# Chromodomain proteins CEC-3 and CEC-6 affect chromatin and small RNA pathways and protect germline immortality

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## 1. Introduction

Germline immortality is required for the continual inheritance of genetic information. *C. elegans* histone methyltransferase mutants defective in proper H3K9 and H3K27 histone methylation show defective fertility or maternal effect sterility, respectively [1]. Similarly, mutants defective in RNAi, piRNA, nuclear RNAi defective (Nrde) pathways and RNAi inheritance pathways also display a mortal germline phenotype [1]. Nuclear RNA interference pathways lead to deposition of histone methylation marks (H3K9me and H3K27me) at the silenced locus, suggesting that small RNA pathways and chromatin pathways may work together to protect germline immortality (Figure 1) [2, 3].

*C. elegans* chromodomain containing protein (CEC)-3 and CEC-6 recognize H3K9me2/3 and H3K27me2/3 marks *in vitro* [4]. When mutated together, *cec-3Δ; cec-6Δ* mutants display a mortal germline phenotype [4]. To understand how CEC-3 and CEC-6 contribute to germline immortality we used several established phenotypic and transgenic reporter assays to evaluate the roles of CEC-3 and CEC-6 in small RNA- and chromatin-based gene silencing.

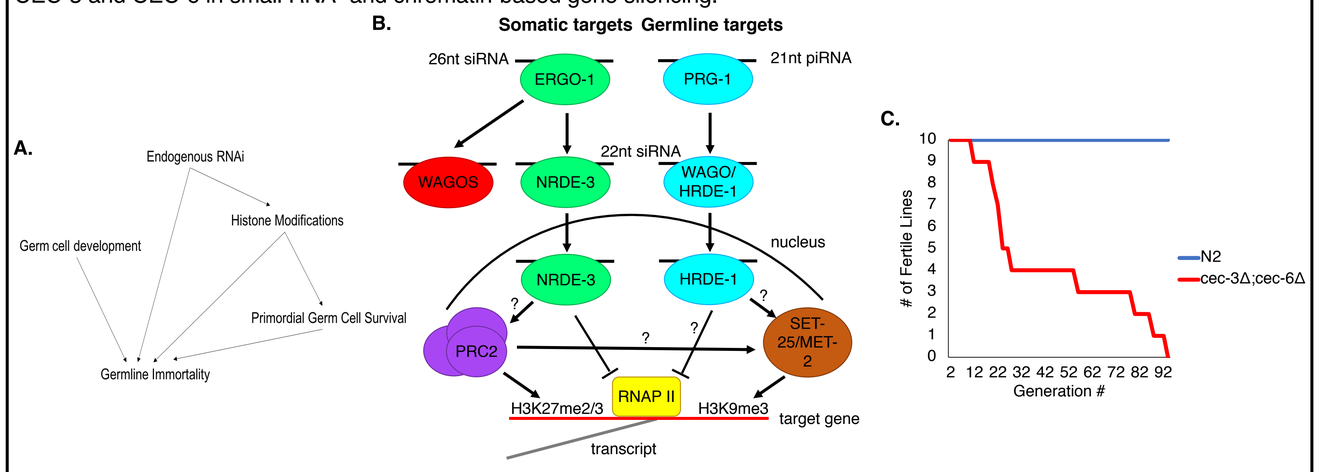



Figure 1: **Small RNA and chromatin pathways contribute to germline immortality.** (A) Diagram of factors and pathways contributing to germline immortality. (B) Diagram of endogenous RNAi pathways. Both pathways lead to inhibition of gene expression and deposition of H3K9 and H3K27 methyl marks. Adapted from [2]. (C) Mortal germline assay with *cec-3Δ; cec-6Δ* mutants. 10 lines of N2 and *cec-3Δ; cec-6Δ* mutants obtained from a mortal germline assay at the 44th generation were observed for sterility every 2 generations. Six larval stage one (L1) worms were picked onto new plates every week. Sterile lines did not produce any progeny after 14 days.

