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Removal of Polycomb Repressive Complex 2 Makes *C. elegans* Germ Cells Susceptible to Direct Conversion into Specific Somatic Cell Types

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SUMMARY

How specific cell types can be directly converted into other distinct cell types is a matter of intense investigation with wide-ranging basic and biomedical implications. Here, we show that removal of the histone 3 lysine 27 (H3K27) methyltransferase Polycomb repressor complex 2 (PRC2) permits ectopically expressed, neuron-type-specific transcription factors ("terminal selectors") to convert *Caenorhabditis elegans* germ cells directly into specific neuron types. Terminal-selector-induced germ-cell-to-neuron conversion can be observed not only upon genome-wide loss of H3K27 methylation in PRC2(−) animals but also upon genome-wide redistribution of H3K27 methylation patterns in animals that lack the H3K36 methyltransferase MES-4. Manipulation of the H3K27 methylation status not only permits conversion of germ cells into neurons but also permits *hlh-1/MyoD*-dependent conversion of germ cells into muscle cells, indicating that PRC2 protects the germline from the aberrant execution of multiple distinct somatic differentiation programs. Taken together, our findings demonstrate that the normally multistep process of development from a germ cell via a zygote to a terminally differentiated somatic cell type can be short-cut by providing an appropriate terminal selector transcription factor and manipulating histone methylation patterns.

INTRODUCTION

A number of transcription factors are known to be absolutely required for the induction of specific cellular differentiation programs. However, such transcription factors are often remarkably inefficient at imposing such a program on other cell types upon ectopic misexpression (Zhou and Melton, 2008). For example, ectopic misexpression of the CHE-1 zinc finger transcription factor, which is normally required to generate the ASE gustatory neuron type in *Caenorhabditis elegans* (Chang et al., 2003; Uchida et al., 2003), converts only a very small number of sensory neurons into ASE-like neurons; all other cell types are immune to the cell-fate-inducing ability of *che-1* (Tursun et al., 2011).

To explore the context dependency of *che-1* activity, we considered the possibility that an inhibitory mechanism may exist to prevent *che-1* from driving the ASE differentiation program in most other cell types. With this possibility in mind, we undertook a loss-of-function screen for genes whose knockdown enables *che-1* to more broadly induce ASE-like fate in other cellular contexts. This RNA interference (RNAi)-based screen identified a phylogenetically conserved histone chaperone, *lin-53* (called Rbbp4 and Rbbp7 in vertebrates), whose removal permitted a direct, *che-1*-mediated conversion of mitotic germ cells into ASE-like neurons (Tursun et al., 2011).

In this work, we explored the mechanistic basis of the conversion process by asking which other genes are involved in this process. We based our analysis on the well-documented observations that in vertebrates and invertebrates, the histone chaperones LIN-53/Rbbp4,7 are components of many distinct multiprotein complexes with various functions in chromatin biology. These complexes include the nucleosome remodeling and histone deacetylation (NURD) complex, the chromatin assembly factor (CAF) complex, the histone deacetylase corepressor complex Sin3, the histone acetyltransferase 1 (HAT1) complex, the nucleosome remodeling factor (NURF) complex, the retinoblastoma-gene-containing repressor complex DP, Rb, and class B synMuv (DRM), and Polycomb repressive complex 2 (PRC2) (Harrison et al., 2006; Loyola and Almouzni, 2004). The presence of LIN-53/Rbbp4,7 in these functionally very distinct complexes has been shown biochemically as well as through genetic analysis. Here, we show that the effect of *lin-53* on germ-cell-to-neuron conversion can be phenocopied by removal of the PRC2 complex, and further characterize features of the cellular conversion process.

RESULTS AND DISCUSSION

Removal of PRC2 Complex Components Allows for Germ-Cell-to-Neuron Conversion

Our initial RNAi screen, which showed that *lin-53* functions as a brake against the conversion of germ cells to neurons (Tursun

