Outline

- Background
  - What is known?
  - What is unknown?
- Paper
  - Central question/hypothesis
  - Techniques and experimental tools
  - Figures #1-3
    - Question, experimental results, conclusion/interpretation
- Conclusion
- Next Steps and Future directions

Outline

- Background
  - What is known?
  - What is unknown?
- Paper
  - Central question/hypothesis
  - Techniques and experimental tools
  - Figures #1-3
    - Question, experimental results, conclusion/interpretation
- Conclusion
- Next Steps and Future directions

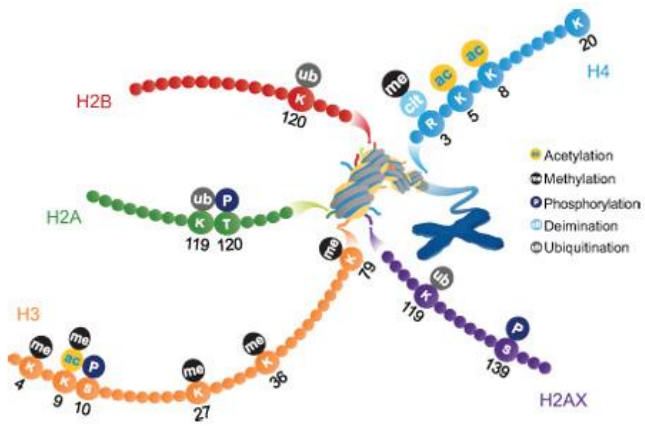
Background: What is known?

Broad repertoire of chromatin regulators involved in nucleosome remodeling, DNA methylation, histone modifications, etc.

The language of covalent histone modifications

Brian D. Strahl & C. David Allis

Department of Biochemistry and Molecular Genetics, University of Virginia Health Science Center, Charlottesville, Virginia 22908, USA



Conversion of 5-Methylcytosine to 5-Hydroxymethylcytosine in Mammalian DNA by MLL Partner TET1

Mamta Tahiliani,[1] Kian Peng Koh,[1] Yinghua Shen,[2] William A. Pastor,[1] Hozefa Bandukwala,[1] Yevgeny Brudno,[2] Suneet Agarwal,[3] Lakshminarayan M. Iyer,[4] David R. Liu,[2]* L. Aravind,[4]* Anjana Rao[1]*

Background: What is known?

These play a large role in establishing and maintaining gene expression states

Dynamics of global histone acetylation and deacetylation in vivo: rapid restoration of normal histone acetylation status upon removal of activators and repressors

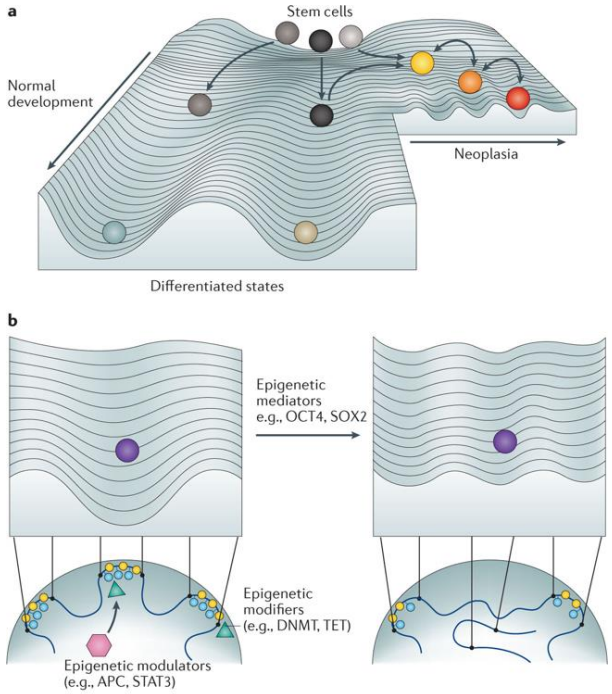
Yael Katan-Khaykovich and Kevin Struhl[1]

DNA methylation patterns and epigenetic memory

Adrian Bird[1]

Background: What is known?

With further implications in cell fate decision-making



Feinberg, Andrew P., Michael A. Koldobskiy, and Anita Göndör. "Epigenetic modulators, modifiers and mediators in cancer aetiology and progression." *Nature Reviews Genetics* (2016).

Outline

- Background
  - What is known?
  - What is unknown?
- Paper
  - Central question/hypothesis
  - Techniques and experimental tools
  - Figures #1-3
    - Question, experimental results, conclusion/interpretation
- Conclusion
- Next Steps and Future directions

Background: What is Unknown?

- Analysis of chromatin regulators control of gene expression **quantitatively** over time in single cells
    - How strong and rapidly can chromatin regulators affect gene expression?

Background: What is Unknown?

- Analysis of chromatin regulators control of gene expression **quantitatively** over time in single cells
    - How strong and rapidly can chromatin regulators affect gene expression?
- Effect of chromatin regulators on **epigenetic memory**
    - How long can these effects persist?

