Epigenetic inheritance uncoupled from sequence-specific recruitment

Kaushik Ragunathan, Gloria Jih, Danesh Moazed

Presented by: Alison Bartkowski, Candice Gard, Dahlia Rohm

Premise of Paper

- Wanted to determine if the inheritance of H3K9 methylation is dependent on DNA sequence
- To determine the effects of DNA sequence on H3K9me
  - Organism: fission yeast, *Schizosaccharomyces pombe*
  - Created an inducible system for heterochromatin establishment
  - Observed maintenance and inheritance of heterochromatin
- Results: Histone modifications can be transmitted independently of specific DNA sequences, DNA methylation or RNA interference [1]

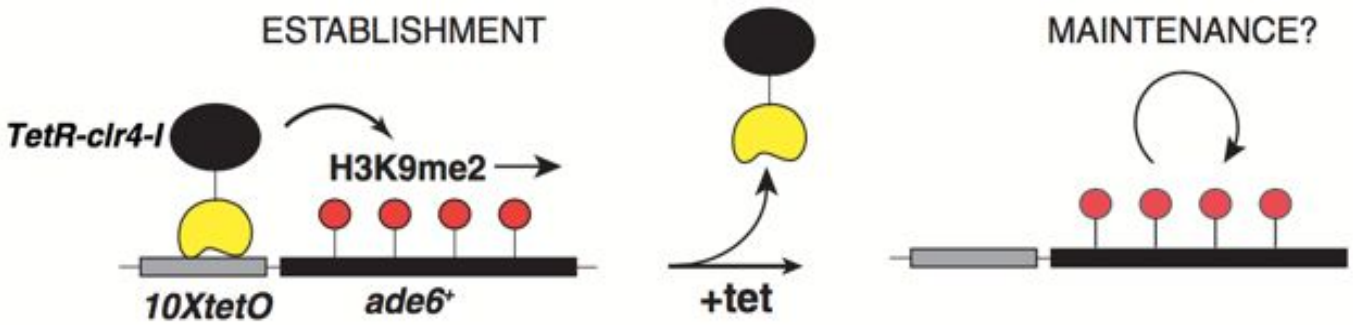
Process of Histone Methylation Inheritance

- Parental histones are retained and randomly distributed to newly synthesized daughter DNA strands during DNA replication [1]
- In *S.pombe* yeast, H3K9 methylation is catalyzed by Clr4
  - Clr4 is also responsible for copying H3K9me to daughter histone
  - RNAi machinery and site specific DNA binding proteins recruit Clr4 to control heterochromatin establishment [2]

Design of Inducible System for Heterochromatin

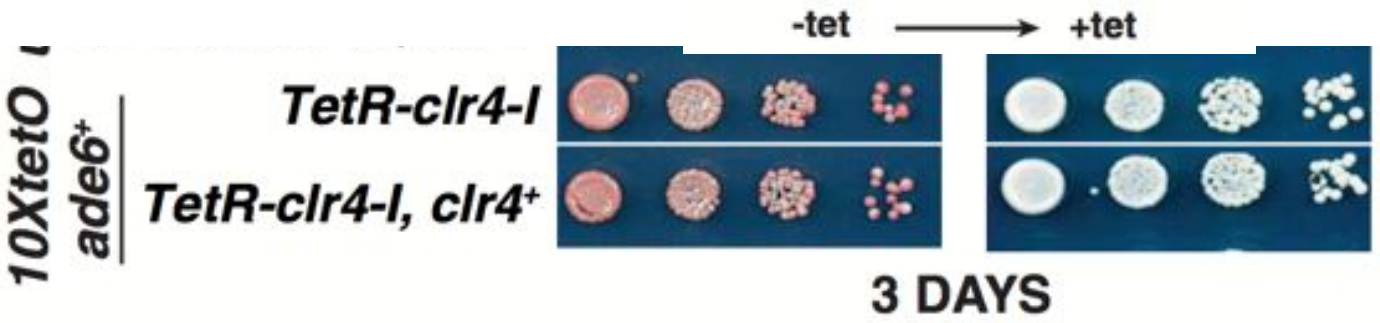
- Fused together TetR and clr4 methyltransferase without chromodomain to create the initiator TetR-clr4-I
- Used a promoter (10XtetO) and a reporter (ade6+) that changes color when expressed[1]
  - pink for silent- H3K9me present,
  - white for expressed- no H3K9me

Fig 1A: Tetracycline (tet) promotes the release of TetR-Clr4-I from tetO sites so that initiator-independent maintenance could be tested



Effects of Clr4 in tet+/tet- medium

Fig1B:



- Heterochromatin establishment does not require the chromodomain of Clr4
- Found loss of 40- to 50-kb domain of H3K9 H3K9me2 and -me3 encompassing the promoter-reporter region after 24 hours (~10 cell divisions) after adding tet[1]
  - Why couldn't wild-type clr4 maintain the methylation of H3K9?