## 2. CEC-6 but not CEC-3 is required for germline silencing of integrated and extrachromosomal multi-copy transgene arrays

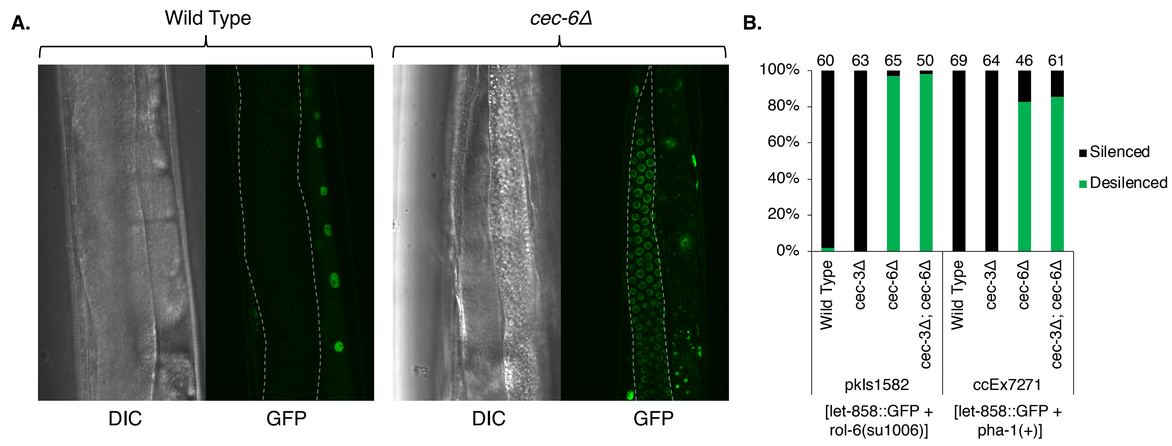



**Figure 2: CEC-6 but not CEC-3 is required for germline silencing of integrated and extrachromosomal multi-copy transgene arrays.** Integrated (*pkIs1582*) [5] and extrachromosomal (*ccEx7271*) [6] multi-copy array transgenes were crossed into *cec-3Δ* and *cec-6Δ* mutants and imaged for *gfp* expression in the germline at adulthood. Representative images are shown in **(A)**. Areas outlined in white indicate distal germline syncytium. In **(B),** % desilencing was totaled for integrated and extrachromosomal transgene array bearing strains. Total worms scored for each strain is shown at the top of each bar.

## 3. CEC-6 is required for initiation and positively regulates maintenance of germline transgene silencing

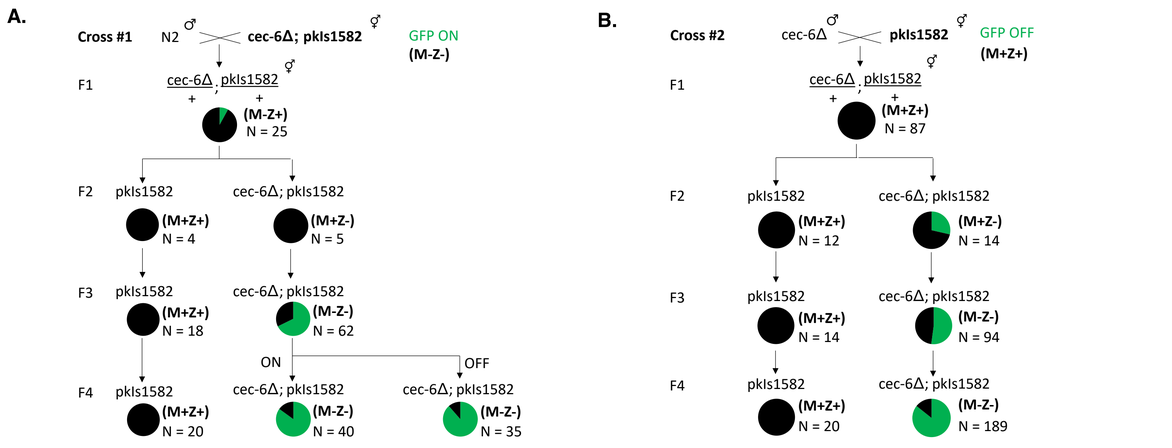



**Figure 3: CEC-6 is required for initiation and positively regulates maintenance of germline transgene silencing.** Two crosses for parental contribution of CEC-6 were scored every generation for proportion of germline desilencing. In **(A)** hermaphrodites carrying a Pmyo-2::GFP-marked cec-6 deletion and the repetitive transgene, *pkIs1582*, were crossed with wild type males. 4 replicates are summarized here. In **(B)** males carrying a Pmyo2::GFP-marked cec-6 deletion were crossed with *pkIs1582* containing hermaphrodites. 2 replicates are summarized here. Initial state of germline transgene silencing was indicated in green. MZ notation was shown to indicate maternal and zygotic contribution of CEC-6.