Background: What is Unknown?

- Analysis of chromatin regulators control of gene expression **quantitatively** over time in single cells
    - How strong and rapidly can chromatin regulators affect gene expression?
- Effect of chromatin regulators on **epigenetic memory**
    - How long can these effects persist?
- **Cell to cell variability** of gene expression through in the context of chromatin regulators
    - How uniformly can chromatin regulators alter gene expression?

Background: What is Unknown?

- Analysis of chromatin regulators control of gene expression quantitatively over time in single cells
    - How strong and rapidly can chromatin regulators affect gene expression?
- Effect of chromatin regulators on epigenetic memory
    - How long can these effects persist?
- Cell to cell variability of gene expression through in the context of chromatin regulators
    - How uniformly can chromatin regulators alter gene expression?

answering these questions is important because:

"We anticipate that integrative models of chromatin dynamics in living cells will be required to understand *how gene regulation is achieved through modulation of chromatin structure*."

Outline

- Background
  - What is known?
  - What is unknown?
- Paper
  - Central question/hypothesis
  - Techniques and experimental tools
  - Figures #1-4
    - Question, experimental results, conclusion/interpretation
- Conclusion
- Next Steps and Future directions

Outline

- Background
  - What is known?
  - What is unknown?
- Paper

EPIGENETICS

Dynamics of epigenetic regulation at the single-cell level

Lacramioara Bintu,[1*] John Yong,[1*] Yaron E. Antebi,[1] Kayla McCue,[1] Yasuhiro Kazuki,[2] Narumi Uno,[2] Mitsuo Oshimura,[2] Michael B. Elowitz[1,3†]

Outline

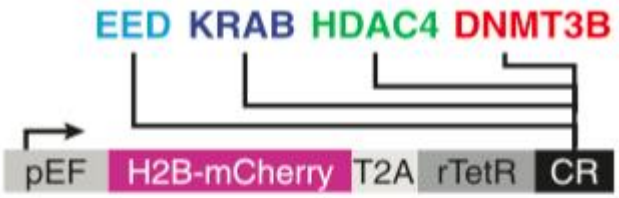
- Background
  - What is known?
  - What is unknown?
- Paper
  - Central question/hypothesis
  - Techniques and experimental tools
  - Figures #1-4
    - Question, experimental results, conclusion/interpretation
- Conclusion
- Next Steps and Future directions

How strongly, rapidly, and uniformly can chromatin regulators alter gene expression, and how long can their effects persist?

Outline

- Background
  - What is known?
  - What is unknown?
- Paper
  - Central question/hypothesis
  - Techniques and experimental tools
  - Figures #1-4
    - Question, experimental results, conclusion/interpretation
- Conclusion
- Next Steps and Future directions

Techniques and Experimental Tools



Constructs used

Chromatin Regulators (CRs)

1. **EED (embryonic ectoderm development)**
   Methylates histone 3 at lysine 27 (H3K27Me3)

Chromatin Regulators (CRs)

1. **EED (embryonic ectoderm development)**
   Methylates histone 3 at lysine 27 (H3K27Me3)

2. **KRAB (kruppel associated box)**
   Associates with CRs that read/write H3K9Me3

Chromatin Regulators (CRs)

1. **EED (embryonic ectoderm development)**
   Methylates histone 3 at lysine 27 (H3K27Me3)

2. **KRAB (kruppel associated box)**
   Associates with CRs that read/write H3K9Me3

3. **DNMT3B (DNA methyltransferase)**
   Causes de novo methylation of CpGs

Chromatin Regulators (CRs)

1. **EED (embryonic ectoderm development)**
   Methylates histone 3 at lysine 27 (H3K27Me3)

2. **KRAB (kruppel associated box)**
   Associates with CRs that read/write H3K9Me3

3. **DNMT3B (DNA methyltransferase)**
   Causes de novo methylation of CpGs

4. **HDAC4 (Histone deacetylase 4)**
   Removes acetyl groups from histones H3 and H4

Chromatin Regulators (CRs)

1. **EED (embryonic ectoderm development)**
   Methylates histone 3 at lysine 27 (H3K27Me3)

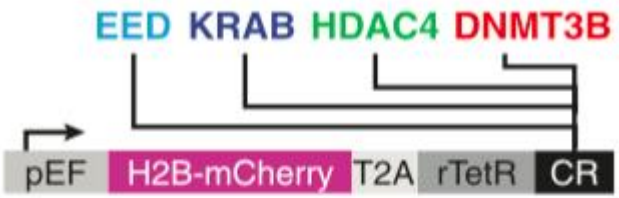
2. **KRAB (kruppel associated box)**
   Associates with CRs that read/write H3K9Me3

