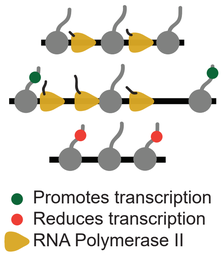
Guiding gene-specific methylation of histones in *C. elegans*

Shavin S Thomas, and Antony M Jose.
Department of Cell Biology and Molecular Genetics, University of Maryland, College Park.

Understanding the impacts of histone modification could aid in disease diagnosis and treatment

Histones are mediators of gene expression that can change DNA availability to transcriptional machinery.



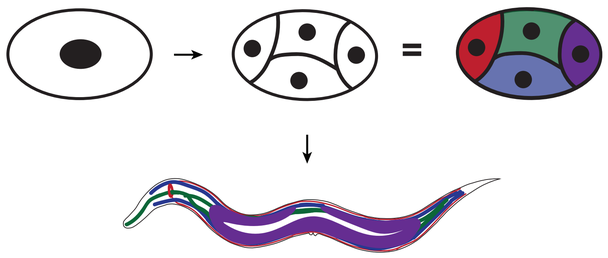
Certain histone modifications are associated with chromatin compaction, leading to transcriptional repression, while others are associated with unwinding the DNA from the histone complex, allowing for increased transcriptional activity [1].

In mice, SMYD2, a methyltransferase of Lysine 36 on Histone 3, was implicated as a mediator of renal cyst growth [2].

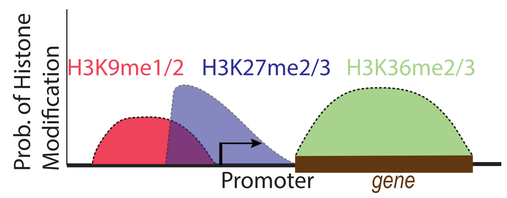
Furthermore, overexpression of enhancer of zeste homolog 2, a catalytic subunit involved in the methylation of Histone 3 Lysine 27, promoted atherosclerosis in mice [3].

These observations suggest that mutations in a histone modifier and histone modifications can influence gene expression and therefore disease progression.

The *C. elegans* histones and their modifiers, which have clear human orthologs, play a role in overall cell differentiation and gene regulation [4].

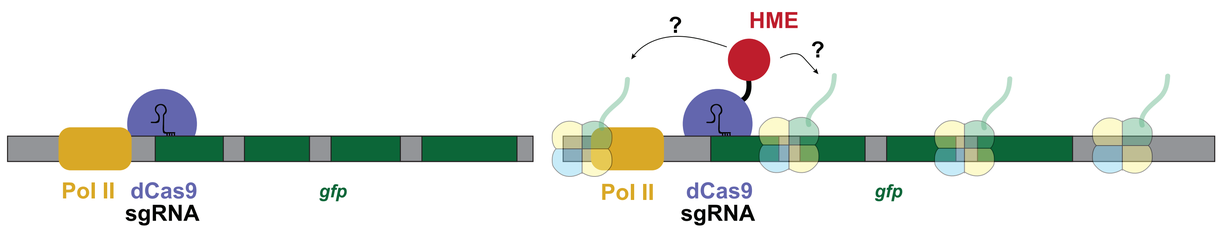


However, while some modification are associated with specific gene regions, the role of individual modifications in driving gene regulation is unknown.