Deletion of Epe1 gene

- Hypothesized demethylase might remove marks before maintenance
- Epe1 = gene that encodes a putative histone H3K9 demethylase
- Deletion of Epe1 prevents demethylation

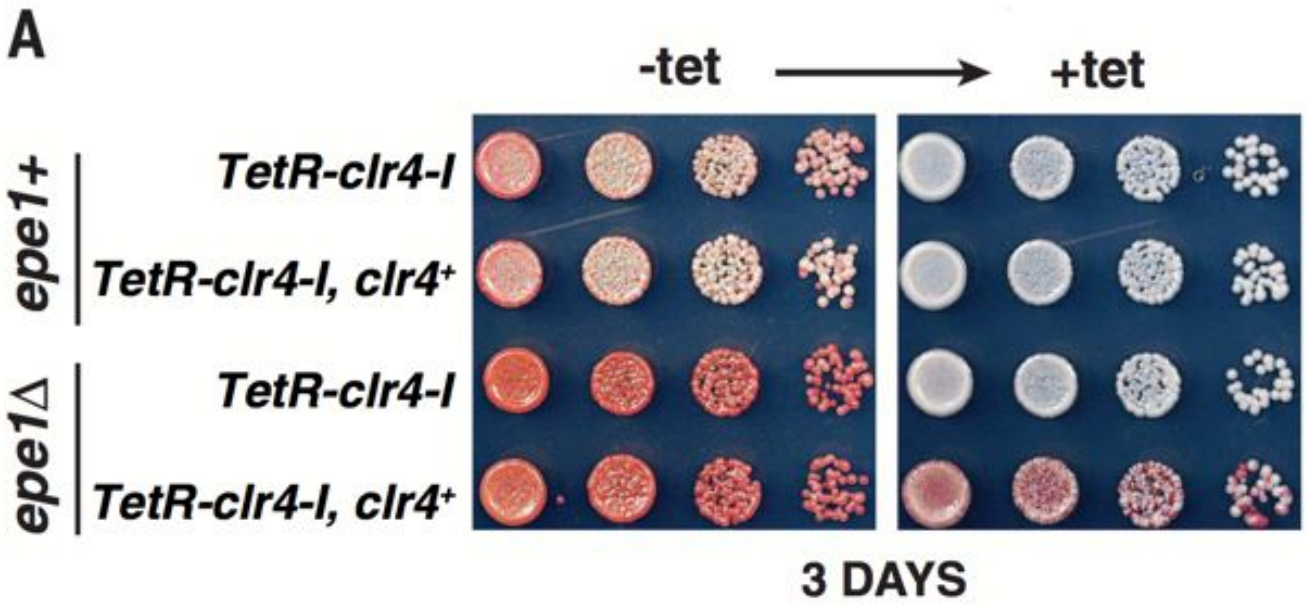
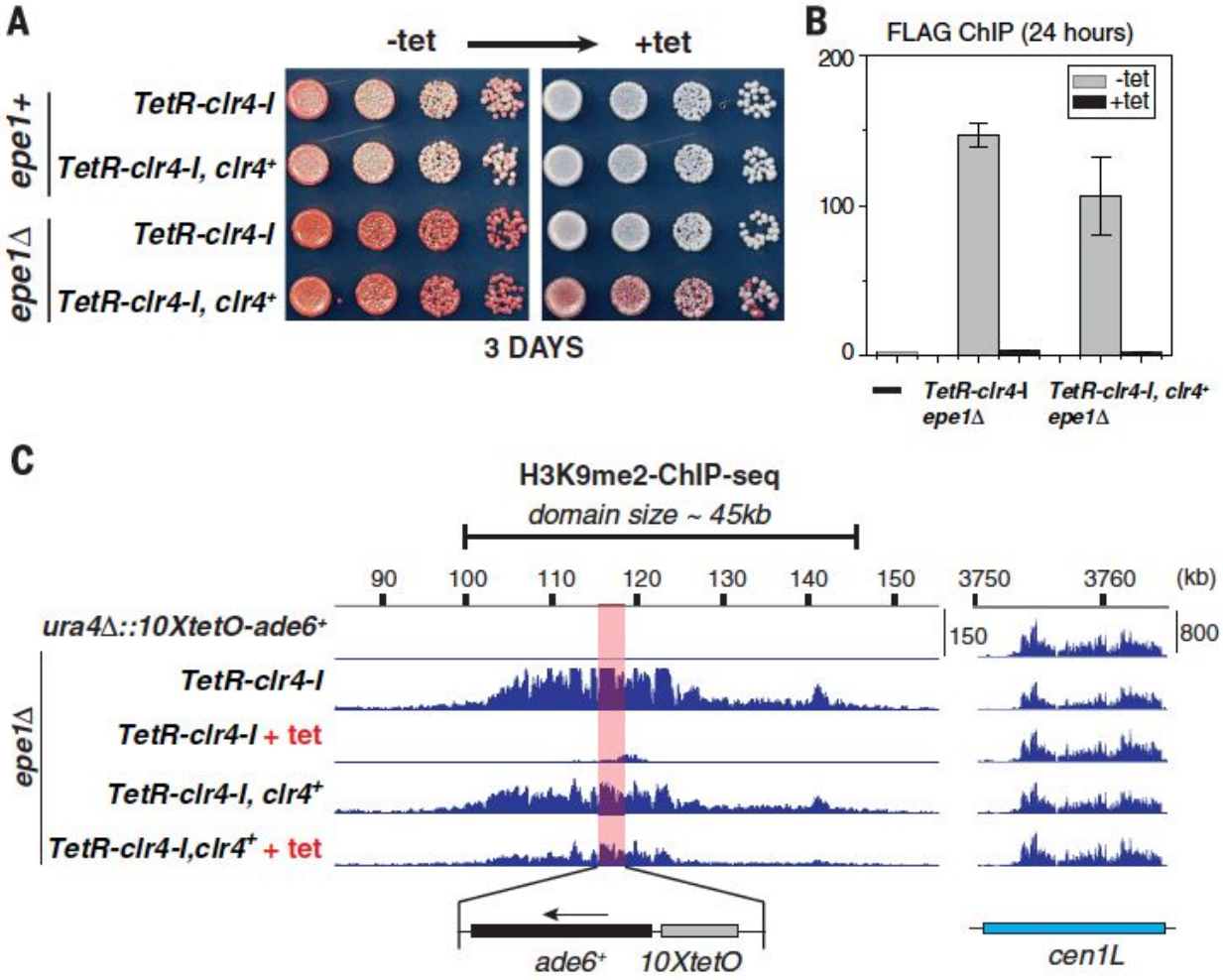