3. **DNMT3B (DNA methyltransferase)**
   Causes de novo methylation of CpGs

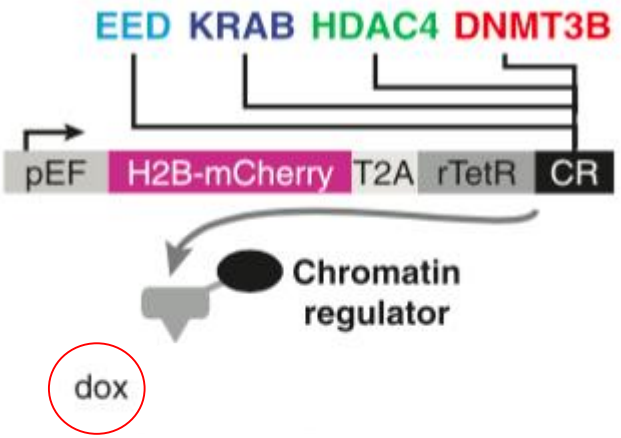
4. **HDAC4 (Histone deacetylase 4)**
   Removes acetyl groups from histones H3 and H4

Purpose: to compare capabilities of distinct regulators - these are 4 repressive CRs that span a broad range of chromatin modifications

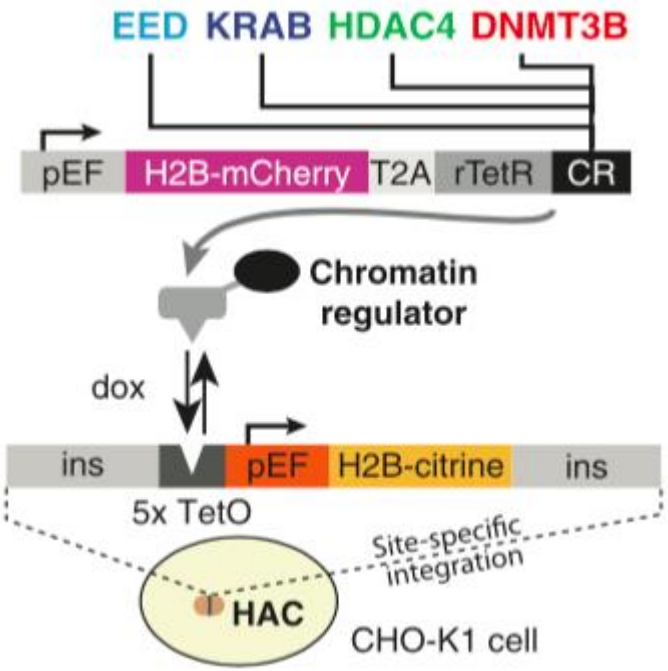
Techniques and Experimental Tools



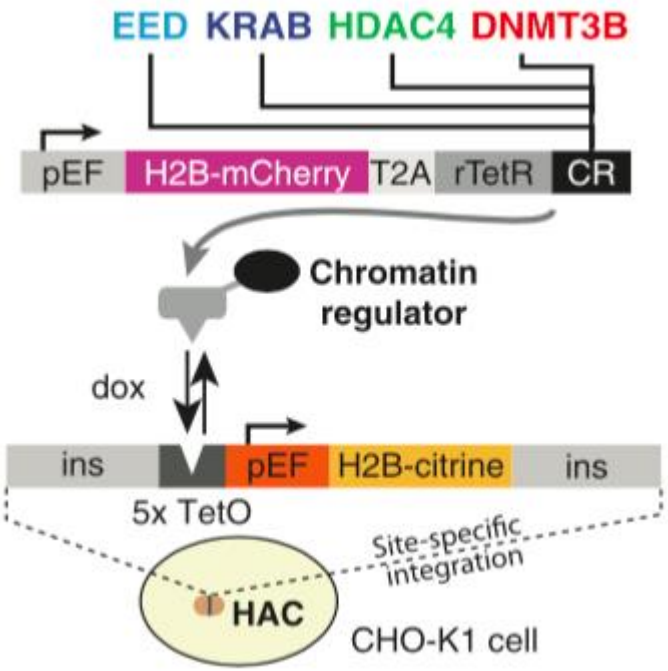
Techniques and Experimental Tools



Techniques and Experimental Tools

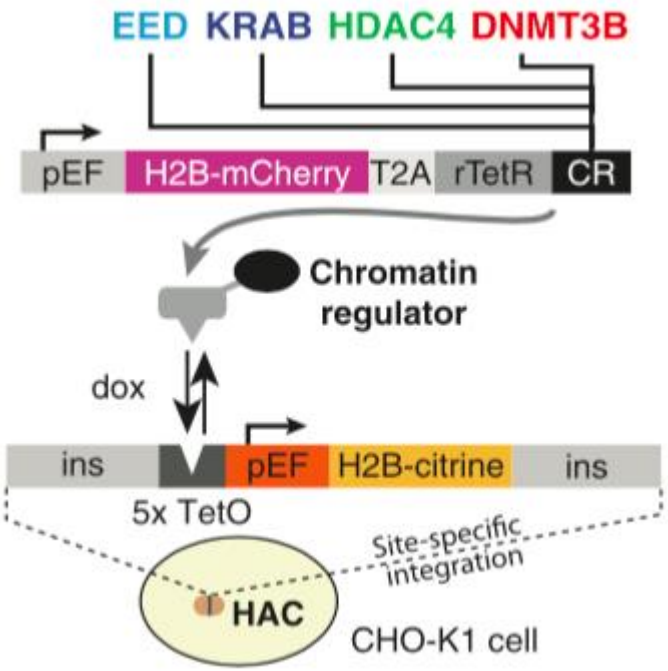


Techniques and Experimental Tools



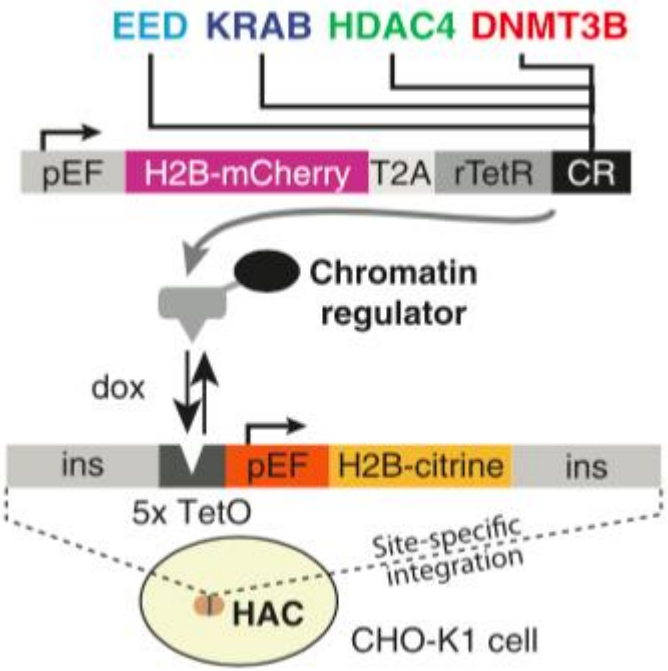
- Model system: CHO-K1 cells (mammalian)

Techniques and Experimental Tools



- Model system: CHO-K1 cells (mammalian)
- Summary of important features:
  - Chromatin regulators tagged with reverse Tet repressor which binds DNA only in the presence of dox
  - Construct allows temporal (timing and duration) control of chromatin regulator recruitment

Techniques and Experimental Tools



- Model system: CHO-K1 cells (mammalian)
- Summary of important features:
  - Chromatin regulators tagged with reverse Tet repressor which binds DNA only in the presence of dox
  - Construct allows temporal (timing and duration) control of chromatin regulator recruitment
- Time lapse microscopy
  - Single cell analysis

