

Difference in phenotypic severity of presumed null alleles of *capg-1*

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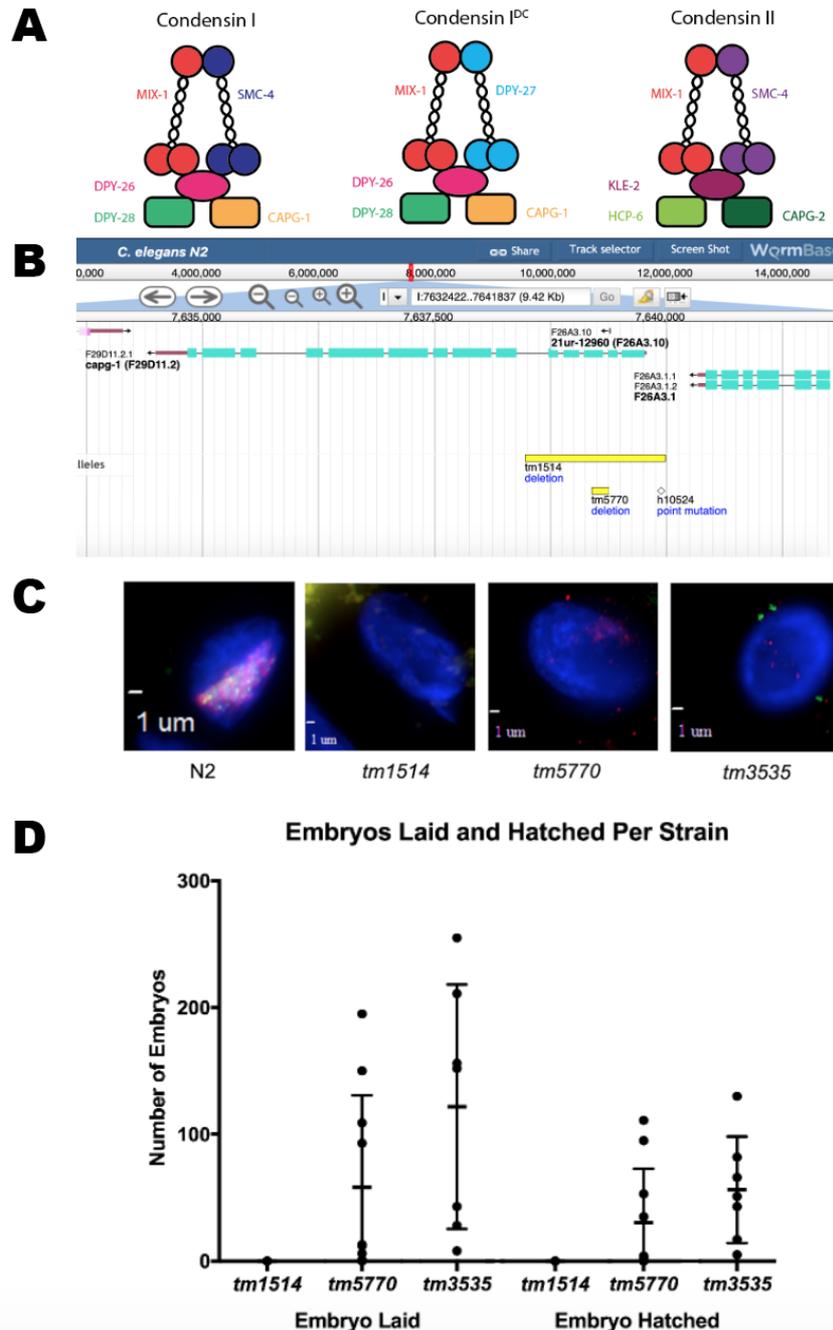


Figure 1: **A:** Subunit composition of condensin I, condensin I^{DC}, and condensin II complexes. DPY-28 (green), DPY-26 (pink), and CAPG-1 (light orange) are found in both condensin I and condensin I^{DC}. **B:** *capg-1* gene information obtained from WormBase, showing the *tm1514* deletion, *tm5770* deletion, and the 21ur-12960 piRNA. **C:** Immunofluorescence images of adult hermaphrodite intestinal nuclei in wild type N2, *capg-1(tm1514)*, *capg-1(tm5770)*, and *dpy-28(tm3535)* stained for DPY-27 (with Cy3, red) and CAPG-1 (with FITC, green), merged with DAPI. X-localized DCC is only seen in N2. **D:** Number of embryos laid and hatched in each strain. Each dot represents the number of embryos laid by an individual worm. The *capg-1(tm1514)* mutants (n=10) did not lay any eggs, while both *capg-1(tm5770)* mutants (n=10) and *dpy-28(tm3535)* mutants (n=7) laid a significant number of eggs, many of which hatched.

