which I achieved the first transformation rescue of *unc-119*. The gene was narrowed down to a 5.7-kbp *Hind*III-*Xba*I fragment cloned in plasmid pDP#MM016 (**Fig. 2**). As expected, the *e2498* allele contained a Tc1 insertion, most of the gene was missing from *eDf2* and *eDp6*, and the point mutants were all GC to AT transitions: *ed9* mutated a splice acceptor resulting in a −1 frameshift, while *ed3* and *ed4* were nonsense mutations within 30 base pairs of each other. By 1994, we knew that *unc-119* encoded an apparently novel protein of 219 amino acids. Making use of a newly-available GFP plasmid from Martin Chalfie (Columbia University, NY), I found that *unc-119*::GFP was expressed broadly throughout the nervous system with some additional expression in the head. The identification of *unc-119*, and its expression using an *unc-119*::lacZ reporter made from an expression plasmid from Andrew Fire (now at Stanford University, CA), were reported in *Genetics* in November of 1995.[3] I also cloned the *C. briggsae* ortholog from a λ genomic library made by Terry Snutch (University of British Columbia, Canada) and David Baillie (Simon Fraser University, Canada), and showed that *Cbr-unc-119* could rescue the *C. elegans* mutant phenotype as an extrachromosomal array.[4] The *C. briggsae* gene was much more compact, owing to a reduction in size of the introns (**Fig. 2**).

Shortly after the first report on *Ce-unc-119*, a sequence ortholog of UNC-119, Human Retinal Gene 4 (HRG4), appeared in Genbank from a paper on genes enriched in the human retina.[5] HRG4 can fully rescue the *unc-119* mutant defects when expressed from the *C. elegans unc-119* promoter.[6] Orthologues are now known in many species including *Drosophila*,[6] zebrafish,[7] mouse,[8] and the protozoan *Naegleria*,[9] suggesting it is found in all animals. An ortholog, POC7, was also identified in proteomic analysis of centrioles from the green alga *Chlamydomonas*.[10] UNC-119 proteins are lipid-binding chaperones that control trafficking of myristoylated G protein α-subunits and Src tyrosine kinases.[11] In *C. elegans*, UNC-119 is known to be important for axon structure[12,13], localization of the G protein α subunits ODR-3 and GPA-13,[14] and other aspects of ciliogenesis.[15]