Fig2A: Color-silencing assays showing that in TetR-clr4-I, clr4+, epe1D cells, silencing is maintained on +tet medium.


A
-tet
+tet
epe1+
TetR-clr4-I
TetR-clr4-I, clr4+
epe1Δ
TetR-clr4-I
TetR-clr4-I, clr4+
3 DAYS
B
FLAG ChIP (24 hours)
200
100
0
-tet
+tet
TetR-clr4-I epe1Δ
TetR-clr4-I, clr4+ epe1Δ
C
H3K9me2-ChIP-seq
domain size ~ 45kb
90
100
110
120
130
140
150
3750
3760
(kb)
ura4Δ::10XtetO-ade6+
150
800
epe1Δ
TetR-clr4-I
TetR-clr4-I + tet
TetR-clr4-I, clr4+
TetR-clr4-I,clr4+ + tet
ade6+
10XtetO
cen1L

Importance of Epe1 gene

Epe1 dominates over clr4

<u>Epigenetic maintenance not unique to *epe1Δ* cells</u>

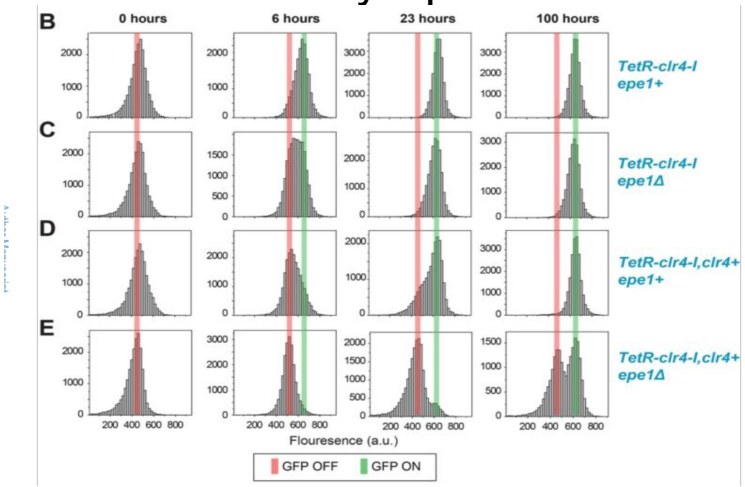
- detection masked by the rapid erasure of H3K9me marks by Epe1.

<u>Mst2*Δ* and Set1*Δ*</u>

- Stronger than wild type establishment
- Weak maintenance

<u>Pozl*Δ* and Dcrl*Δ*</u>

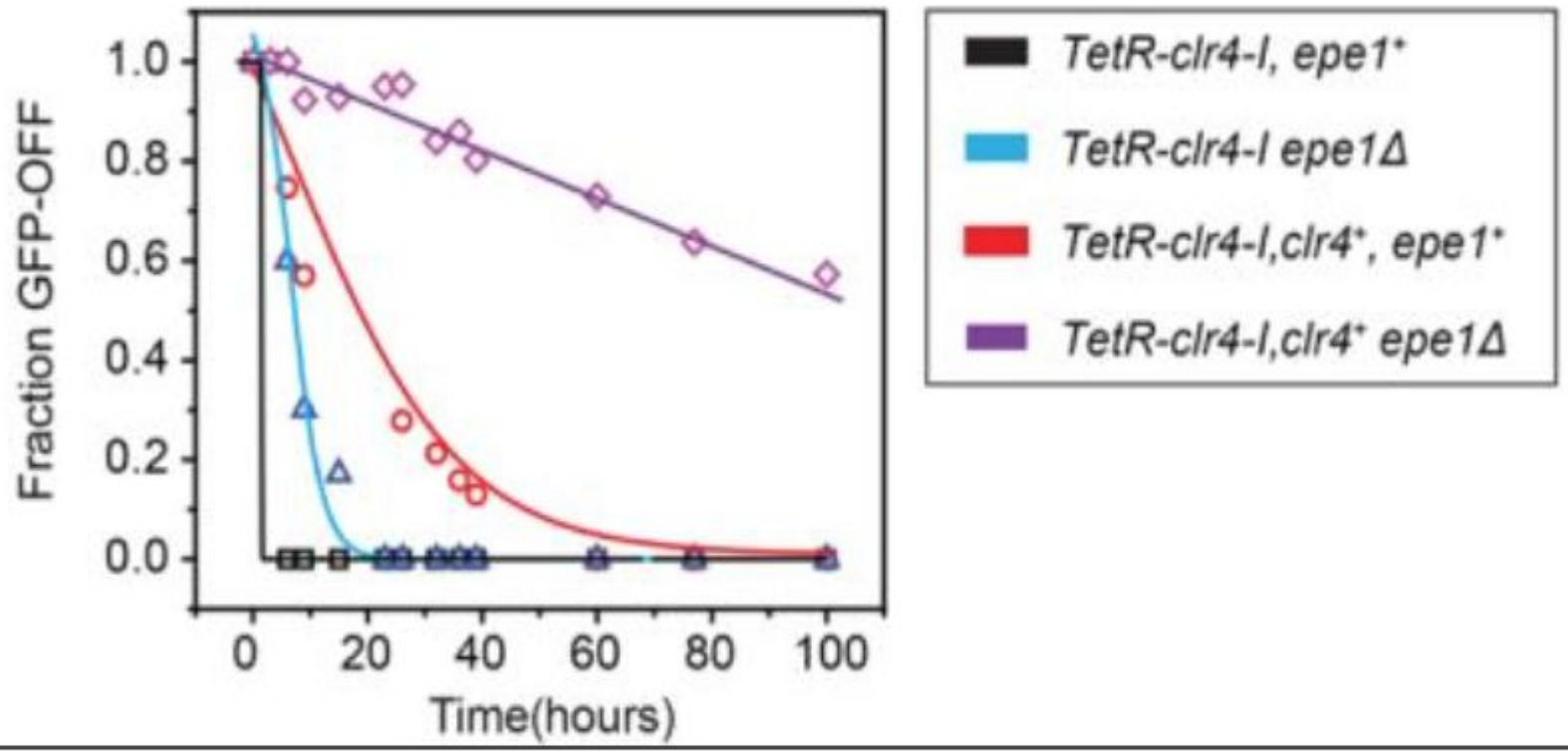
- No change in establishment
- No maintenance Dcrl, weak Pozl