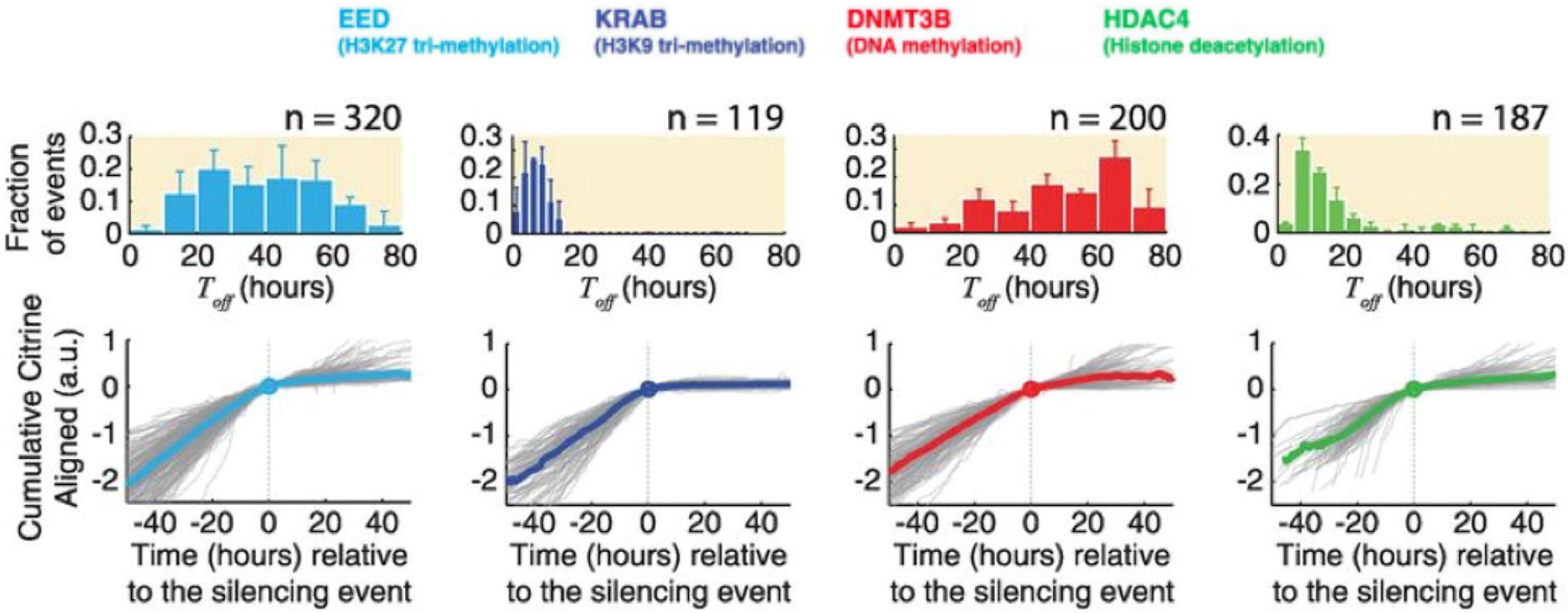
Outline

- Background
  - What is known?
  - What is unknown?
- Paper
  - Central question/hypothesis
  - Techniques and experimental tools
  - Figures #1-3
    - Question, experimental results, conclusion/interpretation
- Conclusion
- Next Steps and Future directions

