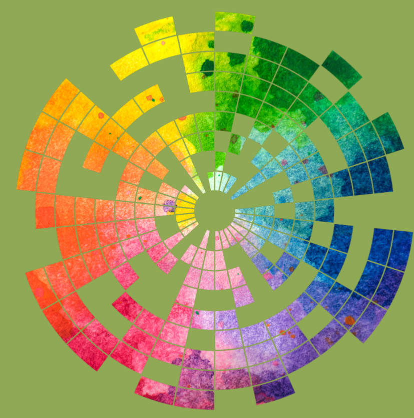

The contributions of Polycomb Repressive Complex 2 and H3K27me3 on gene repression in *Neurospora crassa*

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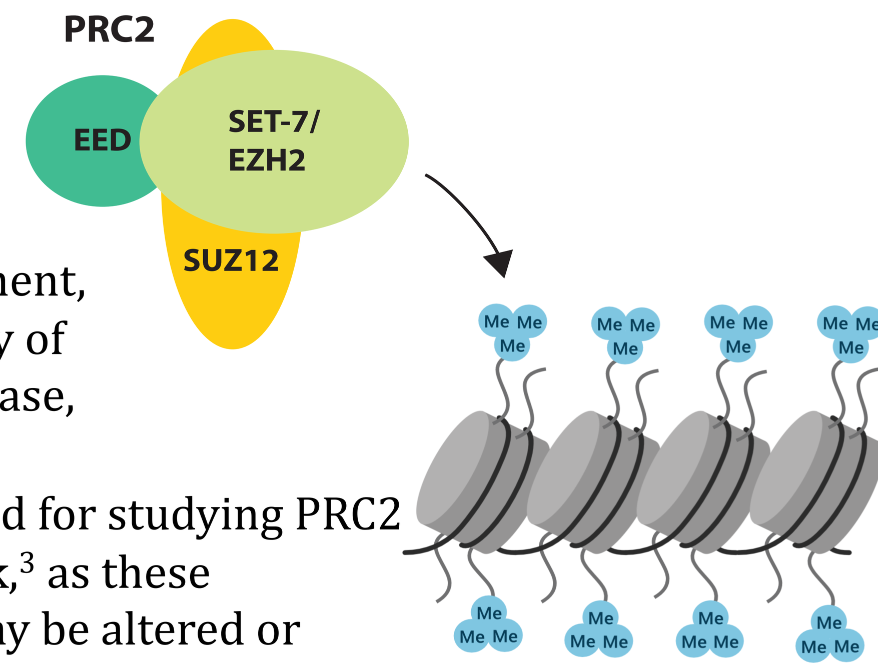
Introduction

- Gene expression can be regulated by the addition of chemical groups to the histone proteins DNA wraps around, such as the trimethylation of lysine 27 of histone 3 (H3K27me3).[1]
- H3K27me3 is catalytically deposited by the protein complex Polycomb Repressive Complex 2 (PRC2) and represses associated genes.[2]
- This repression is crucial for establishing gene expression patterns for proper development, and aberrant activity of PRC2 can cause disease, such as cancer.[2]



- *N. crassa* is well suited for studying PRC2 and its catalytic mark,[3] as these epigenetic factors may be altered or removed to study resulting changes without killing the organism.

Problem:

- Although H3K27me3 is associated with silenced genes, it is thought that H3K27me3 alone does not repress genes,[4] pointing to a gap in understanding the role of PRC2 in repression.

Research Question:
What is the role of PRC2 in repression, independent of its catalytic mark, H3K27me3?

Methods

Mutant Construction to Eliminate H3K27me3:

- Catalytic null SET-7 mutations were made by site-directed PCR mutagenesis and validated by Sanger sequencing and western blot analysis.

```
N.crassa         788  YINHASEN[5]NITPKIIYVNNEYRIKFTALRDIKAGEELFFNYGDNF  837
H.sapiens        686  FANHSVNP   NCYAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQ  730
D.melanogaster   704  FANHSINP   NCYAKVMMVTGDHRIGIFAKRAIQPGEELFFDYRYGP  748
X.laevis         688  FANHSVNP   NCYAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQ  732
D.rerio          700  FANHSVNP   NCYAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQ  744
                         *                                        *
                       H791A                                    Y833F
```

Sequence alignment of the SET domain of SET-7 orthologs, with mutated residues marked.

Assess Repression in SET-7 Mutants:

- Expression of genes normally marked by H3K27me3 were quantified by reverse transcriptase quantitative PCR.