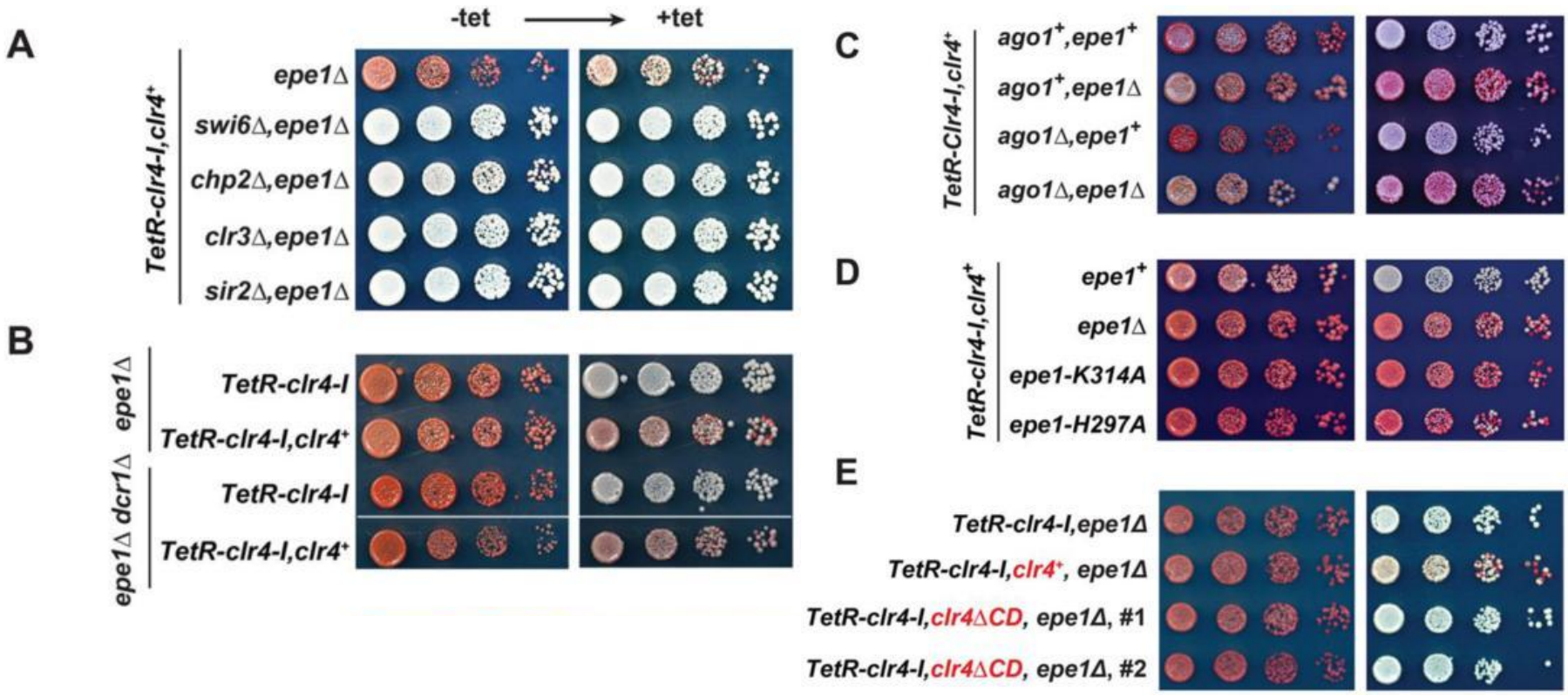
Conclusion:

The decay rate is primarily influenced by demethylation by Epe1

F

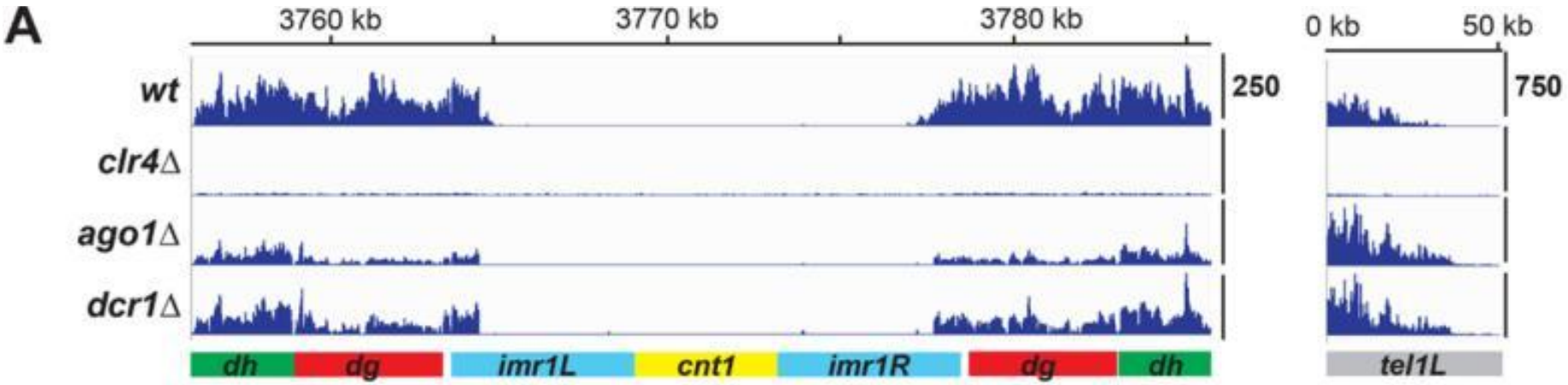

Fraction GFP-OFF
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Time(hours)
TetR-clr4-I, epe1+
TetR-clr4-I epe1Δ
TetR-clr4-I,clr4+, epe1+
TetR-clr4-I,clr4+ epe1Δ

