

Dynamics and Memory of Heterochromatin in Living Cells

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Erzsébet Ravasz Regan

What is epigenetics?

Original definition:

- * mechanisms by which different cellular phenotypes are clonally heritable, without altering the genetic code
- * self-sustaining in the absence of original stimulus

mammalian cell types

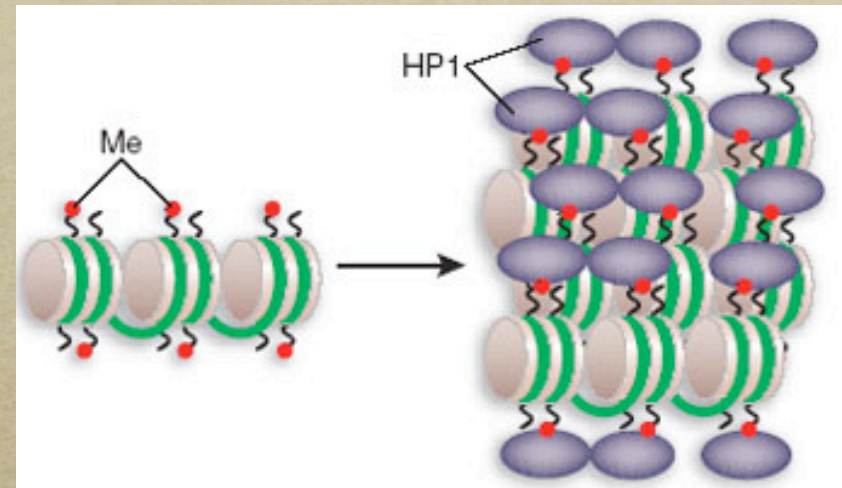
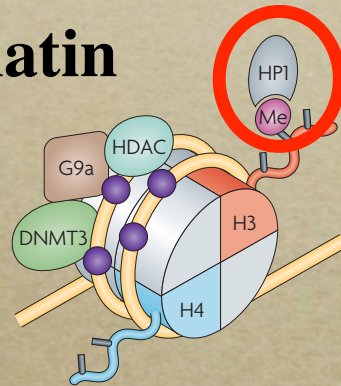
What types of mechanism?

- * DNA methylation
- * nucleosomal histones ???
(* *noncoding RNAs*)

**Field
studying these +
noncoding RNAs =
epigenetics**

Heterochromatin formation - H3K9m3 and binding of HP1

H3K9m3-bound HP1
condenses chromatin



HP1 recruits histone
methyltransferases
(HMTs) => H3K9m3 on
neighboring nucleosomes