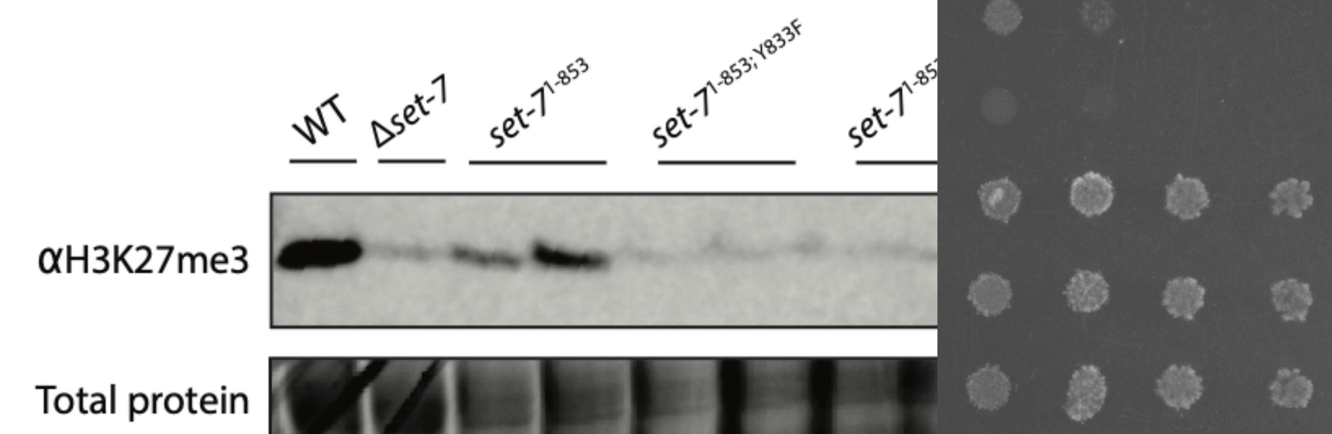
Wild N. crassa, the model organism used in this study.[5]

Assess PRC2 Complex Assembly with Mutant SET-7:

- Complex stability in the presence of catalytic SET-7 mutations was assessed by identification of changes in PRC2 core subunit protein levels in western blot analysis.