## 4. *met-2* suppresses *cec-3Δ* and *cec-6Δ* mediated somatic transgene silencing

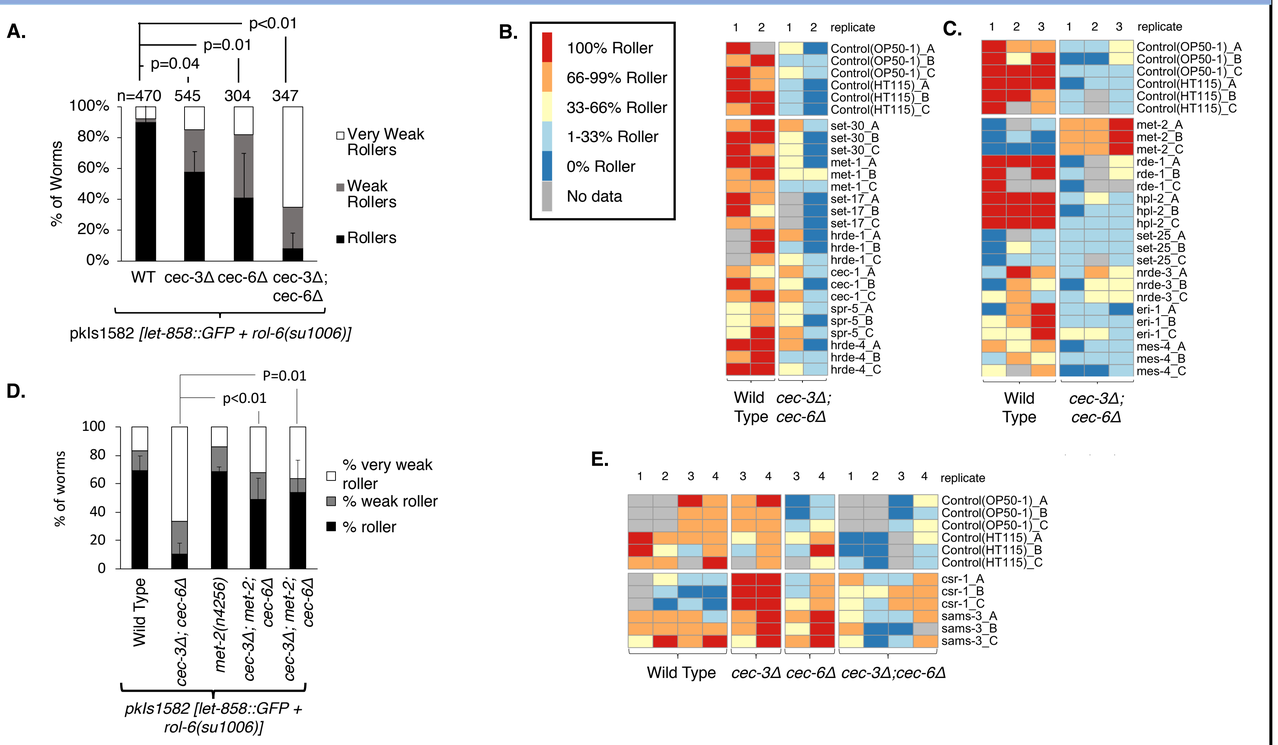



**Figure 4: *met-2* suppresses *cec-3Δ* and *cec-6Δ* mediated somatic transgene silencing.** Somatic transgene silencing was assayed in *cec-3Δ* and *cec-6Δ* mutants with a *pkIs1582* background (typically causing rolling) and is shown in **(A)**. Rolling phenotype was scored as a readout of somatic transgene silencing. 4 biological replicates were averaged. Error bars indicate standard deviation of rollers. Significance was calculated using a t-test. RNAi screens for genetic requirements of *cec-3Δ* and *cec-6Δ* mediated somatic silencing was shown in **(B, C and E)**. Embryos were laid on plates with *E. coli* expressing dsRNA against indicated gene targets, OP50-1 and HT115. Larval stage 4 worms were plated for *csr-1* and *sams-3* in the P0 generation. Plates were qualitatively scored in the F1 generation. Each column represents one biological replicate. Rows represent technical replicates. Legend is shown in B. *met-2* mutants were assayed for suppression of *cec-3Δ* and *cec-6Δ* mediated somatic transgene silencing in **(D)**.