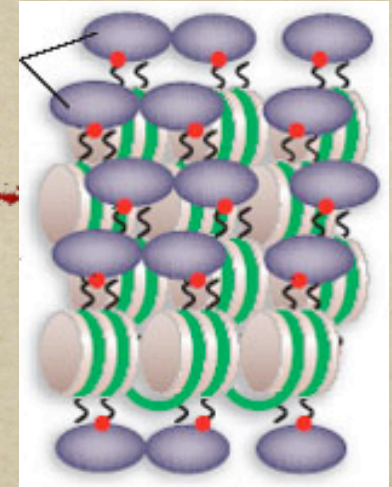
Suv39h1/2

SETDB1

Spreading of
H3K9m3

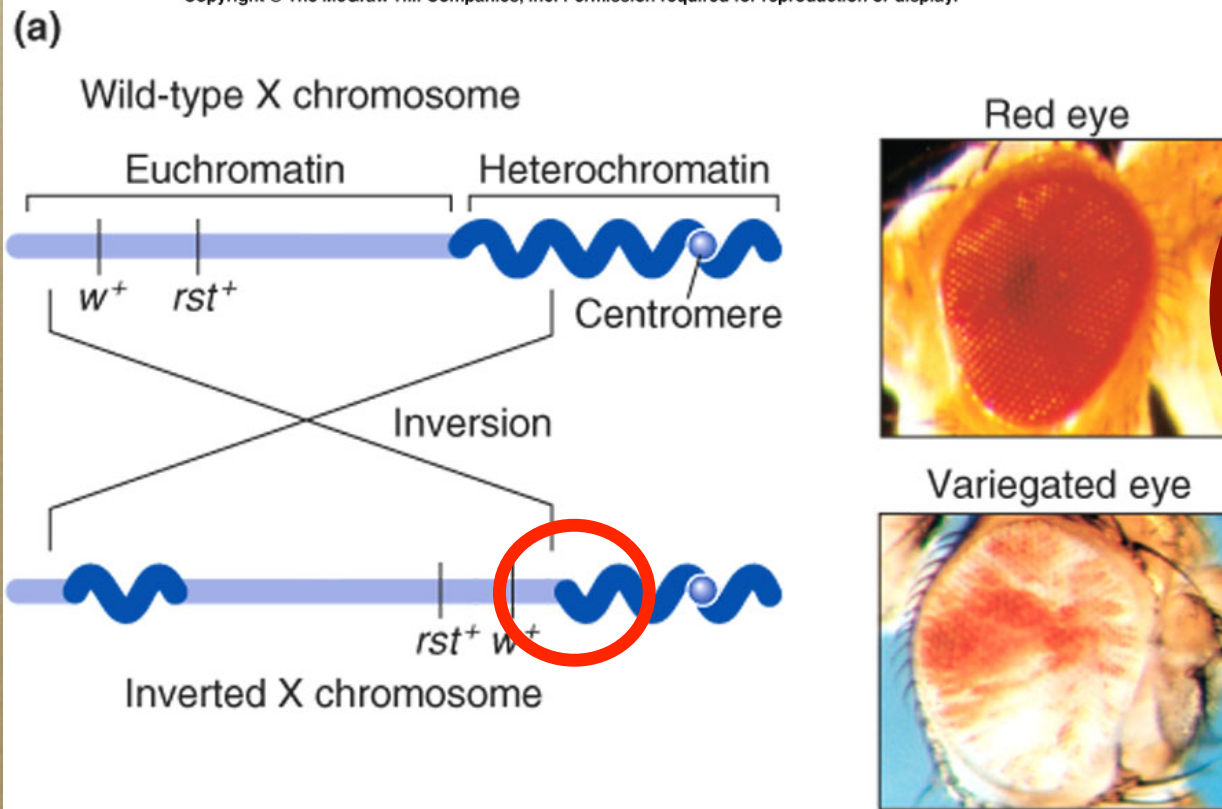
"Copy enzymes"

Is the histone code a heritable epigenetic mark?



Proof of principle: position effect variegation

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stochastic
spreading of
heterochromatin
over w^+

clonally
inherited

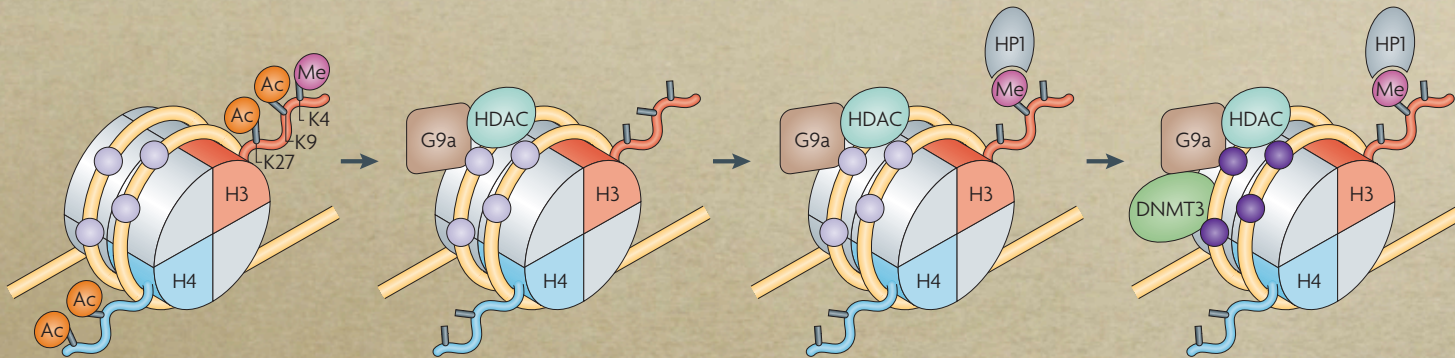
Definite proof of histone code
heritability on a promoter is lacking

Questions:

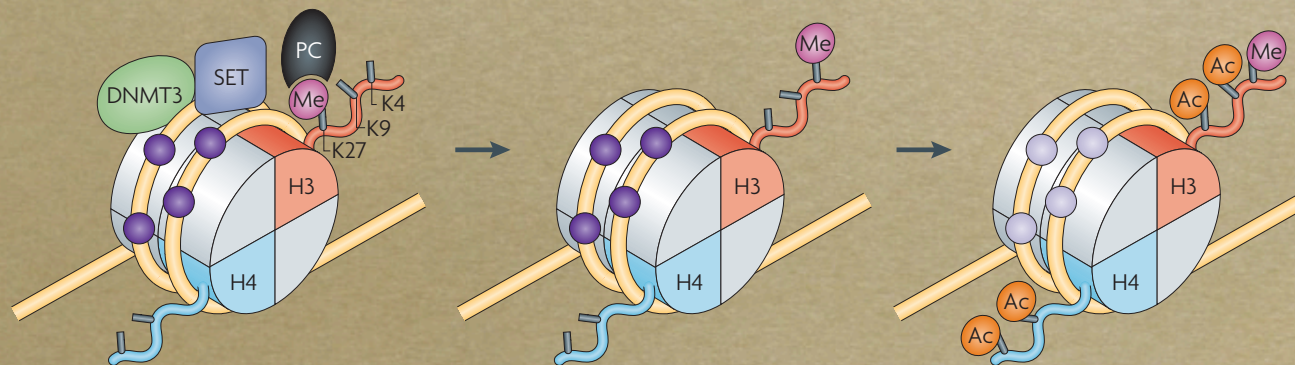
- *are histone modifications heritable?*
- *how do they interface with transcription?*
- *how do they propagate?*
- *how do they interface with DNA methylation?*

The story of Oct4 and reprogramming

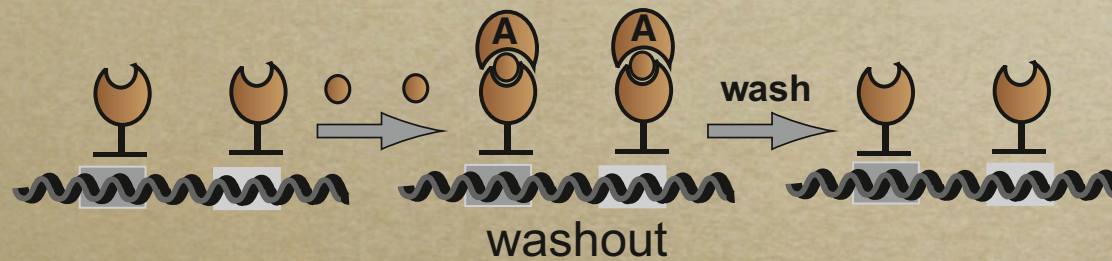
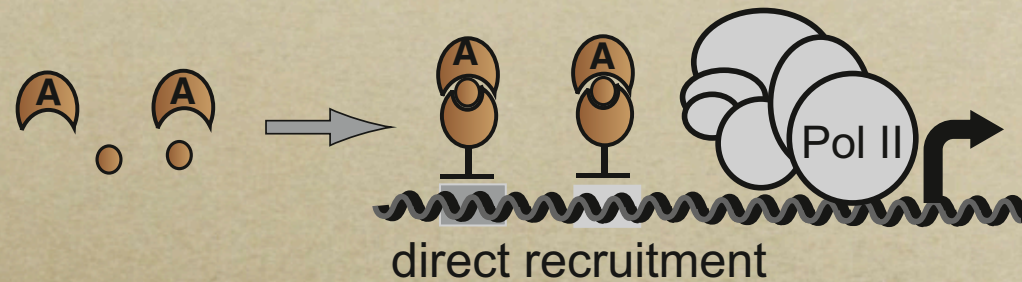
- * **Silencing pluripotency genes (e.g., Oct4)**
-< loss of active histone marks (H3K27^{Ac}, H3K9^{Ac})



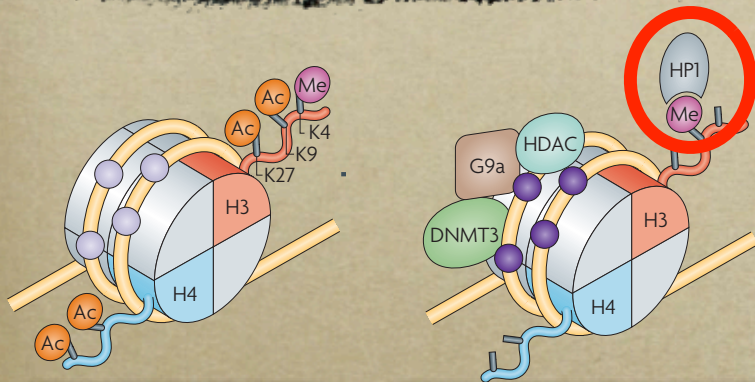
- * **Reprogramming somatic cells to iPS state**
-< loss of repressive histone marks (H3K27^{Me})



Approach: CIP (chemically induced proximity)