Description

Dosage compensation is the mechanism by which organisms correct the sex chromosome imbalance between sexes (e.g. females having two X chromosomes compared to one X in males). In *C. elegans*, dosage compensation is achieved by the downregulation by half of both X chromosomes in hermaphrodites (Albritton & Ercan, 2018). This downregulation is accomplished by the Dosage Compensation Complex (DCC), which is comprised of a condensin I^{DC} subcomplex interacting with other accessory proteins. Condensin I^{DC} has a similar structure to canonical condensins (condensin I and condensin II), which function to compact chromosomes during mitosis and meiosis (Csankovszki *et al.*, 2009). The DPY-27 subunit is unique to condensin I^{DC}, MIX-1 is present in all three condensins, while the proteins CAPG-1, DPY-26, and DPY-28 are found both in condensin I and condensin I^{DC} (Figure 1A).

DCC mutants show maternal effect lethality, since all subunits of condensin I^{DC} and several of the accessory proteins are maternally contributed to oocytes (Plenefisch *et al.*, 1989; Csankovszki *et al.*, 2009). Homozygous DCC mutants derived from heterozygous mothers survive to adulthood due to the maternally provided RNA and/or protein. These mutants are referred to as maternal positive, zygotic negative (M+Z-). M+Z- hermaphrodites are unable to produce a functional gene product; therefore, their progeny have no maternal or zygotic contribution of these proteins (M-Z-). As a consequence, very few M-Z- hermaphrodites survive past the L1 stage; however, males do not require the DCC to survive. It is possible, then, to recover M-Z- male progeny from self-fertilizing hermaphrodites in these conditions.

A previous study (Csankovszki *et al.* 2009) showed that M+Z- *capg-1* null mutants (*tm1514*) are sterile and have severe developmental defects. This phenotype is different from and more severe than what was previously seen for genes encoding other condensin I^{DC} members (Plenefisch *et al.*, 1989). It raised the possibility that the sterility and more severe developmental phenotypes of *capg-1(tm1514)* is the result of another role of CAPG-1 outside of condensin I and I^{DC} function. We acquired another *capg-1* allele (*tm5770*) from the Japanese National Bioresource Project. This allele deletes a smaller portion of the coding sequence than *capg-1(tm1514)* but is also predicted to be null due to the resulting frameshift mutation. Also of interest, the *capg-1(tm5770)* allele removes only the terminal nucleotide from a short piRNA gene deleted entirely in *capg-1(tm1514)* (Figure 1B). If the absence of the CAPG-1 protein function was responsible for the sterility phenotype observed in *capg-1(tm1514)*, the M+Z- hermaphrodites from the *capg-1(tm5770)* strain should also be sterile.

We first confirmed via fluorescence microscopy that the DCC is not recruited to the X chromosome in the *capg-1(tm1514)* adult hermaphrodites, consistent with previously published results (Csankovszki *et al.*, 2009). For additional control, we used a mutation in another condensin I^{DC} member, *dpy-28(tm3535)*, which has similar defects (Hernandez *et al.*, 2018). Fluorescent antibodies specific to CAPG-1 and another condensin I^{DC} subunit, DPY-27, were used to visualize localization of the DCC to the X chromosome compared to wild type (N2) (Figure 1C). N2 hermaphrodites have overlapping signals of CAPG-1 and DPY-27 on both X chromosomes. The X localization of these two condensin I^{DC} proteins is also absent in *capg-1(tm5770)*. This indicates that the *capg-1(tm5770)* mutation also disrupts DCC localization to the X to a similar degree as *capg-1(tm1514)* or *dpy-28(tm3535)*.

The *capg-1(tm5770)* M+Z- hermaphrodites, unlike the *capg-1(tm1514)* mutants, were observed laying embryos, some of which hatched then arrested in L1, showing more phenotypic similarity to the *dpy-28(tm3535)* mutants than the *capg-1(tm1514)* mutants. To quantify this observation, we conducted embryo and lethality counts in *capg-1(tm1514)*, *capg-1(tm5770)*, and *dpy-28(tm3535)* mutants to assess both the number of embryos laid and the number of embryos hatched (Figure 1D). Our results show that while *capg-1(tm1514)* M+Z- mutants did not lay any eggs, the *capg-1(tm5770)* M+Z- and the *dpy-28(tm3535)* M+Z- mutants produced significant numbers of embryos. Many of the embryos laid by the *capg-1(tm5770)* and *dpy-28(tm3535)* mutants hatched then arrested in the L1 stage. Interestingly, there is a high amount of variability in numbers of embryos laid between individual worms in the *dpy-28(tm3535)* and *capg-1(tm5770)* strains. The *dpy-28(tm3535)* and *capg-1(tm5770)* mutants produced a small percentage of M-Z- progeny that survived until adulthood. Phenotypically, these were either males or very Dpy hermaphrodites that had severe developmental defects and were sterile. The *capg-1(tm5770)* mutants (n=10) laid an average of 58 embryos per worm, ranging between 0 and 195. Of the 580 total embryos laid, 297 hatched, of which 291 arrested in the L1 stage, with 1 male and 5 hermaphrodites surviving to adulthood. The *dpy-28(tm3535)* mutants (n=7) laid an average of 122 embryos per worm, ranging between 8 and 255. Of the 853 total embryos laid, 354 hatched, of which 340 arrested in the L1 stage, with 9 males and 5 hermaphrodites surviving to adulthood. The appearance of males in the M-Z- progeny is consistent with a weak Him phenotype reported previously for condensin I mutants (Plenefisch *et al.*, 1989; Hernandez *et al.*, 2018). Overall, these results indicate that the *capg-1(tm5770)* mutation results in phenotypes resembling the phenotypes caused by *dpy-28(tm3535)* and other mutations in condensin I^{DC} subunits (Plenefisch *et al.*, 1989). These condensin I^{DC} mutant phenotypes are different from the phenotypes resulting from the *capg-1(tm1514)* mutation.