## 5. *cec-3Δ* and *cec-6Δ* mutants are hypersensitive to nuclear RNAi but weakly defective in germline RNAi

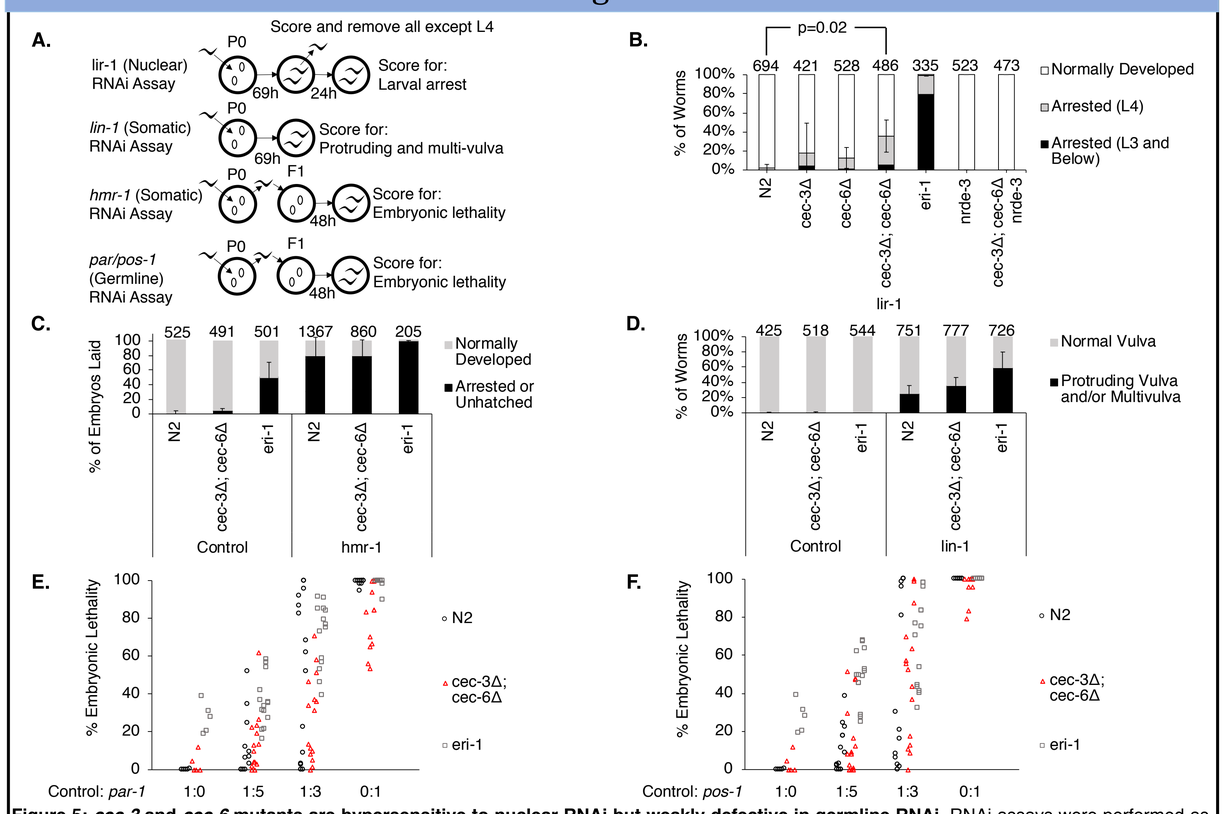
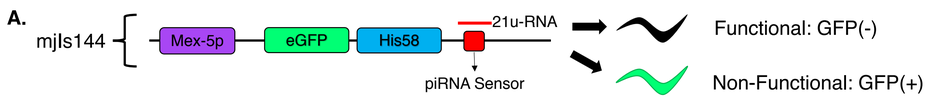



**Figure 5: *cec-3* and *cec-6* mutants are hypersensitive to nuclear RNAi but weakly defective in germline RNAi.** RNAi assays were performed as shown in **(A)**. Briefly, strains of interest were plated on *E. coli* expressing dsRNA against target genes or control RNAi (non-specific target). Worms laid eggs for 2-3 hours (synchronized lay) then removed. Worms were passaged in a similar way. Data for the *lir-1* RNAi assay is shown in **(B)**, summarizing 4 replicates. Data for somatic RNAi assays: *hmr-1* (3 replicates) and *lin-1* (3 replicates) are shown in **(C)** and **(D)**, respectively. Numbers above bars indicate total worms scored. Error bars represent standard deviation between replicates. Significance between genotypes were calculated using a t-test. Data for germline RNAi assays: *par-1* (5 replicates) and *pos-1* (5 replicates) are shown in **(E)** and **(F)**, respectively.

