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# A *C. briggsae* Genetic Suppressor Screen to Identify Dosage Compensation Pathway Components

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## Abstract

Dosage compensation, the process by which the expression of X-linked genes are equalized between males, which have a single X chromosome and females, which have two, is essential in all heterogametic organisms. In *C. elegans*, dosage compensation is a complex process that is regulated by the developmental switch gene, *xol*-1. To better our understanding of the evolution of dosage compensation in nematodes, we use *C. briggsae* which has diverged from *C. elegans* ~15-30 million years ago, as a comparative model organism. In both species, loss of *xol*-1 results in a male specific lethality phenotype. We exploited this phenotype in *C. briggsae* and performed a classic genetic suppressor screen and identified nine suppressor mutations that are likely to represent components in the *C. briggsae* dosage compensation pathway.

**Keywords:** Genetic suppressor screen; *C. briggsae*; xol-1

## Introduction

Proper development requires the precise regulation of gene expression. In addition to individual genes being required to be expressed at the right time and place during development, chromosome-wide gene regulation events, such as dosage compensation, are also required. Dosage compensation is a specialized mechanism of chromosome-wide gene regulation, by which the expression of X -linked genes are equalized between males, which have a single X chromosome and females, which have two. This process is essential in all heterogametic organisms and failure to properly equalize gene dose between heterogametic organisms often results in sex-specific lethality. Dosage compensation also provides us with an interesting situation in which to study evolution since numerous unrelated organisms have evolved different mechanisms to achieve this global regulation of chromosome activity. This process occurs differently in mammals (XX/XY), flies (XX/XY) and worms (XX/XO). Equalization of the X-linked gene products in mammalian males and females is achieved by shutting down one of the two X chromosomes in the somatic cells of female mammals [1]. In flies, the level of transcription of the single set of X-linked genes in the male (XY) is increased [2]. In worms, decreasing the level of transcription of both sets of each X-linked gene in hermaphrodites relative to males equalizes X-linked gene expression between the sexes [3].

The existence of different mechanisms indicates there is a high level of developmental variation for dosage compensation between different organisms. Each of these groups (mammals, flies, and worms) co-opted a different module of genes for dosage compensation, which resulted in three very different dosage compensation solutions mammals [1-3]. Therefore, to understand how this essential chromosome-wide gene regulation process has evolved, characterization and comparison of more closely related species is required. The two *Caenorhabditis* species, *Caenorhabditis briggsae* and *Caenorhabditis elegans* provide an ideal system for this analysis. *C. elegans* and *C. briggsae* separated ~15-30 million years ago, and their sequence divergence is about 0.3 substitutions per site, slightly greater than human and mouse [4].

In *C. elegans*, dosage compensation is achieved by sex-specific targeting of the DCC to the hermaphrodite X chromosome those results in a reduction of X chromosome transcript levels by one-half [3]. As a result, the gene dose from the two hermaphrodite X chromosomes and the single male X chromosome are equal. In *C. elegans*, the DCC is comprised of proteins that function in several essential cellular processes such as, dosage compensation, mitosis and meiosis [5]. For example, five DCC components (MIX-1, DPY-27, DPY-26, DPY-28, and CAPG-1) are homologous to subunits of condensin, a conserved protein complex that promotes the compaction, resolution, and segregation of chromosomes during mitosis and meiosis [6-10]. Additional DCC subunits, SDC-1, SDC-2, and SDC-3, confer sex-specificity to the dosage compensation process and recruit the DCC to the X chromosomes of hermaphrodites [11,12] resulting in a two-fold reduction of X chromosome transcript levels [13,14] and hermaphrodite fates. This sex-specific targeting of the DCC is controlled by the expression of the developmental switch gene, *xol*-1 [15]. *xol*-1 is also responsible for regulating *C. elegans* sex determination [13]. High levels of XOL-1 result in a male fate (dosage compensation off), whereas low levels of XOL-1 results in a hermaphrodite fate (dosage compensation on) **(Figure 1)**.

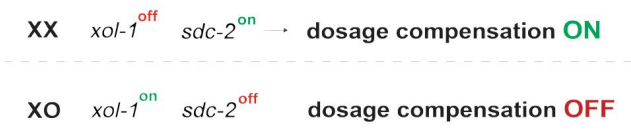


**Figure 1** Simplified *C. elegans* dosage compensation pathway. *xol-1* and *sdc-2* regulate dosage compensation. In hermaphrodites (XX), *xol-1* is off and *sdc-2* is on resulting in the activation of dosage compensation. In males (XO), *xol-1* is on and *sdc-2* is off and dosage compensation is not activated.