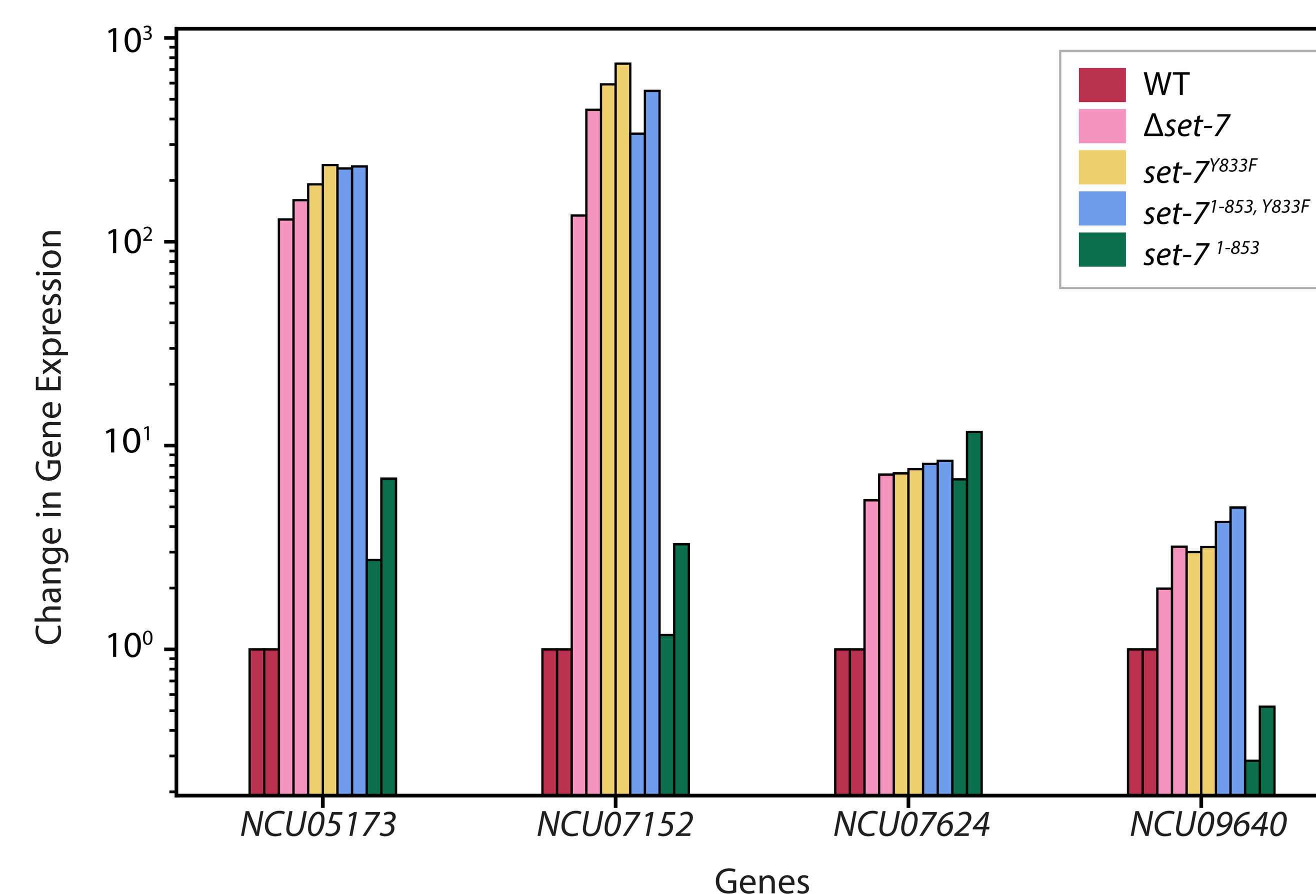
Results

Catalytic-null SET-7 mutations eli … me3



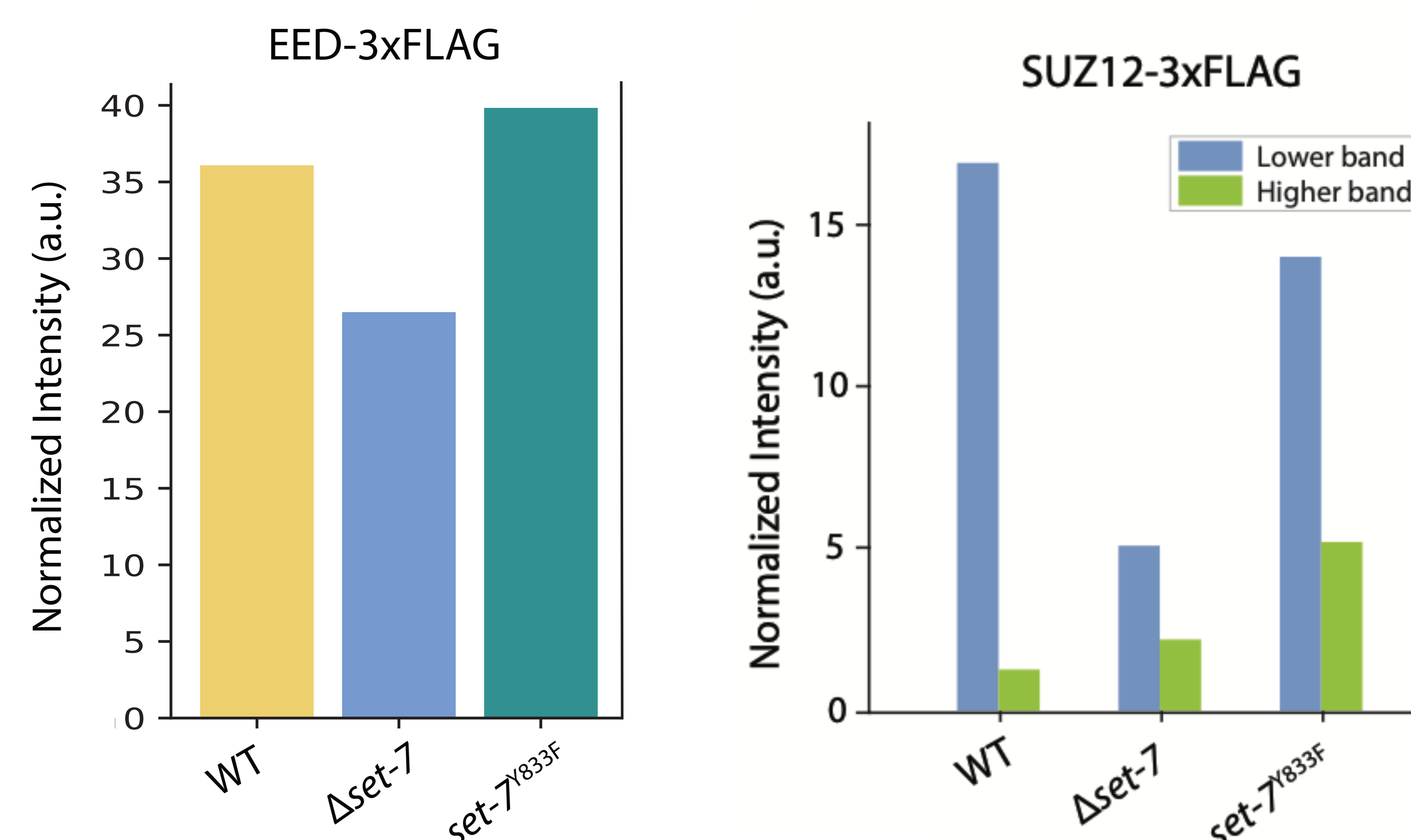
- Both mutations eliminated H3K27me3, phenocopyin … monstrating that the mutations catalytically inactivated SET-7.