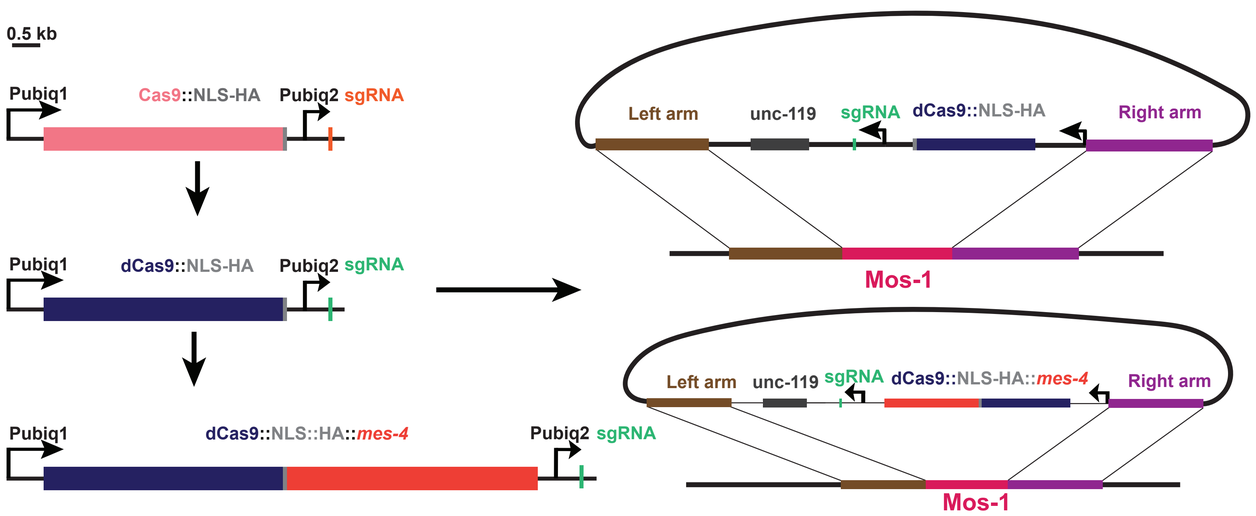


dCas9-mediated histone methylation can be used to induce specific histone modifications

Inactivated Cas9(dCas9) [5] can be used to guide histone modifying enzymes (HME) to target regions [6].



Experimental design for expressing dCas9 or dCas9-H3K36-methyltransferase in *C. elegans*

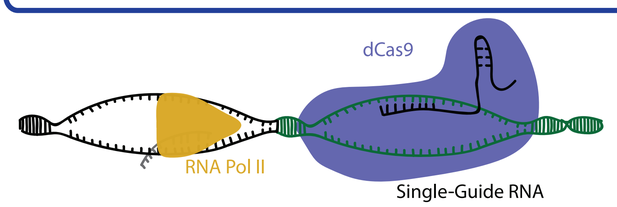


The dCas9 transgene was created from a template plasmid (pDD122) [7] by Gibson Assembly. Overlapping primers were used to mutate Cas9 to dCas9, and to transfer the sequence to a homology repair template (HRT). The HRT was inserted into *C. elegans* using Mos1-mediated Single Copy gene Insertion (MosSCI) [8]. A transgene with *mes-4*, a H3K36 methyltransferase, was also created and inserted using the same methods.

References

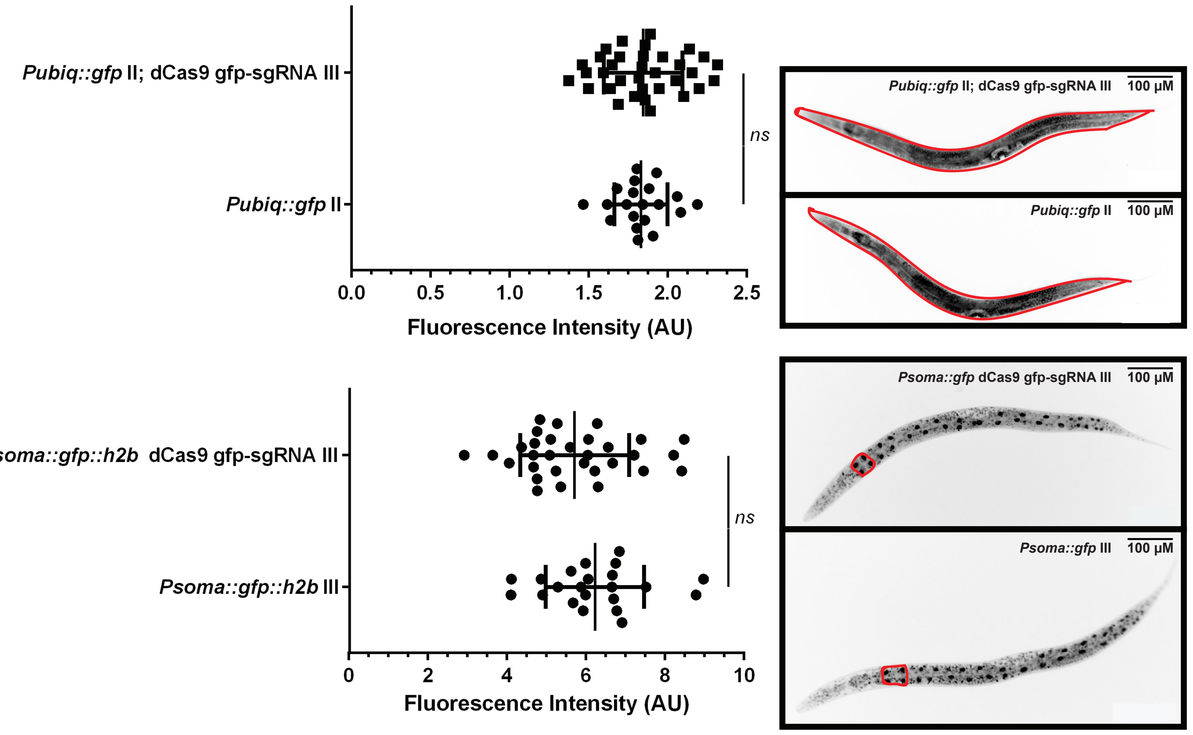
1. Venkatesh and Workman, Nature Rev. 2015
2. Yu and Zhuang, Front Pharmacol 2019
3. Xu et al., Trends in Endocrinology & Metabolism 2018
4. Cui and Han, WormBook 2007
5. Qi et al., Cell 2013
6. Agne et al., Synth. Bio. 2014
7. Dickinson et al., Nat Methods 2013
8. Frøkjær-Jensen et al., Nat Methods 2012