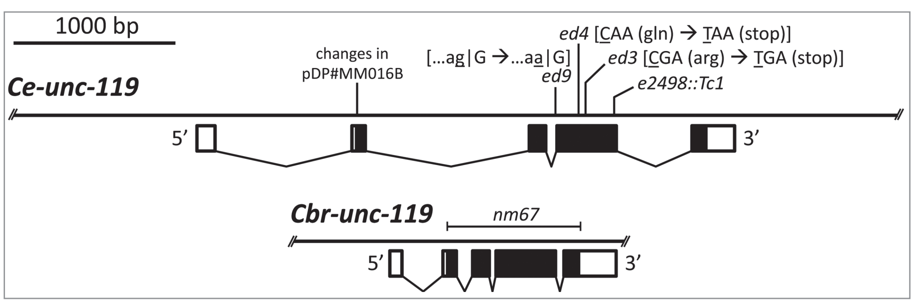

Figure 2. The *C. elegans* and *C. briggsae unc-119* loci.[3, 4] The *Ce-unc-119* genomic region is the 5.7-kbp *Hind*III-*Xba*I rescuing fragment found in the pBluescript KS- clone pDP#MM016, while the *Cbr-unc-119* genomic region corresponds to the 2.1-kbp rescuing fragment found plasmid pCFJ151.[28] The derived clone pDP#MM016B contains 2 point mutations that introduce a *Bgl*II restriction site at the start of the coding region: ATG AAG GCA GAG CAA CAA ATG AAG GCA GAt CtA CAA (*Bgl*II site underlined, changed bases in lowercase). The *ed9* allele changes the 3′G of an intron, such that the subsequent G becomes used as a splice acceptor and results in a (-1) frameshift.[3] The coding region in the *Ce-unc-119* transcript shown corresponds to M142.1b (Wormbase WS246). A longer gene model found in WormBase (M142.1a) contains an exon upstream of the region shown here. The coding region for the *C. briggsae* transcript corresponds to CBG18291 (Wormbase WS246). The *C. briggsae nm67* allele was made in the laboratory of Eric Haag (University of Maryland College Park, MD)[37]; 3 additional deletion alleles, not shown, were made in the laboratory of Robert Waterston (University of Washington, Seattle, WA).[38]

Use of *unc-119* for Extrachromosomal Transformation

Transgenesis in *C. elegans* got its start in the mid-1980s when Andrew Fire (now at Stanford University, CA) reported success with gonadal injection of a plasmid carrying an amber suppressor tRNA, *sup-7*, to suppress a *tra-3* nonsense mutation.[16] Soon after, Craig Mello (now at University of Massachusetts Medical School in Worcester, MA) reported the use of the dominant *rol-6(su1006D)* allele, carried on plasmid pRF4, for injection of the gonad syncytium.[17] Cloning of genes, and generation of reporter fusions (at first to *lacZ*,[18] then to GFP[19]), became widespread as the physical map and subclones of the *C. elegans* genome became available.[20] Use of *rol-6D* to make arrays by gonadal microinjection became the "dominant" way to make reporter transgenes for many years, because it could be introduced into otherwise wild-type worms to cause a strong Roller phenotype. However, this phenotype affects late larval and adult body shape, strongly reduces male mating efficiency, and reduces fecundity relative to non-transgenic siblings with wild-type locomotion.[21]

Widespread use of *unc-119* rescue as a marker started in the late 1990s as a direct result of the community's sharing of information over the emerging Internet. The USENET newsgroup CELEGANS/*bionet.celegans*, started in 1994, became a popular form of communicating and posting questions directly to researchers. (It still exists today in Google Groups, http://groups.google.com.) In March of 1996, in response to an inquiry about uncoordinated mutants that might be useful alternatives for transformation, I posted a description of the suitability of *unc-119* rescue for transgenesis. Within a few months, I had sent a modified rescuing clone (pDP#MM016B) and an *unc-119* mutant to over 50 *C. elegans* labs around the world. Although the original *e2498* allele was sent out initially, different labs may have received *ed3, ed4* or *ed9*. Aside from the first papers on *unc-119* and its *C. briggsae* ortholog,[3, 4] the first to report use of *unc-119*-rescued transgenes began to appear in 1998.[22–25] The observation that