Catalytic-inactivation of SET-7 derepressed H3K27me3-marked genes



- Expression of H3K27me3-marked genes consistently increased in mutant *set-7* and *set-7* knockout strains.

PRC2 complex assembly is maintained despite catalytic inactivation of SET-7



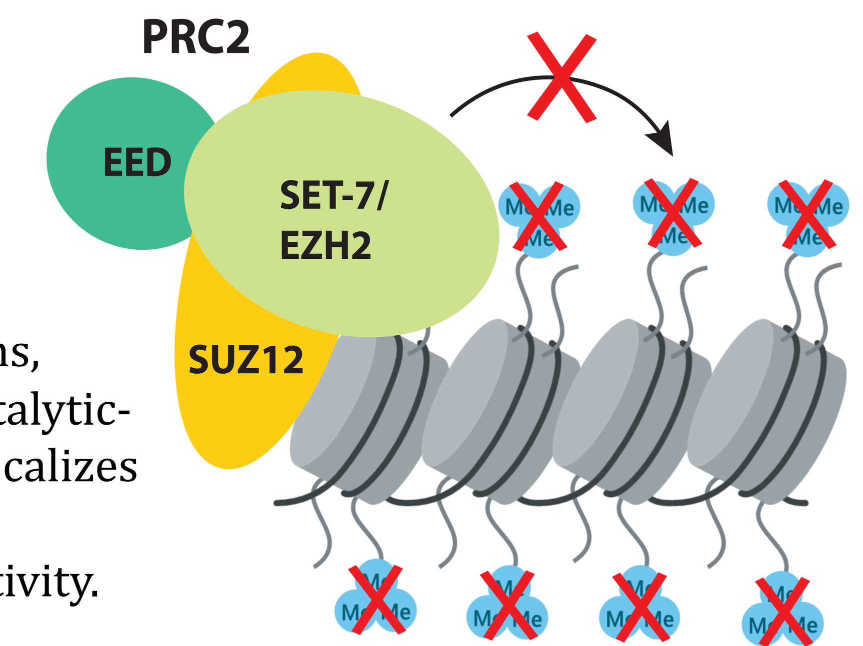
- The presence of mutant SET-7 maintains EED stability. Mutant *set-7* enriches the higher molecular weight band of SUZ12, yet elimination of *set-7* greatly reduces the quantity of lower molecular weight SUZ12 compared to mutated *set-7*.

Conclusions

- We found that PRC2 does not repress genes independently of its catalytic mark, H3K27me3.
- Loss of *set-7* destabilizes PRC2, whereas catalytic inactivation of SET-7 maintains the complex while repressing H3K27me3-marked genes.
- PRC2 itself is not acting repressively, eliminating a variable in the mechanism of H3K27me3 repression.
- This contributes to our understandings of a pertinent regulator of gene expression to development and disease.

Future Directions

- Co-immunoprecipitation between EED and SUZ12 in a mutant SET-7 background could further demonstrate PRC2 stability.
- Chromatin-immunoprecipitation with PRC2 subunits may reveal normal interactions with target genomic regions, indicating that the catalytic-null PRC2 correctly localizes despite loss of methyltransferase activity.



- Investigating the functional importance of the differently sized SUZ12 proteins would illuminate mechanisms of PRC2 localization.

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