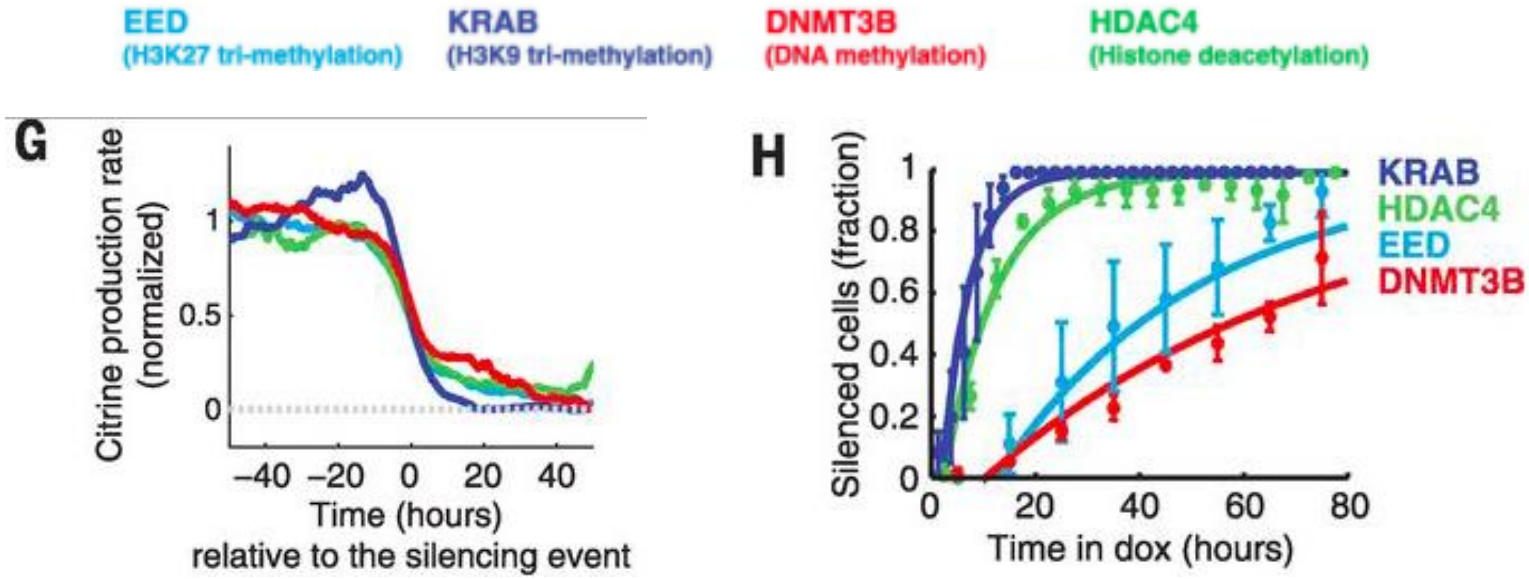
#1 Time-Lapse Analysis of CR silencing

Time lapse microscopy of EED silencing

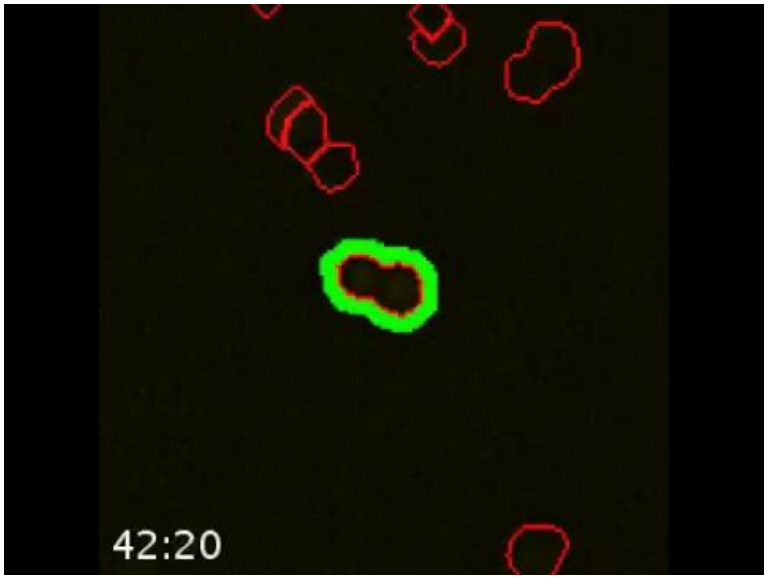
#1 Time-Lapse Analysis of CR silencing



#1 Time-Lapse Analysis of CR silencing

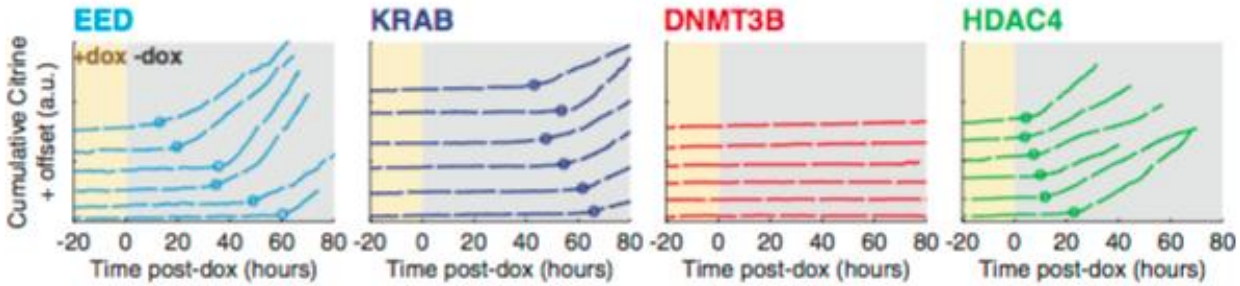


#2 Effect on epigenetic memory by terminating CR recruitment