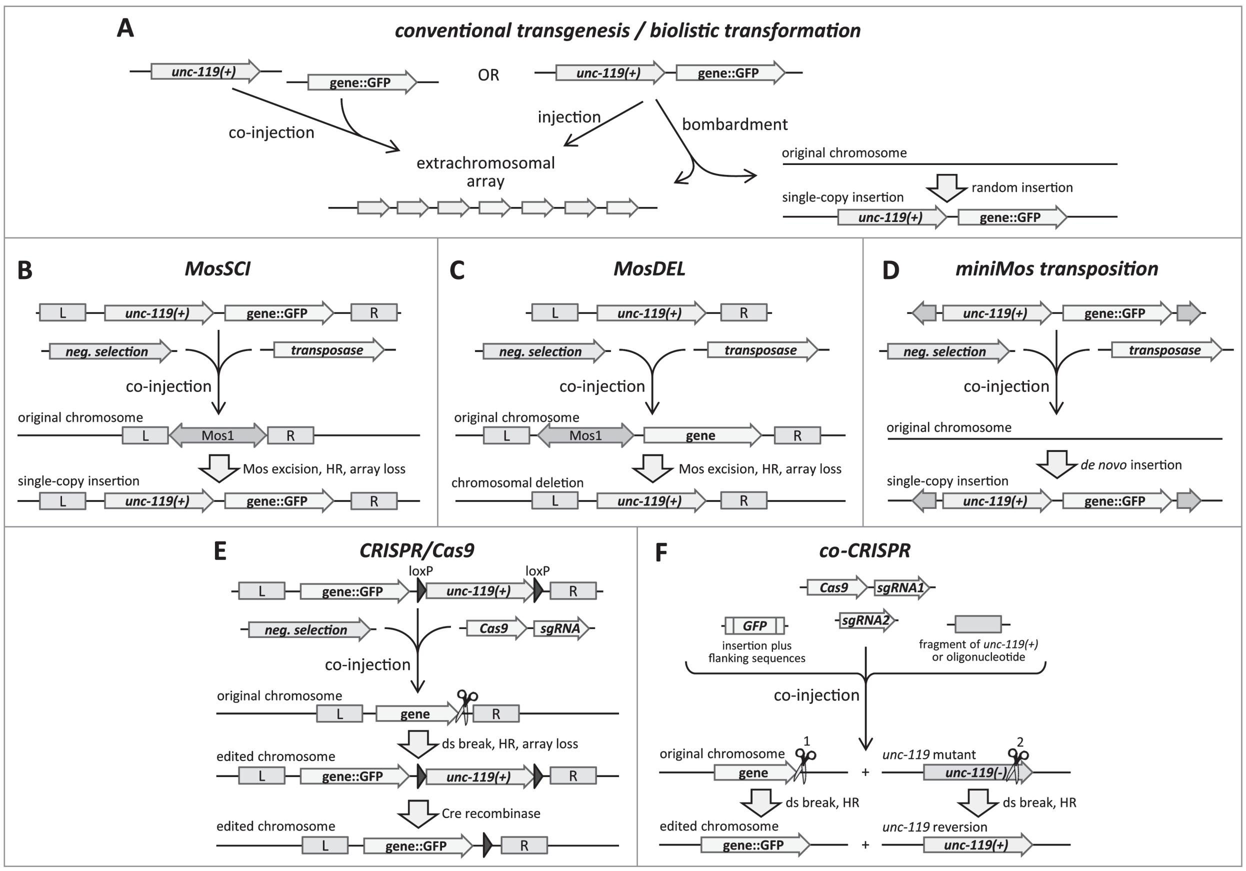

Figure 3. Multiple applications that use *unc-119* rescue. All approaches involve transgene delivery into *unc-119* mutant animals. While injected DNA can be linear or circular, features of injected DNA are shown as linear for simplicity. In all of the applications except co-CRISPR, "*unc-119(+)*" can be either *Ce-unc-119(+)* or *Cbr-unc-119(+)*. (**A**) For conventional transgenesis, *unc-119* mutants are coinjected with *unc-119(+)* and a transgene (e.g. gene::GFP) to generate extrachromosomal arrays. Both *unc-119(+)* and the transgene of interest can be on the same plasmid. In biolistic transformation, microparticle bombardment is used with a single plasmid, although it is possible to use 2 different plasmids.[39] Bombardments produce extrachromosomal lines, single- and low-copy insertions, a small fraction of which can occur at an endogenous locus.[40] (**B**-**D**) Methods that use excision of the Mos1 transposon. Note that if a single plasmid containing *unc-119(+)* and a transgene of interest is constructed for MosSCI or miniMos transposition, it can be also used for conventional transgenesis or bombardment. (**B**) In MosSCI, Mos1 excision from a known chromosomal site creates a double-stranded break that gets repaired by homology-directed repair (HR). This is guided by the left and right flanking regions around the break, typically ~1.5 kbp in the original MosSCI protocol.[28] The original injected DNA can form an array that must be selected against, usually through inclusion of negative selection (neg. selection) markers such as mCherry reporters and/or an inducible toxic *hs-peel-1* plasmid.[29] (**C**) In MosDEL, *unc-119(+)* is inserted inside a deleted region.[30] (**D**) In miniMos transposition, a modified Mos transposon inserts *de novo* at a random genomic site.[31] (**E**) In one version of a CRISPR/Cas9-mediated approach,[33] a GFP reporter and *unc-119(+)* are inserted by HR at a double-stranded break created by Cas9. An optional step is the removal of the *unc-119(+)* marker through germline expression of Cre recombinase, which recombines loxP sites flanking *unc-119(+)*[33]. Recent work suggests that the homology can be as short as 30 base pairs.[41] (**F**) In a co-CRISPR strategy, an *unc-119* point mutant can be reverted, while a gene of interest is edited in its endogenous site, using 2 guide RNAs (sgRNA1 and sgRNA2).[34, 36, 42]