While there is a wealth of knowledge about *C. elegans* dosage compensation and the role of *xol*-1 in regulation dosage compensation in *C. elegans*, far less is known about *C. briggsae* dosage compensation and the role of *xol*-1 in *C. briggsae*. Using a loss of function *C. briggsae xol*-1 deletion mutant (gift courtesy of B. J. Meyer), that results in male specific lethality we performed a genetic suppressor screen to identify components in the *C. briggsae* dosage compensation pathway including components of the *C. briggsae* dosage compensation complex itself.

# Methods

## Strains

TY5006 *C. briggsae xol-1(y430)*

RE921 *C. brigssae him-8(v188)*

TWL005 *C. briggsae him-8(v188); xol-1(y430)*

## *C. briggsae him-8(v188); xol-1(y430)* strain construction

Standard *C. elegans* genetic techniques [16] were used to build the *C. briggsae him-8(v188); xol-1(y430)* strain used in the suppressor screen. Briefly, *Cbr-him-8(v188)* males were crossed to *Cbr-xol-1(y430)* hermaphrodites. *Cbr-him-8(v188)/+; +/xol-1(y430)* heterozygous F1 progeny were allowed to produce self-progeny. F2 progeny were screened via PCR and Sanger sequencing to identify double homozygous *C. briggsae him-8(v188); xol-1(y430)* hermaphrodites.

## *C. briggsae xol-1* suppressor screen

*C. briggsae him-8(v188); xol-1(y430)* L4s were mutagenized using a traditional EMS mutagenesis screen [17]. L4 hermaphrodites were incubated in 47mM EMS for four hours. After EMS incubation, hermaphrodites were washed five times with M9 buffer and allowed to recover for one hour on an NGM plate with OP50 bacteria. After recovery, L4 hermaphrodites were cloned out (1/plate) and allowed to have progeny at 20°C until F1s reached the L4 stage and could be screened for the presence of males. F1 progeny of mutagenized hermaphrodites were also moved to new plates (3/plate) so that their F2 progeny could be screened for males.

## Male rescue assay

Male rescue was determined by measuring the percentage of male and hermaphrodite progeny. Single L4 worms were cloned to individual NGM plates with OP50 and allowed to produce progeny at 20°C. Worms were moved to new NGM plates with OP50 every 24 hours until the worm was no longer laying embryos. Once the F1 progeny reached the adult staged they were scored as either hermaphrodites or males. For each individual L4 the percent male rescue=males/total progeny (males + hermaphrodites) produced by the individual. *C. briggsae him-8*, and *him-8*; *xol*-1 animals were used as controls. *C. briggsae him-8* mutants produce 30% male progeny, and him-8; *xol*-1 mutants produce 0% male progeny.

# Results

We utilized a suppressor screen **(Figure 2)** to identify downstream targets of *C. briggsae xol*-1. In both *C. elegans* and *C. briggsae*, the loss of XOL-1 results in a sex-specific male lethality. To facilitate this screen and remove the necessity of mating mutagenized *C. briggsae xol*-1 hermaphrodites to generate XO male progeny, we created a *C. briggsae* him-8; *xol*-1 double mutant.

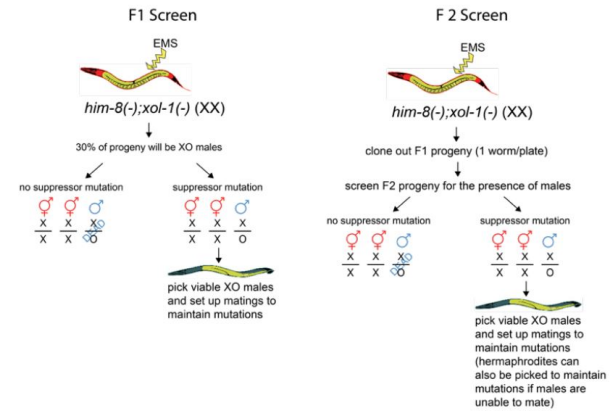


**Figure 2** *C. briggsae xol-1* suppressor screen**.** Male specific lethal phenotype can be exploited to identify *xol-1* suppressors. *him-8(-); xol-1(-)* hermaphrodites (XX) were mutagenized with EMS. The *him -8(-)* mutation results in a high incidence of male (XO) progeny (30%), however, in the presence of the *xol-1(-)* mutation, these male (XO) progeny are lethal. In the F1 screen, progeny (F1) of the mutagenized *him -8(-); xol-1(-)* hermaphrodites are screened for the presence of viable males. In the F2 screen, F1 progeny of mutagenized *him-8(-); xol-1(-)* hermaphrodites are cloned out and the F2 progeny (grandchildren of the mutagenized hermaphrodite) are screened for the presence of males.