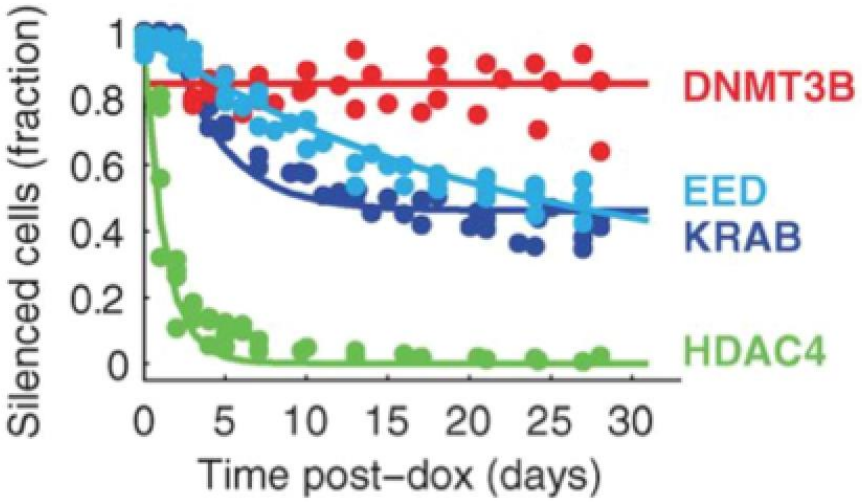


Time lapse microscopy of reactivation (EED)

#2 Effect on epigenetic memory by terminating CR recruitment



#2 Effect on epigenetic memory by terminating CR recruitment

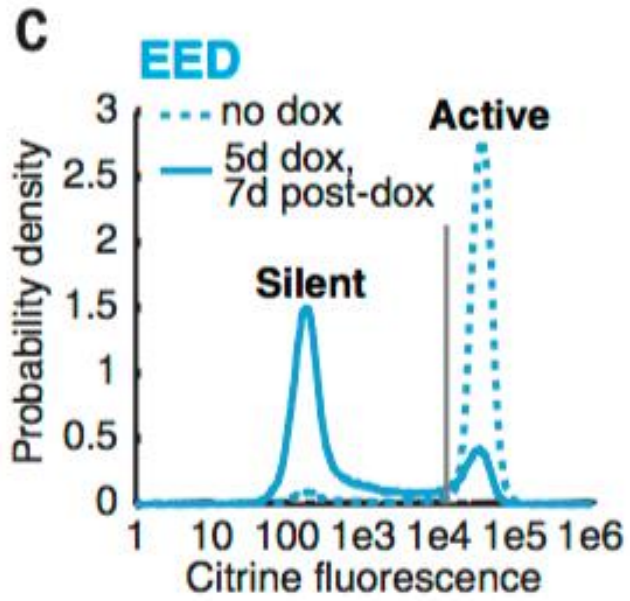


DNMT3B: **Long** term memory. No Reactivation.

