Apr 21st, 5:10 PM - 6:10 PM

Phenotype Characterization of sas-7(or1945) C. elegans

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Abstract

The ability to form a bipolar spindle is crucial for accurate cell division. In the nematode *C. elegans* several genes have been described with roles in spindle assembly including *sas-7*. The centriole is a key organizer of mitotic spindles. The *sas-7* protein is a centriole component that regulates centriole duplication, elongation, and assembly. To date, most work on *sas-7* was using a conditional non-null allele[1]. In this study, phenotypes associated with the loss-of-function *sas-7(or1945)* null allele were characterized. Homozygous *sas-7(or1945)* hermaphrodites have reduced brood sizes with no viable embryos compared to wild-type and heterozygotes. When they do produce embryos, they become multinucleated suggesting failures in spindle formation and cell division. As adults, homozygous hermaphrodites also appear to have protruding vulvas with the eventual gut explosion from the vulva. Adult homozygous hermaphrodites also appear to have uncoordinated movement. Homozygous *sas-7(or1945)* males have misshapen tails, and they appear unable to mate. Whether they produce viable sperm is unknown at this time. We conclude that *sas-7(or1945) C. elegans* have phenotypes suggesting possible sterility. As cell division and developmental processes are well conserved in animals, what we learn from our studies in *C. elegans* may help us to understand these processes in other organisms as well.

Background

- C. elegans are a good genetic model
  - They have a rapid life cycle
  - It is easy to examine embryos
  - Their genome is already sequenced
- Cell division is highly conserved
  - The ability to form a bipolar spindle is essential
  - Improper cell division results in improper cell growth and death
  - *sas-7* gene has been identified as an allele that affects the ability to form a bipolar spindle
- Previous work focused on the conditional non-null allele *sas-7(or452ts)*
- Our work was on the complete-loss-of-function allele *sas-7(or1945)*

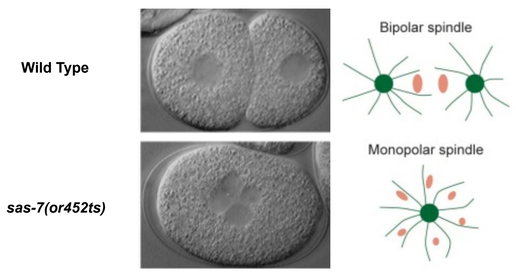

Figure 1: Improper spindle formation in *sas-7(or452ts)* compared to wild type.
Wild type *C. elegans* form a normal bipolar spindle while *sas-7(or452ts)* form a monopolar spindle. Microtubules are in green and chromosomes are in salmon.

Genotype	Mutations	Origin	Monopolar Spindle
or452ts	53 a.a. deletion	EMS	+++
or1945	premature stop codon at 104 a.a.	CRISPR/Cas9	+

Figure 2: *sas-7* gene models and mutant alleles.
Location of the sas-7 mutant alleles being studied and their specific mutations.

Results

Table 1: Brood Sizes and Crosses of Different Strains of *C. elegans*

	Hermaphrodites			Males
Worm Genotype	**Percentage Layed Eggs**	**Average Number of Embryos On Plate**	**Percentage of Dead Embryos**	**Percentage of Working Crosses**
Wild Type	100% (n=7)	238 ± 13 (n=8)	1.6% ± 0.4 (n=8)	n.d.
Heterozygotes (*sas-7(or1945)* / hT2)	100% (n=7)	245 ± 25 (n=7)	3.2% ± 0.8 (n=7)	86% (n=7)
Homozygotes (*sas-7(or1945)* / *sas-7(or1945)*)	74% (n=30)	32 ± 7 (n=29)	100% (n=29)	0% (n=7)

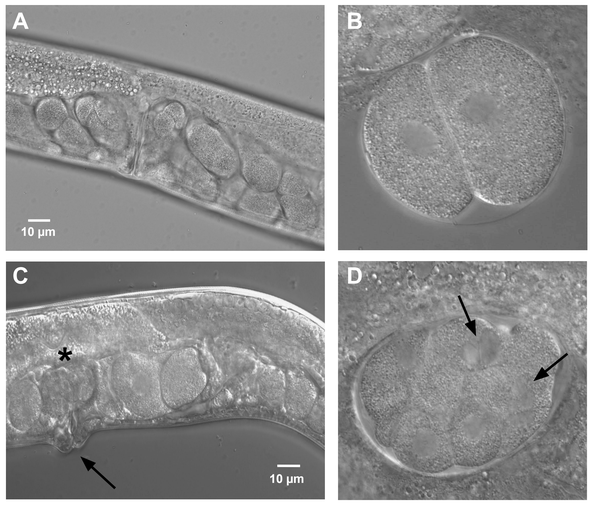

Figure 3: Reduced and abnormal embryo formation in adult *sas-7(or1945)* hermaphrodites.
The heterozygote (A) has normal embryos. The homozygote (C) has reduced embryos and abnormal formation. Asterisk shows multinucleated embryo. Arrowhead shows protruding vulva. While *sas-7(or1945)* embryos typically look fine at the 2-cell stage (B), cells in slightly older embryos are multinucleated (arrows in D).

