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01/03/2019 – *Open Access*

A CRISPR/Cas9-generated *cdc-7* loss of function mutation does not cause temperature-dependent fertility defects

Heather N. Currey[1], Nicole F. Liachko[1,2]*

1. Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, USA.
2. Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA 98104, USA.

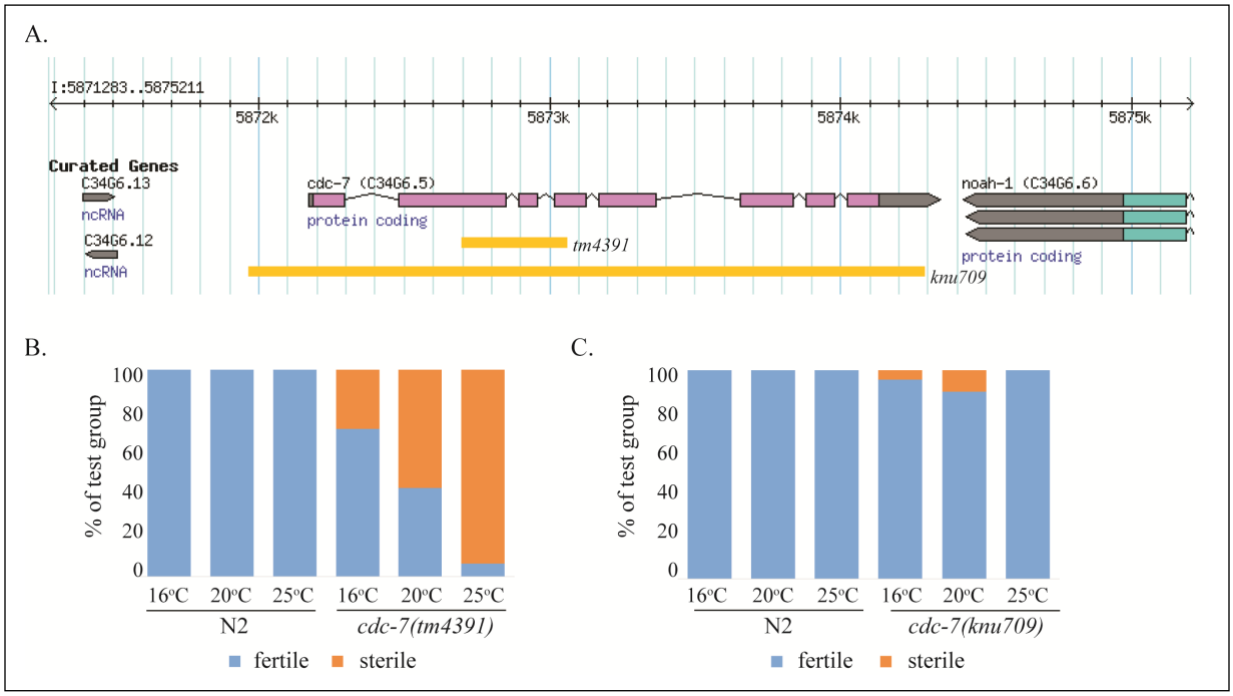

Figure 1. *cdc-7(tm4391)* but not *cdc-7(knu709)* is temperature-sensitive sterile. (**A**) Schematic of *cdc-7* gene locus and location of *tm4391* and *knu709* deletions. *knu709* deletes 2,284 nucleotides, beginning 163 nucleotides upstream of the *cdc-7* ATG start and ending 136 nucleotides downstream of the *cdc-7* TAA stop, within the *cdc-7* 3'UTR. This deletion does not impact upstream or downstream genes. **(B)** *cdc-7(tm4391)* worms are temperature sensitive sterile, with higher temperatures being progressively worse for fecundity. At 25ºC, 90% of individual worms tested were sterile (Total n= 48. Number of plates with progeny = 3, number without progeny = 45). There was an intermediate effect at 20ºC with 56% sterility (Total n= 49. Number of plates with progeny = 21, number without progeny = 28). *cdc-7(tm4391)* was most fertile when kept at 16ºC, with only 30% sterility (Total n= 49. Number of plates with progeny = 35, number without progeny = 14). N2 controls had no observed sterility at any temperatures (for 25ºC, 20ºC and 16ºC respectively, total n= 50, 50, 50. Number of plates with progeny = 50, 50, 50). **(C)** *cdc-7(knu709)* were substantially less likely to be infertile and infertility did not correspond to temperature (for 25ºC, 20ºC and 16ºC respectively, percent sterile = 0%, 10%, 4%. Total n= 50, 49, 45. Number of plates with progeny = 48, 44, 43. Number of plates without progeny = 0, 5, 2).