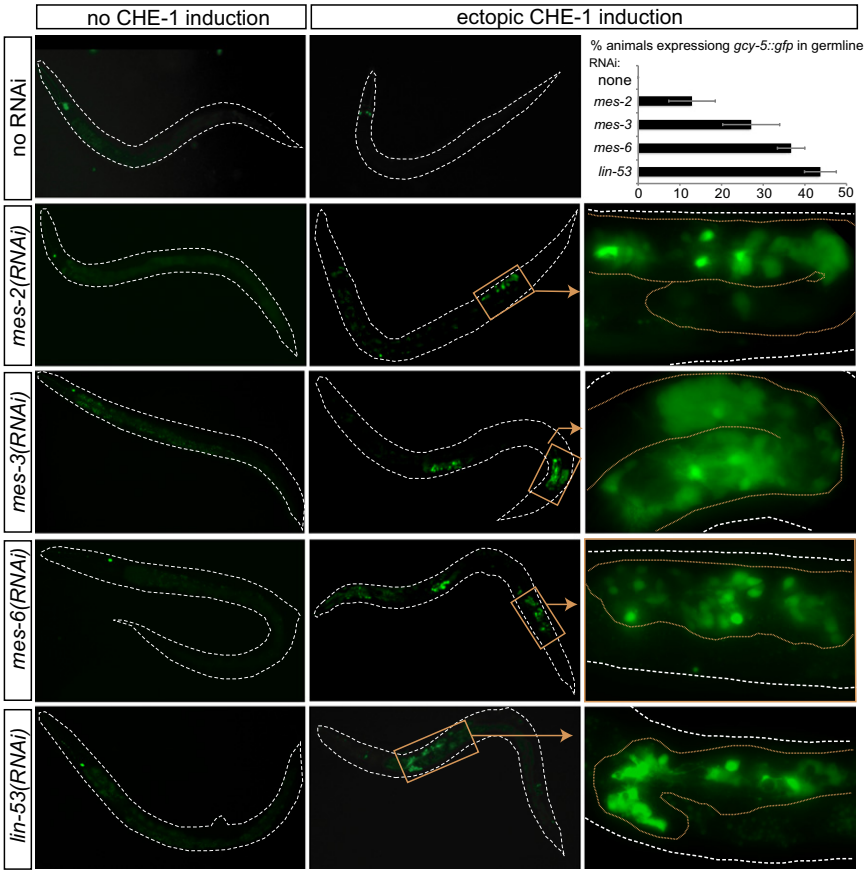

Figure 1. Knockdown of Members of the PRC2 Complex Allow *che-1*-Mediated Conversion of Germ Cells to Neurons

Larval progeny of RNAi-treated animals were analyzed for *gcy-5::gfp* (ASE fate marker; *ntIs1* transgene) expression ~24 hr after heat-shock induction of *che-1* (*otIs305* transgene). Right panels show blowups of boxed regions in middle panels, with germlines outlined by brown stippled lines. The top-right panel shows penetrance of conversion phenotypes after *che-1* induction at the ~L4 stage (at least three independent experiments, n = 90–300 for each RNAi). Error bars for each data set represent the SEM. The incomplete penetrance is most likely due to the incomplete effect of RNAi (as quantified in Figure S1). We show with antibody staining that the germline conversion phenotype cannot be explained by improved germline expression of *che-1* from the heat-shock vector (Figure S2). See also Experimental Procedures for more comments on transgene expression in the germline.

See also Figures S1, S2, S4, S5, and S6.

et al., 2011), did not reveal any obvious *lin-53*-like phenotypes for individual members of the many complexes with which the LIN-53/Rbbp4,7 protein is known to associate. As a result, the mechanism by which LIN-53 operates to prevent a germ-cell-to-neuron conversion remained an open question. However, negative results from this screen were difficult to interpret, mainly because RNAi of many of the various LIN-53 complex components resulted in infertility or early developmental defects, thereby precluding analysis of the germline.

We focused our analysis on the well-characterized Polycomb repressive complex 2 (PRC2), which in vertebrates and *Drosophila* contains the LIN-53 orthologs Rbbp4,7 (CAF1 in *Drosophila*), the H3K27 methyltransferase Ezh2 (Enhancer of Zeste in *Drosophila*), the WD40 protein Eed (Extra sex combs in *Drosophila*), and other associated proteins (Kuzmichev et al., 2002; Margueron and Reinberg, 2011). Similarly, in *C. elegans*, the PRC2 complex has been shown to contain the H3K27 methyltransferase MES-2/Ezh2 and two accessory proteins, MES-3 and the WD40 protein MES-6/Eed (Bender et al., 2004; Xu et al., 2001). Ectopic CHE-1 expression in *mes-2* and *mes-3* null mutants that lack both maternal and zygotic gene activity did not induce neurons in the germline (data not shown), but this is because the germline of such animals degenerates during larval stages (Capowski et al., 1991). In contrast, partial knockdown of *mes-2*, *mes-3*, and *mes-6* by RNAi in a genetic background that was not sensitized for RNAi improved the fertility and viability of double-stranded RNA (dsRNA)-treated animals, allowing the production of more germ cells, and these germ cells appeared superficially normal (Figure S1). After feeding animals with dsRNA against *mes-2*, *mes-3*, and *mes-6*, we induced *che-1* expression in the progeny of dsRNA-fed animals in all tissues through the heat-shock promoter, at approximately mid-larval stages. Feeding of control dsRNA or no dsRNA resulted in heat-shock-induced *che-1* being able to ectopically induce the ASE fate marker *gcy-5* exclusively in a small number of head neurons. In contrast, RNAi of each member of the *C. elegans* PRC2 complex (*mes-2/Ezh2; mes-3* and *mes-6/Eed*) resulted in $che\text{-}1^{heat\text{-}shock}$ -dependent *gcy-5* expression in the germline (Figure 1), providing the first hint that, as in *lin-53(RNAi)* animals, the germ cells may have converted into ASE-like neurons. This effect is not merely the result of improved germline expression of *che-1*, as shown by antibody staining (Figure S2). Neuron-like conversion is not observed in zygotic *mes* null mutant animals that still have a maternal *mes* gene contribution (M+Z−), suggesting that partial (but not complete) elimination of maternal *mes* by RNAi allows germ cell survival and generates susceptibility to conversion.

To study the cell-fate conversion in more detail, we performed RNAi against *mes-2*, *mes-3*, and *mes-6*, and induced *che-1* in a number of transgenic animals that express several reporter gene constructs. These included a second marker of ASE fate (*ceh-36*) and two panneuronal markers (*unc-33* and *snb-1*). We found that all of these markers were induced in the germline under these circumstances (Figure 2A). Neuronal marker induction was not only observed at the level of reporter transgenes but was also confirmed by single-molecule fluorescence in situ hybridization (smFISH; Raj et al., 2008), which revealed induction of endogenous neuronal genes, normally expressed