Deletion of Other Genes



RNAi at native heterochromatin

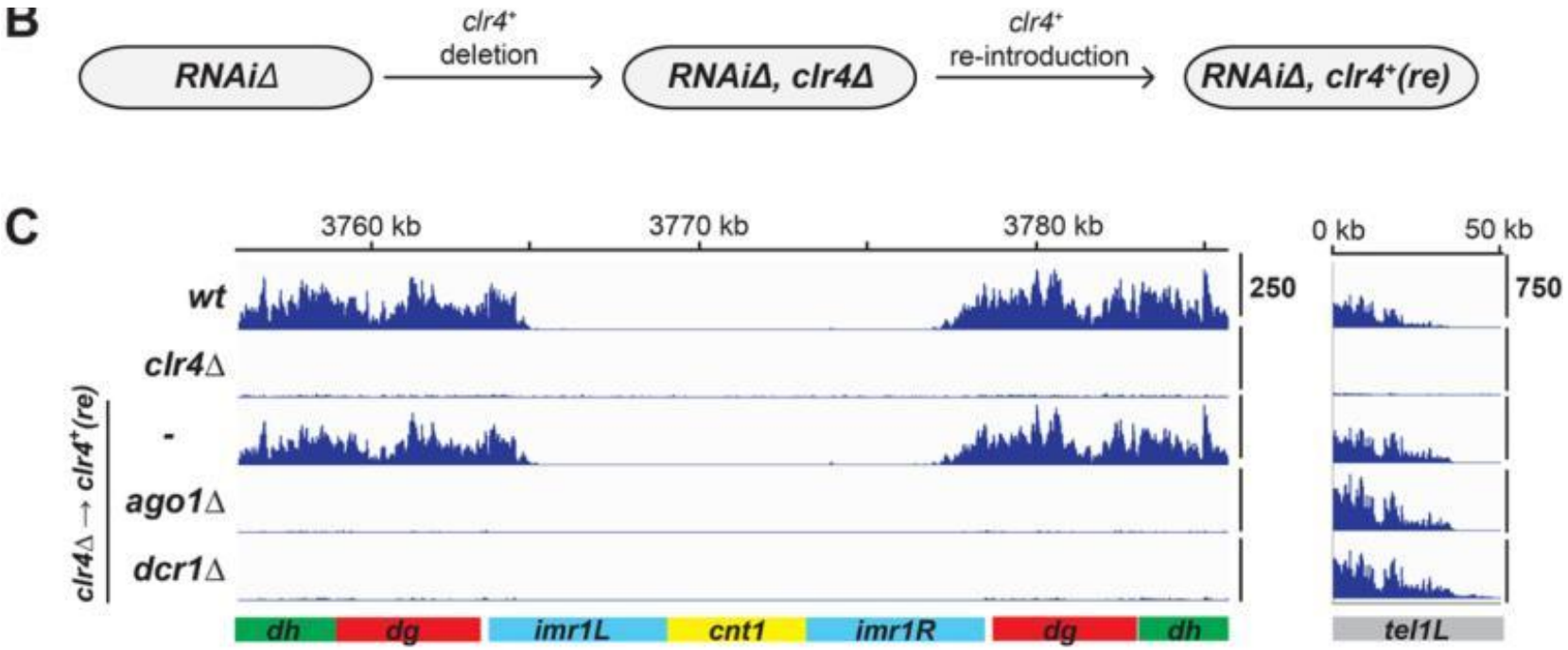
- Investigated the effect of RNAi on inheritance of H3K9me at native heterochromatin
- In *S. pombe,* RNAi is necessary for silencing in centromeric regions but not in other regions (e.g. mating-type) [3]
- siRNAs might recruit proteins necessary for heterochromatin formation (e.g. Clr3, Clr4, Clr6)
- Found that even when RNAi components were deleted, some residual H3K9me remained



A: residual methylation in RNAi-deletion cells

RNAi

- Q: are there RNAi-independent mechanisms for methylation at pericentromeric repeats? Can H3K9me be established *de novo* in the absence of RNAi influence?
- No *de novo* establishment of H3K9me in RNAi-deleted cells
- Conclusion: RNAi is main mechanism for *de novo* methylation near centromeres (sequence-specific)[1]
- the residual H3K9me is likely due to maintenance, not establishment[1]



B: schematic of reintroduction of clr4 into RNAi(del), clr4(del) cells
C: restoration of H3K9me upon reintroduction of clr4, only in clr4-deletion cells but not the double knockouts

Conclusions Summary

- Clr4 chromodomain not required for *de novo* H3K9me but IS required for maintenance
- Main mechanism of loss of methylation at ectopic locus (independent of sequence) is demethylation by Epe1
- Silencing at the ectopic locus is not influenced by RNAi
- RNAi seems to be the main mechanism for establishing H3K9me in pericentromeric regions but is not required for maintenance
- Overall: H3K9me histones can act “as carriers of epigenetic information[1]”

Dynamics and Memory of Heterochromatin in Living Cells

- Another paper exploring H3K9 methylation
- Used HP1a to induce methylation and observe kinetics and chromatin formation
- Done at Oct4 locus so could not be sequence independent
- Found methylation to propagate symmetrically at an average rate of 0.18 nucleosomes per hour
- Data allows for steady state dynamics predictions of on / off for H3K9 methylation

Current Work

- DNA sequence-dependent epigenetic inheritance of gene silencing and histone H3K9 methylation
- 1 shared author, Danesh Moazed
- Main Conclusion: There are sequence dependant ATF / CREB transcription factors required for inheritance of silencing.

So is this the reason why only 60% remained silenced? Lack of these sequence specific silencers?