## 6. CEC-3 and CEC-6 are not required for maintenance of piRNA mediated silencing



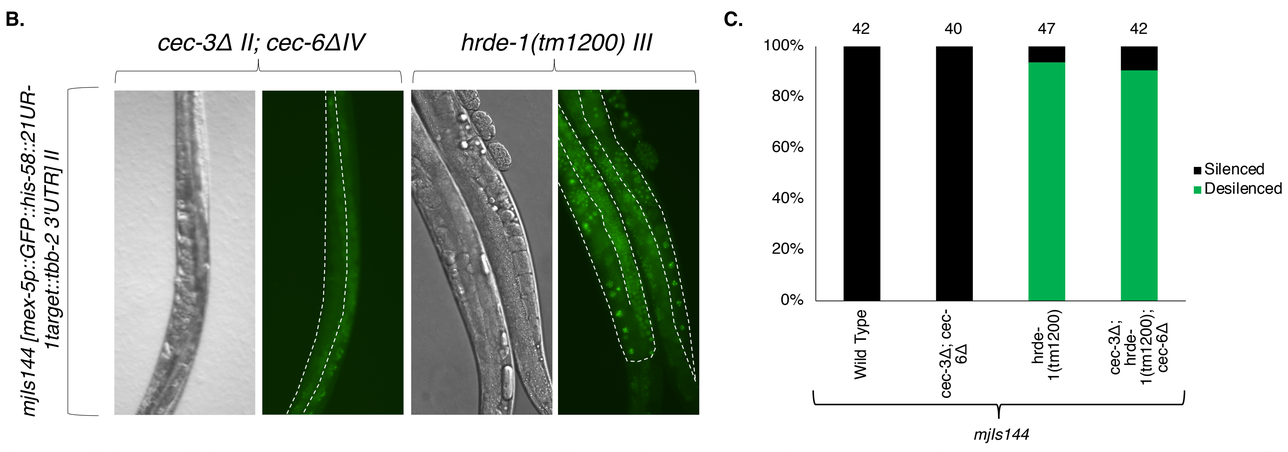




**Figure 6: CEC-3 and CEC-6 are not required for maintenance of piRNA mediated silencing.** Schematic of the piRNA sensor, *mjIs144* [7] is shown in **(A)**. The *mjIs144* transgene contains a sequence complementary to an endogenous piRNA. If the piRNA pathway is functional, the transgene will be targeted for silencing. Conversely, if it is non-functional, the piRNA sensor will be reactivated and the worm will fluoresce green in the germline. *cec-3Δ*, *cec-6Δ*, and *hrde-1(tm1200)* mutants were crossed into the piRNA sensors line, *mjIs144*, and imaged for reactivation of the piRNA sensor in the adult germline. Representative images are shown in **(B)**. White outlines indicate parts of the germline. In **(C),** % desilencing was totaled for *mjIs144* bearing strains. Total worms scored for each strain is shown at the top of each bar

## 7. Conclusion and Future Directions

In conclusion, we find that CEC-3 and CEC-6 affect both small RNA and chromatin pathways. Germline transgene desilencing has been linked to dysfunction in both RNAi and chromatin pathways [5]. In addition, somatic transgene silencing has been linked to enhanced RNAi phenotypes [8]. One potential model is that CEC-3 and CEC-6 may act downstream of MET-2 to inhibit nuclear RNAi on a chromatin level. Loss of inhibition may redirect resources resulting in somatic transgene silencing and germline transgene desilencing. CEC-6 may also function downstream of H3K27 histone methyltransferases to protect germline transgene silencing [9].

To further study the roles of CEC-3 and CEC-6 in germline immortality we plan to:

- Expand the somatic transgene silencing suppressor screen
- Determine if CEC-3 and CEC-6 are required for proper inheritance of germline RNAi by performing a germline RNAi inheritance assay
- Explore the role if CEC-3 and CEC-6 in endogenous RNAi pathways using a 22G siRNA reporter [10]

## 8. References and Acknowledgements

**References:**

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