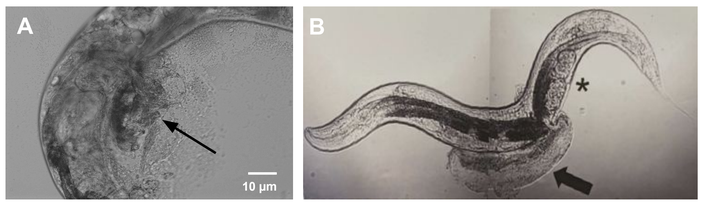

Figure 4: Gut explosion from vulva in adult *sas-7(or1945)* hermaphrodites.
Adult homozygous hermaphrodites commonly have the gut explode out the vulva (A-B). Arrowheads show gut explosion. Asterisk shows reduced embryo formation in a homozygous hermaphrodite.

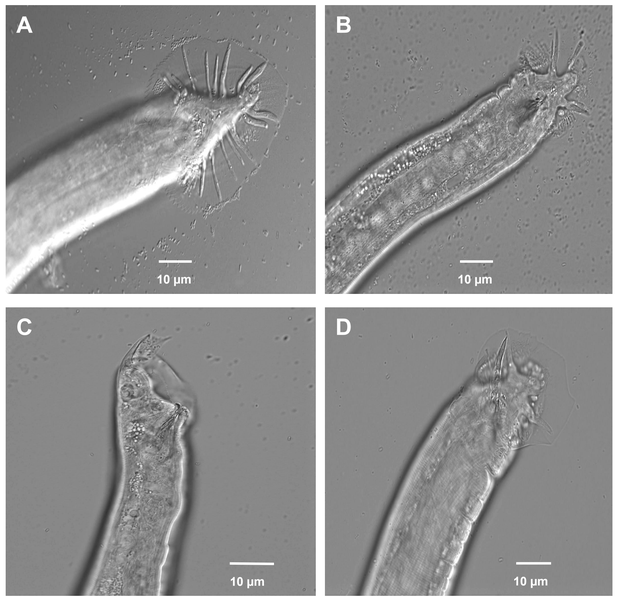

Figure 5: Misshapen tails in *sas-7(or1945)* males.
Heterozygous males have normal fan-shaped tails (A). Homozygous males display a range of tail misshapenness (B-D). Some homozygous males have only slightly misshapen tails with some rays and spicules (B). Other homozygous males have very stumpy tails that lack these mating structures (C-D).

Discussion

- Homozygous *sas-7(or1945)* hermaphrodites are subfertile, often producing no or few embryos. In addition, any embryos they produce are not-viable.
- Adult homozygous *sas-7(or1945)* hermaphrodites were observed to have protruding vulva (puv) most of the time with the eventual explosion of the gut through the vulva. Slight protruding vulva and the occasional explosion of the gut through the vulva was observed in some heterozygous *sas-7(or1945)* hermaphrodites.
- Adult homozygous *sas-7(or1945)* hermaphrodites appear uncoordinated (unc) especially as they age. Heterozygous *sas-7(or1945)* hermaphrodites were not observed to be uncoordinated at any point in their life cycle.
- Both *or452ts* and *or1945* alleles of sas-7 have cell division defects. In *or452ts*, the defects are visible in the first or second cell divisions, whereas *or1945* mutants fail slightly later presumably as a result of maternal contributions of the gene product from their heterozygous mother.
- Homozygous *sas-7(or1945)* males have misshapen tails and are unsuccessful in mating. It is unknown if homozygous *sas-7(or1945)* males produce viable sperm.

References and Acknowledgements

[1] Sugioka, K., Hamill, D. R., Lowry, J. B., McNeely, M. E., Enrick, M., Richter, A. C., Kiebler, L. E., Priess, J. R., & Bowerman, B. (2017). Centriolar SAS-7 acts upstream of SPD-2 to regulate centriole assembly and pericentriolar material formation. *eLife*, *6*, e20353. https://doi.org/10.7554/eLife.20353

Previous students in the Hamill lab, especially Lauren Kiebler and Alyssa Richter contributed to this project.