Appendix

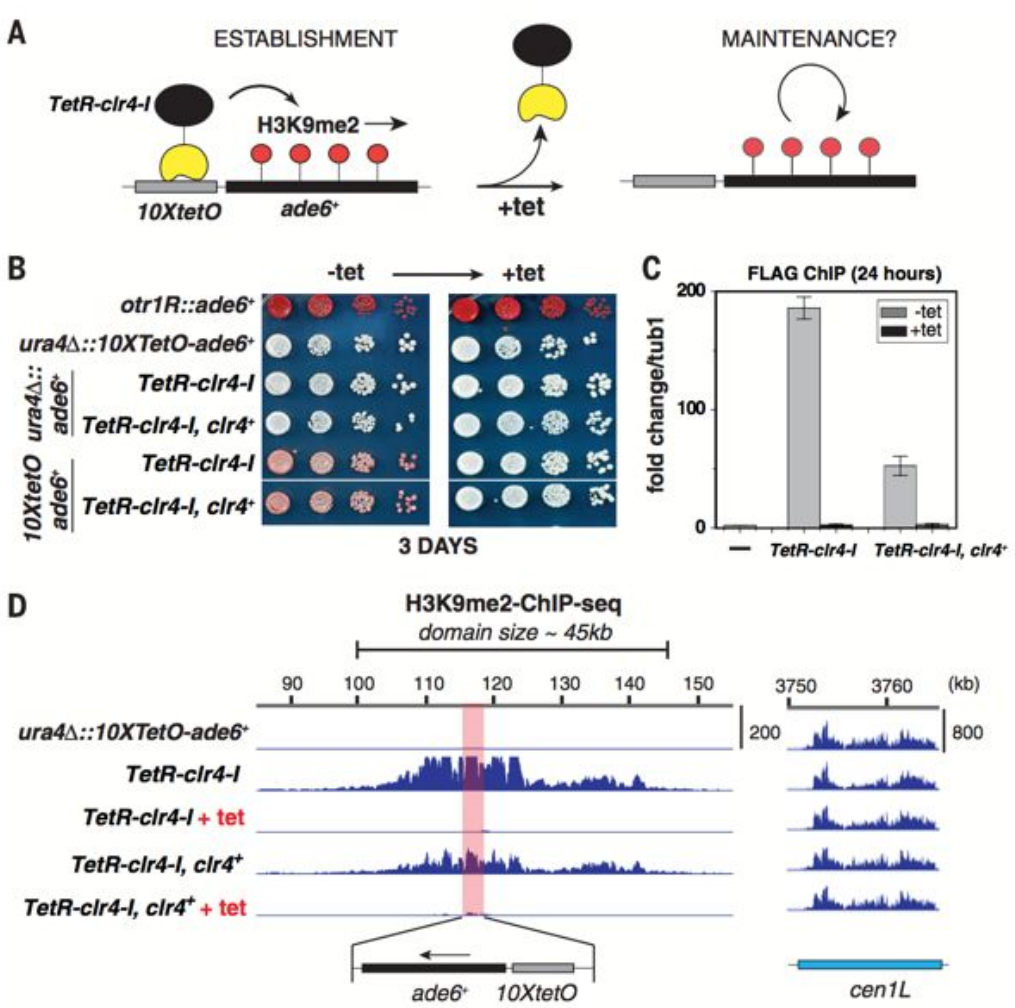
Experimental Setup

- The experiments were performed using plasmids in order to eliminate the effects of silencers (sequences that promote heterochromatin formation in adjacent loci; required for inheritance of a repressed state)
- Generated cells in which TetR-Clr4-I replaced the wild- type Clr4 (TetR-clr4-I) or ,if wild type Clr4 was needed, TetR-Clr4-I was inserted at the trp1+ locus

Methods

- PCR-based gene-targeting or random spore analysis: to produce deletions of the various RNAi and chromatin components
- ChIP: Confirmed the methylation of H3K9
- FLAG ChIP: tag initiator complex to complex and did ChIP PCR to find association
- GFP fluorescence measured via FACScalibur instrument

Fig. 1. Ectopic heterochromatin is lost after sequence-specific establishment upon tetracycline addition.



References

(1)Ragunathan et al. Epigenetic inheritance uncoupled from sequence-specific recruitment. *Science* 348, 1258699 (2015).

(2) Wang, Moazed. DNA sequence-dependent epigenetic inheritance of gene silencing and histone H3K9 methylation. *Science* (2017).

(3) Hansen et al. Global effects on gene expression in fission yeast by silencing and RNA interference machineries. *Molecular and Cellular Biology.* 590-601 (2005).

(4)Hathaway, Nathaniel A. et al. “Dynamics and Memory of Heterochromatin in Living Cells.” *Cell* 149.7 (2012): 1447–1460. *PMC*. Web. 2 May 2017.