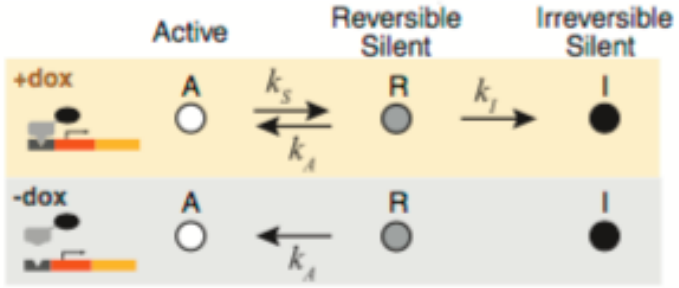
EED & KRAB: **Hybrid** memory. A fraction reactivated (2-3 weeks).

HDAC4: **Short** term memory. Reactivated (5 days).

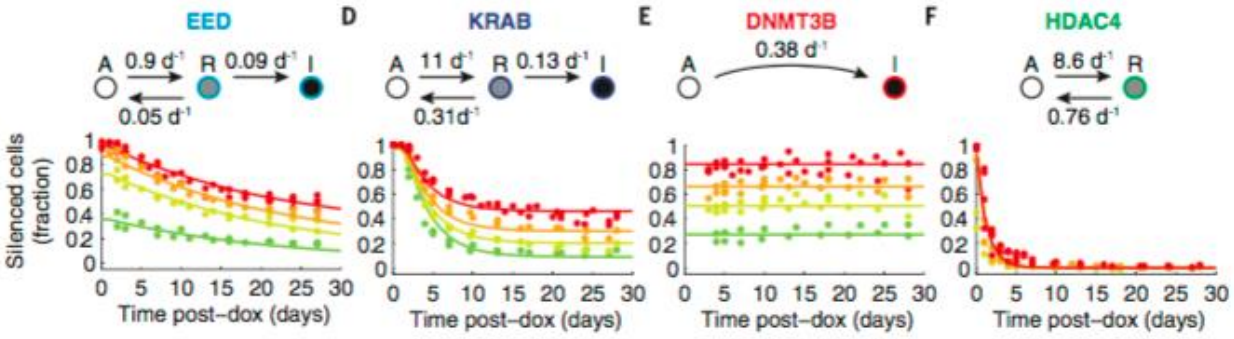
#2 Effect on epigenetic memory by terminating CR recruitment



#3 Hybrid State: 3 State Model



Hypothesis: Longer durations of recruitment will result in a larger fraction of irreversibly (I) silenced cells (cells silent after 30 days).



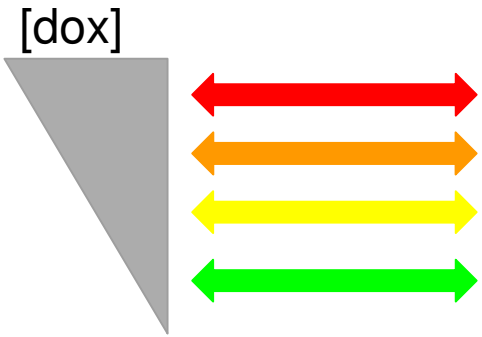
Hypothesis confirmed.

Days of recruitment:

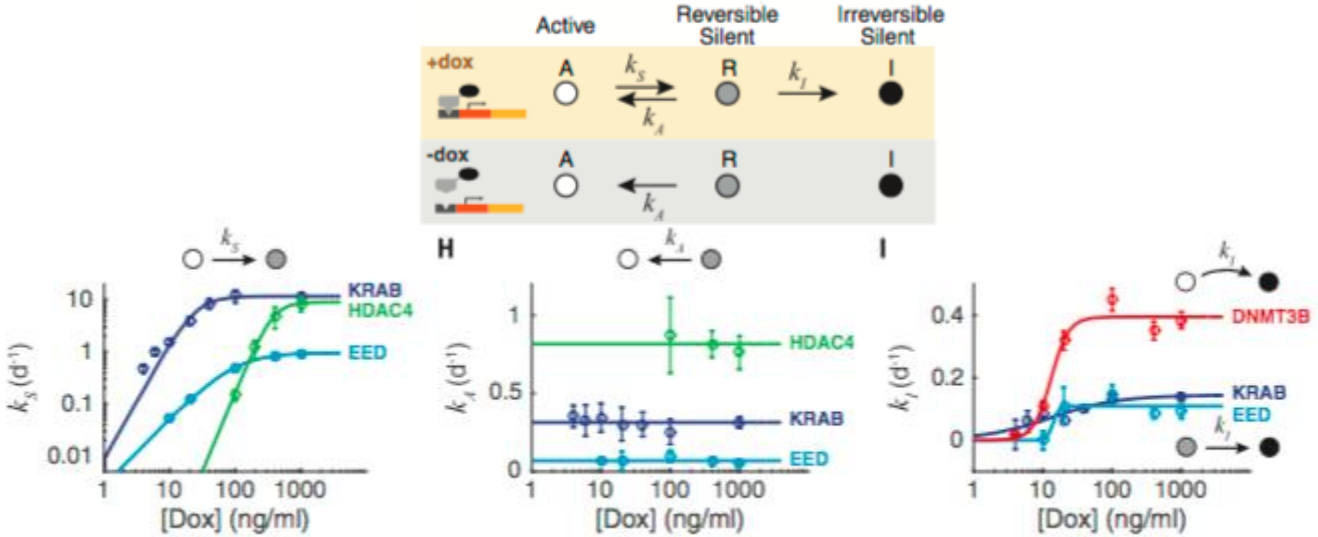
5 days 3 days 2 days, 1 day

#3 Recruitment strength on silencing dynamics and epigenetic memory

Analyzed the effects varying dox concentrations on fraction of cell silenced and rates.