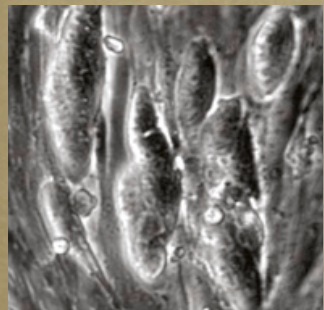
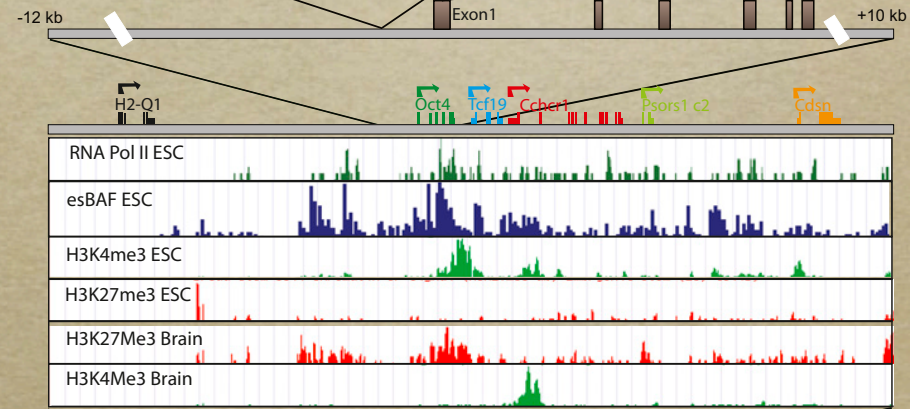
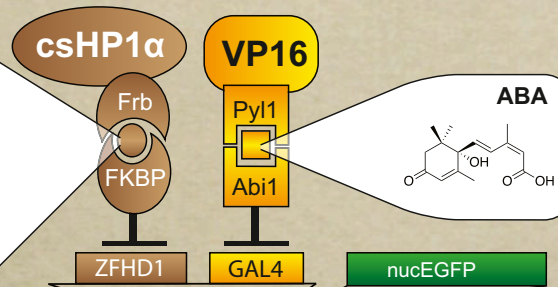
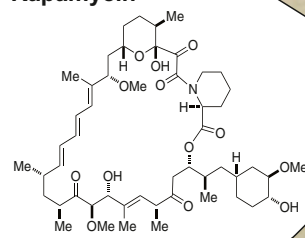
Tethering Hp1 in combination with a strong transcriptional activator