These data suggest that the more severe phenotype in the *capg-1(tm1514)* mutants is not due to disruption of CAPG-1 function. There are several potential alternative explanations. The phenotype may be due to the deletion of the piRNA gene near the 5' end of the *capg-1* gene (Figure 1B). It is also possible that the severe phenotype observed in *capg-1(tm1514)* is due to a disruption of the *trans*-splice site between genes. The *capg-1* gene is last in its operon, and the *capg-*

1(*tm1514*) deletion includes a *trans*-spliced acceptor site (Worm Base). This would result in defective *trans*-splicing between *capg-1* and the upstream gene, F26A3.1. Our data does suggest, however, that the severity of the *capg-1(tm1514)* phenotype is not due to an alternative role of CAPG-1 outside of condensin I and condensin I^{DC} function.

Methods

Strains: All *C. elegans* strains were maintained using standard methods and fed *E. coli* (OP50) on NG agar plates and maintained at 20°C. The strains used included N2 Bristol strain (wild-type) as a negative control, EKM4 *capg-1(tm1514) I/hT2 [qIs48]* (I;III), EKM86 *capg-1(tm5770) I/hT2[qIs48]* (I;III), and EKM40 *dpy-28(tm3535) III/hT2[qIs48]* (I;III). M+Z- hermaphrodites were identified by selecting GFP-negative progeny of GFP-positive hermaphrodites.

Immunofluorescence Imaging: Young adult worms were dissected with needles in 10µL of 1X sperm salts (50mM Pipes pH7, 25 mM KCl, 1 mM MgSO₄, 45 mM NaCl, with 1 mM levamisole as a sedative), fixed in 2% paraformaldehyde in 1X sperm salts for five minutes in a humid chamber moistened with PBST (PBS with .1% Triton X-100), and frozen on dry ice with a coverslip for at least 15 minutes. After freezing, the coverslip was carefully separated with a razor blade and the slides were washed three times for 10 minutes each in PBST. This was followed by overnight incubation in a humid chamber with 40µL of a solution of primary antibodies rabbit anti-DPY-27 and rat anti-CAPG-1 (Csankovszki *et al.*, 2009) diluted 1:250 in PBST. One primary antibody targeted DPY-27, which is part of Condensin I^{DC} in the Dosage Compensation Complex, and was raised in rabbit (Csankovszki *et al.*, 2009). The other primary antibody targeted CAPG-1, which is part of both Condensin I and Condensin I^{DC}, and was raised in rat. Incubation with primary antibody was overnight in a humid chamber at room temperature. The next day, slides were washed three times for 10 minutes each in PBST, incubated for 1 hour at 37°C with 30µL of a solution of secondary antibody (Jackson Immunochemicals Cy3 conjugated anti-rabbit for DPY-27 and FITC conjugated anti-rat for CAPG-1 at 1:100), and washed again three times for 10 minutes each in PBST with the final wash containing 1µL of DAPI (1mg/mL). Slides were then mounted with Vectashield (Vector Laboratories).

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References

- Albritton, S. E., & Ercan, S. (2018). *Caenorhabditis elegans* Dosage Compensation: Insights into Condensin-Mediated Gene Regulation. *Trends in genetics: TIG*, 34(1), 41–53. PMID: 29037439.
- Csankovszki G., Collette K., Spahl K., Carey J., Snyder M., Petty E., Patel U., Tabuchi T., Liu H., McLeod I., Thompson J., Sarkesik A., Yates J., Meyer B.J., Hagstrom K. (2009). Three distinct condensin complexes control *C. elegans* chromosome dynamics. *Curr Biol*, 19:9–19. PMID: 19119011 .
- Hernandez, M. R., Davis, M. B., Jiang, J., Brouhard, E. A., Severson, A. F., & Csankovszki, G. (2018). Condensin I protects meiotic cohesin from WAPL-1 mediated removal. *PLoS genetics*, 14(5), e1007382. PMID: 29768402.
- Plenefisch, J. D., DeLong, L., & Meyer, B. J. (1989). Genes that implement the hermaphrodite mode of dosage compensation in *Caenorhabditis elegans*. *Genetics*, 121(1), 57–76. PMID: 2917714.

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