The Role of H3K27 Methylation on Gene Silencing

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Introduction

- Polycomb repressive complex 2 (PRC2) is a chromatin-modifying enzymatic complex involved in gene silencing[1]
  - Chromatin modification involves covalent changes to histone tails, otherwise known as the histone code (Figure 1)
  - PRC2 uses its active site to trimethylate histone H3, lysine 27 (H3K27) and is plausibly used to recruit PRC1 and other proteins to modify chromatin density (Figure 2a)
- The active site of PRC2 is known as the SET domain
- vSET is a protein containing an isolated SET domain from *Paramecium bursaria* chlorella virus 1[2]
  - It acts specifically on H3K27 to trimethylate (Fig. 3), and we will test its ability to recuit PRC1 and/or PRC2 (Fig 2b)
  - Fusion protein LacI-HA-vSET causes widespread H3K27 trimethylation and extreme chromatin condensation when expressed *in vivo* in *Drosophila* (Fig. 4)

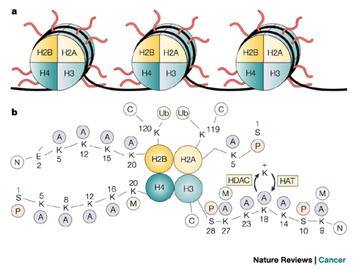

Fig 1. The Histone Code[3]

Nucleosomes are histone protein octamers used to package DNA within a chromosome. Histone tails can be covalently modified to alter chromatin structure. In the case of PRC2, Lysine 27 of Histone 3 is trimethylated, which is associated with gene silencing.

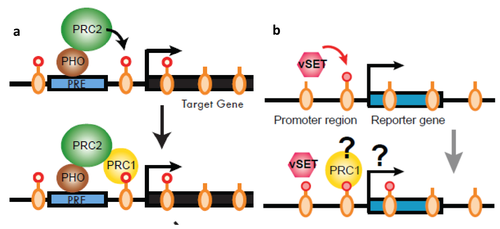

Figure 2. Proposed Effects of H3K27me3

A)PRC2 targets a gene through the Polycomb Response Element and its binding partner PHO.repressive complex. The SET domain of PRC2 trimethylates H3K27, and recruits PRC1 and may act to modify chromatin density. B) vSET also trimethylates H3K27 and is hypothesized to recruit proteins such as PRC1 to silence genes and may also increase chromatin density.

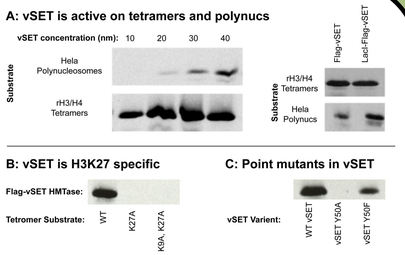

Figure 3. vSET Action on Histones

A)vSET methylation activty is shown with Histone Methyltransferase assays with polynucleosomes (top) isolated from Hela cells and H3H4 tetramers (bottom). B) Using wild-type histone H3 and histones modified at K27 and K9, it is shown that vSET is specific to H3K27. C) Point mutations in vSET identify the importance of Tyrosine 50 within vSET.

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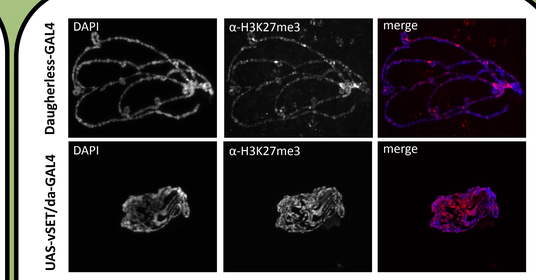

Figure 4. Chromatin Condensation by vSET

The heterologous expression of UAS-vSET within cells causes hypermethylation of H3K27. Without LacI-vSET expressed in cells, chromosomes are open with wild-type chromatin condensation and H3K27me3 banding patterns. However, with said hypermethylation, overall chromatin condensation increases dramatically.

Objective

- Determine the function of the H3K27me3 histone mark placed independently of PRC2
- Determine cause for lethality with ubiquitous hypermethylation at H3K27
- Identify unique developmental phenotypes caused by vSET expression
- Hypothesis: vSET global H3K27me3 prevents key developmental systems from being activated, causing lethality. When driven in specific regions, and at specific times it may cause targeted cell death or changes to the developmental pathway.