many transgenes seemed to work well with *unc-119* as a coinjection marker suggested that in general, *unc-119* rescue does not interfere with the vast majority of applications. This was no doubt an important consideration for labs initiating transgenesis experiments, and those developing newer technologies.

Use of *unc-119* for Low-Copy Chromosomal Insertions

In the lab of Judith Austin (University of Chicago, IL), Vida Praitis (now at Grinnell College, IA) and colleagues developed microparticle bombardment methods for *C. elegans*[26] around the year 2000. Initially they had planned to suppress the phenotype of *rol-6* mutants with a transgene containing the dominant *sqt-1(sc1)* mutation.[21] After contacting the lab of Jim Kramer at the same institution, they were told by Brian Ackley (in the Kramer lab at the time, now at the University of Kansas, KS) about *unc-119* rescue. The Kramer lab had originally obtained *unc-119* mutants and rescuing DNA directly from David Pilgrim in the late 1990s. For bombardment, the inability of mutants to form dauer larvae meant that rare transformants, rescued for *unc-119*, would be able to outgrow the thousands of non-transformed mutants on a plate and be recovered after the bacteria were depleted.

Bombardment was initially performed using a single plasmid containing both *unc-119* rescue and the transgene of interest (**Fig. 3A**).[26] Praitis *et al.* inserted transgenes directly into pDP#MM016, a plasmid in which the size of the *C. elegans* gene made routine cloning somewhat of an inconvenience: The *unc-119* rescuing fragment is 5.7 kbp (**Fig. 2**), and the plasmid backbone 2.9 kbp, making the starting vector already 8.6 kbp in size. Recalling that the *C. briggsae unc-119* gene has smaller introns, Andrew Singson and Barth Grant (both at Rutgers, NJ) asked me to send a subclone of *Cbr-unc-119* to try for bombardment experiments around the year 2003. I sent them pMM571, a plasmid containing a 2.1-kbp *Cbr-unc-119(+)* fragment (**Fig. 2**). Both groups demonstrated single-copy rescue of *C. elegans unc-119* mutants with pMM571. Likely hearing about their work from Andrew Singson, Andy Golden (NIH, Bethesda, MD) requested pMM571 from me and it was used by Christopher Richie in his lab to generate the plasmid pCR39 for their bombardment experiments.