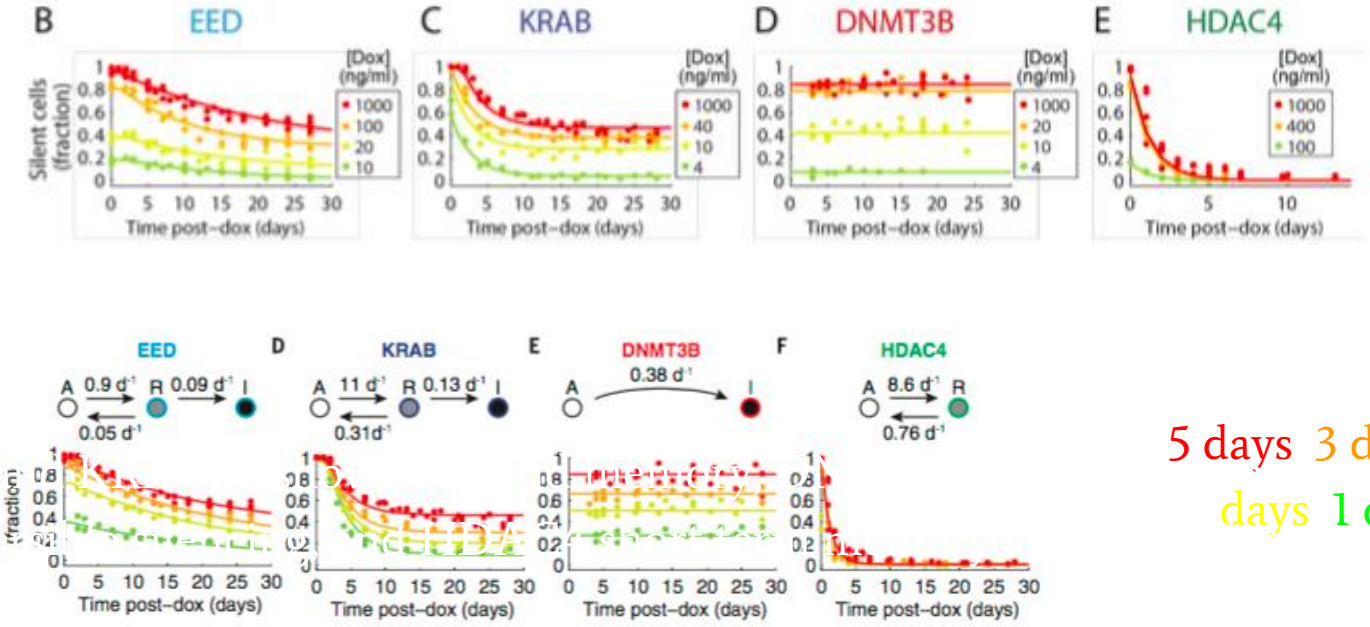


#3 Recruitment strength on silencing dynamics and epigenetic memory



Recruitment strength ([dox]) modulated silencing rates but not the reactivation rates. Reactivation rates only depended on type of CR.

#3 Recruitment strength on silencing dynamics and epigenetic memory



5 days 3 days 2 days 1 day

Outline

- Background
  - What is known?
  - What is unknown?
- Paper
  - Central question/hypothesis
  - Techniques and experimental tools
  - Figures #1-3
    - Question, experimental results, conclusion/interpretation
- Conclusion
- Next Steps and Future directions

Conclusions

- Chromatin regulators can be mathematically model through a three-state model with active, reversible, and irreversible silent
- These different kinetic signatures lead to distinct control modes both in **strength** and **duration** of silencing
- Silencing strength is not reflected by the graded levels of gene expression of the cell; rather silencing alters gene expression in an **all-or-none stochastic** fashion which can be characterized by the fraction of cells silenced

Outline

- Background
  - What is known?
  - What is unknown?
- Paper
  - Central question/hypothesis
  - Techniques and experimental tools
  - Figures #1-3
    - Question, experimental results, conclusion/interpretation
- Conclusion
- Next Steps and Future directions

Next Steps + Future Directions

Important to develop underlying molecular states and biochemical processes for each CR

Critical to account for:

promoter architecture

chromatin state of the locus

Specific set of chromatin regulatory components expressed in different cell types

Findings enable building of synthetic circuits that take advantage of each CRs effect on temporal control and memory capabilities