dCas9 binding alone may not impact gene expression



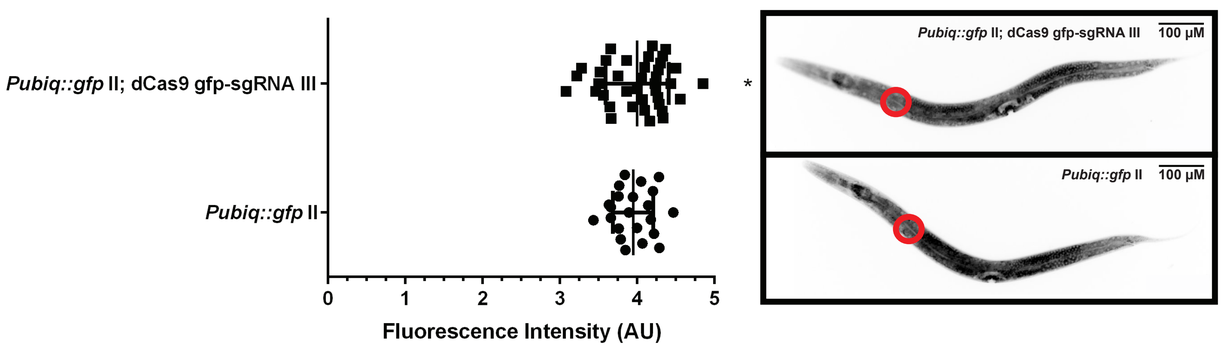
dCas9 binding may sterically hinder the progression of RNA Pol II, thereby decreasing transcription efficiency.

To evaluate the effects of dCas9 binding on gene expression, dCas9 guided by *gfp*-sgRNA was mated with worms containing *gfp* transgenes.



dCas9 binding to a ubiqutously expressing gfp (*gtbp-1*::*gfp*) and a somatic gfp (*Pdpy-30*::*gfp*) did not signficantly influence fluorescence intensity (n≥ 30). Red outline denotes area where fluorescence was quantified.

dCas9 binding may modestly increase variability in gene expression



dCas9 binding to a ubiqutously expressing *gfp* (*gtbp-1::gfp)* did modestly increase(*) the variance of fluorescence in somatic tissue(p=0.067; n≥30), but this was not found in worms solely expressing *gfp* in somatic tissues. Red outline denotes area where fluorescence was quantified.

Future Directions

-Confirm expression of the dCas9 transgene
-Quantify effects of dCas9 fusion products on gene expression
-Quantify the effects of site specific binding
-Quantify the effects of strand-dependent binding
-Determine the specificity of the binding of dCas9 using chromatin immunoprecipitation as described below.

Acknowledgements

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