The *C. briggsae* ortholog would become the fragment of choice for rescuing *unc-119* as newer methods were developed. Christian Frøkjær-Jensen and colleagues in the lab of Erik Jorgensen (University of Utah, UT) were modifying the MosTIC (Mos1 excision-induced transgene-instructed gene conversion) procedure developed by Valerie Robert and Jean-Louis Bessereau (Biologie cellulaire de la synapse, Paris, France),[27] to use rescue of *unc-119* as a marker in a technique they named MosSCI (Mos1-mediated single-copy insertion).[28] This approach uses a targeting plasmid carrying both *unc-119(+)* and a transgene of interest between flanking genomic sequences to direct homologous targeting and insertion (**Fig. 3B**). Wayne Davis in the Jorgensen lab used pCR39, obtained from Christopher Richie, as a source to amplify *Cbr-unc-119(+)*; this fragment subsequently became incorporated into many vectors used for MosSCI.[28] As shown in **Figure 3B**, *unc-119* mutants are injected with a mixture of DNAs that provide a Mos transposase source, the targeting plasmid and various negative selection markers. While the reproductive advantage of chromosomally-rescued *unc-119* animals alone helps in recovery of *bona fide* insertion strains, the negative selection markers enable identification (and/or elimination) of undesired animals rescued by extrachromosomal arrays derived from the injection mixture.[28, 29] Rescue of *unc-119* has been subsequently used in newer Mos-based methods, including MosDEL, in which *Cbr-unc-119(+)* is inserted while generating a targeted deletion of a gene[30] (**Fig. 3C**), and miniMos, in which DNA segments up to 45 kbp are inserted into random locations in the genome[31] (**Fig. 3D**).

Future Applications of *unc-119* Rescue

The development of the CRISPR/Cas9 system in *C. elegans* may have removed the need for transformation markers for most experiments, as genome editing events can be recovered at high efficiency even in the absence of array transmission.[32] Rescue of *unc-119* can still be used in specialized CRISPR/Cas9-based approaches. Daniel Dickinson and colleagues in the lab of Bob Goldstein (University of North Carolina, NC) developed a version of CRISPR/Cas9 mutagenesis that uses *unc-119* rescue to identify gene-targeted events similar to MosSCI[33] (**Fig. 3E**). In their approach, LoxP sites flanking the *Cbr-unc-119(+)* insertion enable subsequent removal of the rescue marker through germline expression of *Cre* recombinase, in what could be called an 'un-rescue' of *unc-119*. Using the method of Dickinson *et al.*, insertion of *unc-119(+)* could also be used to insertionally disrupt genes similar to MosDEL.

Rescue of *unc-119* can be used to aid recovery of any CRISPR/Cas9-mediated targeting event. It was recently shown that pairs of chromosome editing events driven by CRISPR/Cas9 are more likely to occur concomitantly, such that successful editing for one gene predicts a higher recovery of editing of a second locus in the same progeny animals.[34] Using this co-CRISPR strategy, recovery of chromosomally-targeted gene edits could be facilitated by simultaneous reversion of *unc-119* (**Fig. 3F**). An *unc-119(ed3)* mutant has been successfully reverted using CRISPR/Cas9 and DNA oligonucleotides.[35, 36] As reversion can be achieved with a short region of *unc-119(+)* DNA as a repair template and not the intact gene, non-Unc progeny would represent *bona fide* chromosomal reversions, eliminating the need for negative array selection. Furthermore, as the 3 point mutations *ed3, ed4* and *ed9* are found within a 167-bp region, a single sgRNA and repair template could potentially revert any of the 3 alleles. Hence, through co-CRISPR, *unc-119* rescue may continue to find applications in modern *C. elegans* genome editing methods.

Reagent Availability

The *unc-119* alleles shown in **Figure 2** are available from the *Caenorhabditis* Genetics Center (CGC, http://www.cgc.cbs.umn.edu). Plasmids that rescue *unc-119* can be obtained from Addgene (https://www.addgene.org/). Advice on growing *unc-119* mutants and optimizing injection can be found on the Wormbuilder site (http://www.wormbuilder.org).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

I thank Brian Ackley, Wayne Davis, Christian Frøkjær-Jensen, Andy Golden, Barth Grant, Erik Jorgensen, Vida Praitis, Christopher Richie and Andy Singson for helping me reconstruct the history of the use of *unc-119* rescue; Christian Frøkjær-Jensen for additional suggestions; Eric Haag for information about the *nm67* allele of *Cbr-unc-119*; and David Pilgrim, for having given me the opportunity to clone *unc-119* those many years ago.

Funding

Work in the Maduro Lab is currently funded by NSF IOS#1258054.

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