Mutations in him-8 result in a high incidence of male (XO) progeny from a self-fertile hermaphrodite. When combined

with the *xol*-1 mutation, the *C. briggsae* him-8; *xol*-1 double mutant produces 30% XO male progeny that die as embryos due to *xol*-1 XO specific lethality [18]. We exploited this phenotype and performed a traditional EMS mutagenesis suppressor screen [17] to identify mutations that are able to suppress this *xol*-1 male lethal phenotype **(Figure 2)**.

We screened both F1 and F2 progeny of mutagenized worms. Screening both the F1 and F2 progeny allows for the ability to identify both dominant and recessive mutations. An additional advantage of screening F2 progeny is that it allowed for the identification of mutations that result in partial rescue of male lethality. For example, if the rescued male is unable to mate, the F2 screen allows for the isolation of the fertile hermaphrodite parent of a non-mating rescued male.

We screened 4447 haploid genomes and identified 9 suppressors that rescue the male lethal phenotype associated with loss of *C. briggsae xol*-1 function. We identified five suppressors (*lot10*, *lot11*, *lot19*, *lot20*, and *lot23*) from the F1 screen and four suppressors (*lot21*, *lot22*, *lot24*, and *lot25*) from the F2 screen. All suppressors were backcrossed (4X) and assayed for their ability to rescue the male lethal phenotype associated with loss of the *C. briggsae xol*-1 gene function **(Figure 3)**. 10 L4s were cloned out for each suppressor strain and moved daily until worms stopped laying eggs. Once the F1 progeny reached adulthood, worms were scored as either hermaphrodite or male and percent male rescue was determined. Male rescue percentages ranged from 9% to 33% **(Figure 3)** demonstrating that there is variability between suppressors suggesting that our suppressors could represent alleles of more than one gene. The mutagenized strain, *Cbr-him-8(v188)*; *xol*-1(y430) produces 0% viable male progeny **(Figure 3)**, therefore, the presence of any males indicates rescue. We used the *Cbr-him-8(v188)* strain as the positive control. This mutation results in approximately 30% male progeny **(Figure 3)**, therefore, suppressors with 30% male progeny are considered to be fully rescued **(Figure 3)**.

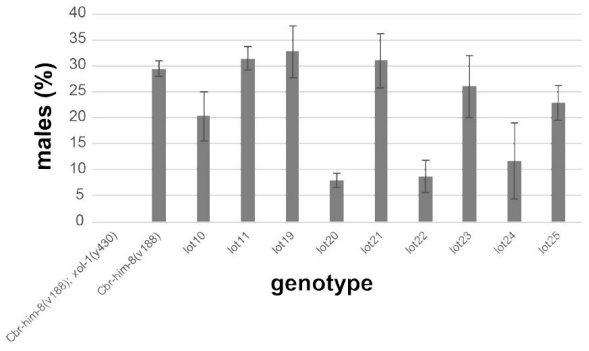


**Figure 3** Male rescue assay**.** *C. briggsae xol-1* suppressors vary in their ability to suppress male lethality. For each genotype, N ≥ 427. The positive control, *Cbr-him-8(v188)* produces approximately 30% male progeny and the negative control, *Cbr-him-8(v188); xol-1(y430)* produces 0% male progeny. Percentage of male progeny for the isolated suppressors varies from 9.0% to 33.0%. Error bars=STDEV.

## Discussion and Conclusion

Using a traditional genetic suppressor screen **(Figure 2)**, we isolated nine mutants that suppress the *C. briggsae xol*-1 male lethal phenotype. The nine suppressors vary in their ability to rescue the male lethal phenotype suggesting the isolation of different mutations (**Figure 3**). We will use SNP mapping and whole genome sequencing to identify the molecular lesions responsible for *C. briggsae xol*-1 suppression. We predict that suppressors will fall into three classes.

- Homologs of known *C. elegans* dosage compensation pathway components
- Novel components unique to *C. briggsae* that have homologs in *C. elegans* that have no known role in *C. elegans* dosage compensation
- Novel components unique to *C. briggsae* that have no known homologs in *C. elegans*.

All classes with further our understanding of the evolution of dosage compensation, however, the second and third classes (novel components) are particularly interesting. Novel components could either reveal divergence between mechanisms in *C. elegans* and *C. briggsae* or represent conserved components that have yet to be discovered in *C. elegans* allowing us to better understand dosage compensation in both species.

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