Results

- Lethal: Pan-neuronal, Muscle, Apterous, Engrailed, Pan-disc drivers lethal
- Wild Type: Vestigial, Sevenless, GMR, Timeless drivers
- Unique Phenotypes: Eyeless, Scalloped, A9 drivers (Figures 5 & 6) (table 1)

Driver	Expression	Mutant Phenotype
Sev-GAL4	eye	Relatively WT
GMR-GAL4	eye	Relatively WT
ey-GAL4	eyeless/eye	Eye transformations to wing disc, other structures
Tim-GAL4	eye/brain	WT
elav-GAL4, on X	pan-neuronal	Female lethal
24B-GAL4	Muscle	Lethal
GAL4 69B	salivary gland + discs	Lethal
ap-GAL4/cyo	Haltere, wing, larval brain	Lethal
yw;en-GAL4	wing and haltere	Lethal
MS1096, on X	wing disc	Females have fluid-filled wings
Vg-M-GAL4	wing margin	Mostly WT, some deaths
Sd-GAL4, on X	wing	Some females have small-fluid-filled wings
A9-GAL4	wing, haltere	Small, developmentally delayed wings

Table 1. Driver/LacI-vSET Cross Results

Shown above is a full list of *Drosophila* crosses and their results. The majority yielded either wild-type progeny, or lethality. In the cases of drivers found on the X chromosome, relevant phenotypes are seen in the female progeny as they have a copy of both the driver and LacI-vSET.

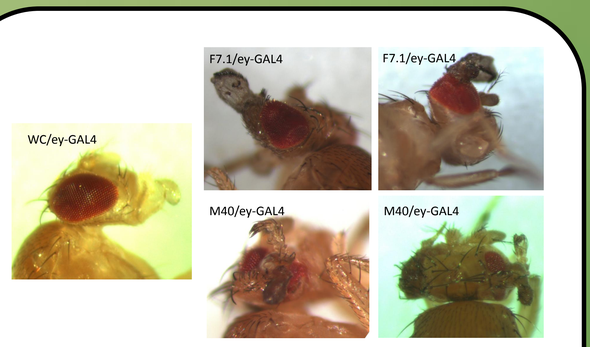

Figure 5. Eyeless-GAL4 Driven

Shown above are the unusual eye phenotypes resulting from driving LacI-vSET expression through the eyeless gene. The cross with WC control flies shows a normal eye (left), but when ey-GAL4 was crossed with either LacI-vSET lines, progeny showed unusual segment transformations. These transformations are hypothesized to be eye to wing, eye to leg, or eye to antenna.

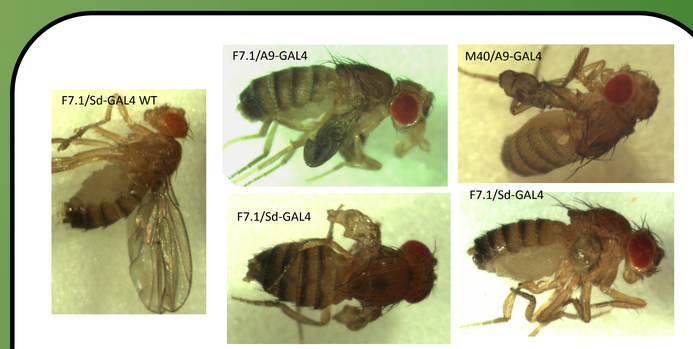

Figure 6. Wing Drivers

The LacI-vSET lines were crossed to a number of wing-specific drivers, which lead to a number of unique phenotypes. With the A9 driver, the wings appeared to be delayed in development, while with the Scalloped driver, the wings of some flies were considerably shrunken and fluid-filled, or were possibly transformed to wing haltere. On the left, an example of a wild-type wing is shown.

Discussion

- Notable non-lethal phenotypes appeared solely with drivers in non-essential structures
- When using drivers that operate at late times, unique or non-wild-type phenotypes were minimal or non-existant
  - Timing may be an important feature to the effect of hypermethylation

Future Directions

- Antibody stains and In-situ hybridization using developmental markers to determine the cause of developmental transformations
- Heat-Shock experiments to determine the role of timing
- Testing of additional GAL4 drivers

Materials and Methods

- Two lines of UAS-vSET *Drosophila melanogaster*
  - F7.1 and M40
- Battery of GAL4 drivers that act upon the UAS promoter
- Cross drivers with vSET lines and White C control
  - Observe adult phenotypes

References

1. Simon, J.A., and Kingston, R.E. (2009). Mechanisms of polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol *10*, 697–708.
2. Manzur, K.L., Farooq, A., Zeng, L., Plotnikova, O., Koch, A.W., Sachchidanand, and Zhou, M.-M. (2003). A dimeric viral SET domain methyltransferase specific to Lys27 of histone H3. Nat Struct Biol *10*, 187–196.
3. Marks, P., Rifkind, R.A., Richon, V.M., Breslow, R., Miller, T., and Kelly, W.K. (2001). Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer *1*, 194–202.

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