Description

CDC7 regulates both DNA replication initiation and checkpoint-regulated progression of the cell cycle during the G1/S phase, contributes to DNA recombination and damage repair, and is an essential gene in many species (YAMADA *et al.* 2014). In *C. elegans*, there has been a single characterized deletion allele available, *tm4391*, impacting the 8-exon coding sequence of *cdc-7* (Figure 1A). *tm4391* is missing 315 bp including part of exon 2, all of exon 3, and part of exon 4, and resulting in a downstream frame shift. *cdc-7(tm4391)* worms are temperature sensitive sterile, with worse fertility at higher temperatures (Figure 1B). We generated a novel deletion allele *knu709*, which is a 2,284 nucleotide deletion and an 18 nucleotide insertion, removing the entire *cdc-7* coding sequence (Figure 1A). We tested *knu709* for temperature sensitive sterility. Surprisingly, this strain is not temperature sensitive sterile (Figure 1C), indicating that the fertility defects of *tm4391* may be due to a closely

linked mutation in another gene or *tm4391* might be a dominant negative or neomorphic allele of *cdc-7* not recapitulated by a true loss-of-function mutation.

Reagents and Methods

Strains

CK609 *cdc-7(tm4391)* I - outcrossed 4x to N2.
NLS1 *cdc-7(knu709)* I - outcrossed 3x to N2. Strain will be sent to CGC.

Genotyping

Oligos used for genotyping *tm4391:* Mix all 3 primers in a single reaction. Expect sizes 757bp and 270bp WT, 400bp mutant.
NL93 (F): tcagtgcaacatgcagaaca
NL94 (R): tgacacaaaccaatcccaaa
NL187 (internal deletion): ATGCGACAGCATAAAGCAAA

Oligos used for genotyping *knu709:* Mix all 3 primers in a single reaction. Expect sizes 2848bp and 823bp WT, 565bp mutant.
NL429 (F): cccgtatcacacactcatcg
NL430 (R): attgctaaaacccgcagaaa
NL431 (internal deletion): ggaaacgtaccctcgcctat

Fertility Assays

Synchronized populations of *C. elegans* were established by treating with alkaline hypochlorite solution (bleaching) (PORTA-DE-LA-RIVA *et al.* 2012). Eggs were grown at 16ºC to L4 stage at which point worms were singled onto 60mm NGM plates seeded with OP-50. Plates with singled worms were then divided into 3 groups of approximately 50 per strain. Groups were transferred to the assay temperature: either 25ºC, 20ºC or 16ºC. After 1 week, plates were scored for the presence of progeny. The absence of progeny was scored as sterile.

CRISPR/Cas9

COP1803 *cdc-7(knu709)* was generated by Knudra Transgenics/ NemaMetrix (Eugene, OR), and the deletion end-points confirmed by sequencing. 3-frame stop insertion sequence at breakpoints of deletion: TAAATAAATAAACTCGAG.

References

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Acknowledgements

We thank Drs. Brian Kraemer and Tom Bird for expert advice. We thank the National Bioresource Project (Japan) for providing strains. We thank WormBase for *C. elegans* model organism information. We thank the Department of Veterans Affairs for funding.

Funding Department of Veterans Affairs Career Development Award 2 #I01BX007080 and Merit Review Grant #I01BX004044 to N.L.

Author Contributions: N.L. was involved in this project conceptualization, formal analysis, methodology, project administration, resources, supervision, visualization and writing. H.C. was involved in project conceptualization, formal analysis, investigation, methodology, project administration, visualization and writing.

Reviewed by Kelly Liu

Received 12/28/2018, **Accepted** 01/03/2019. **Published Online** 01/03/2019.

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01/03/2019 – *Open Access*

Citation: Currey, H. N., & Liachko, N. F. A CRISPR/Cas9-generated *cdc-7* loss of function mutation does not cause temperature-dependent fertility defects. microPublication Biology. https://doi.org/10.17912/MICROPUB.BIOLOGY.000085