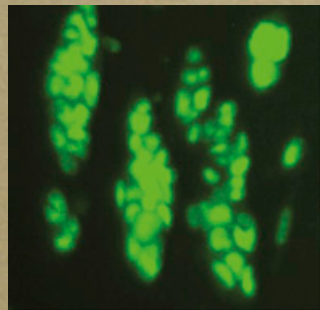
ES cells
Oct4 ON

Non-ES cells
Oct4 OFF

Rapamycin



Phase

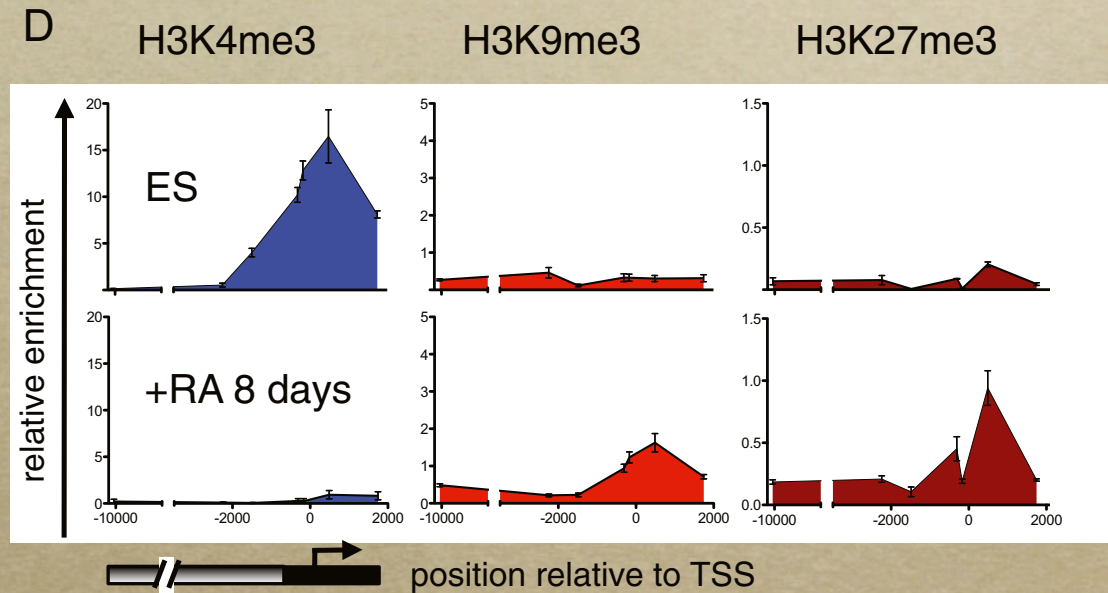
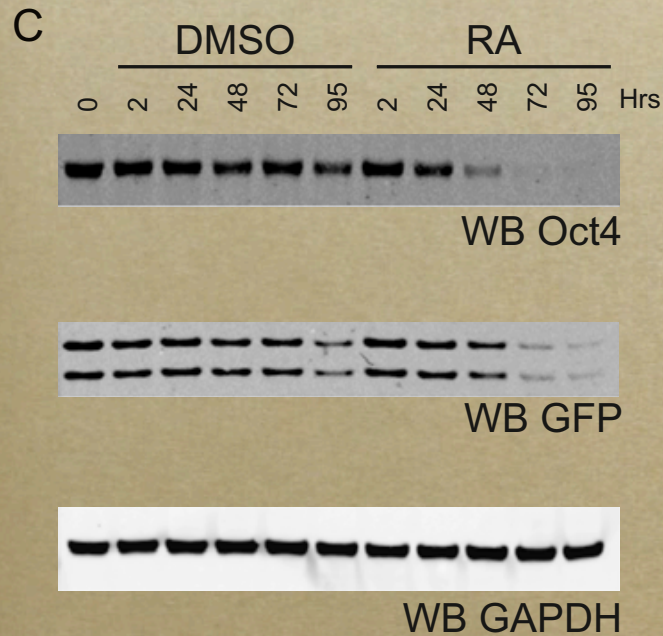


GFP

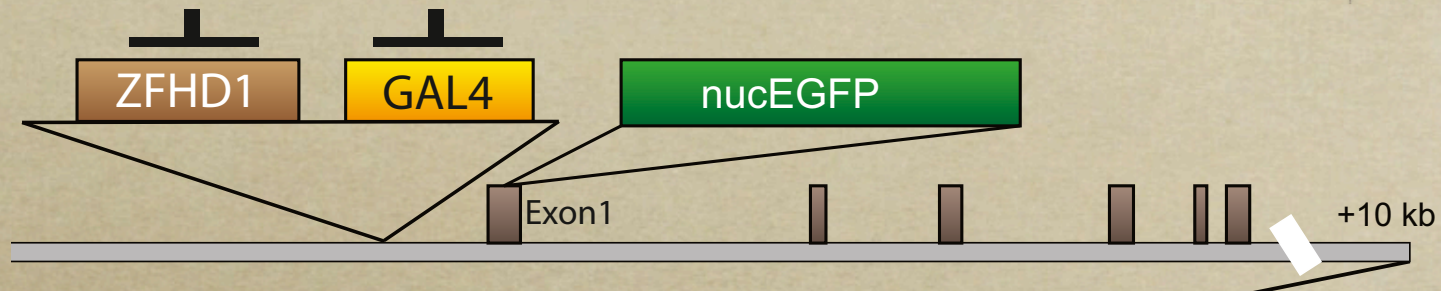


CiA:Oct4
ES cells w. EGFP

Differentiation of *Cia:Oct4* ES cells turns off the *Oct4* locus



Recruitment of HP1 to the Oct4 promoter turns OFF transcription (test)



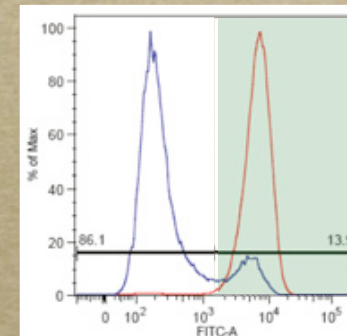
Gal4 *HP1*
**full-length
HP1**

Gal4 *csHP1 α*

CSD (chromatin shadow) domain only:

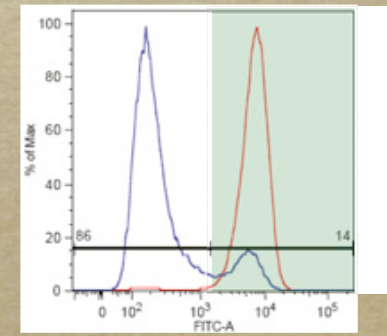
- cannot bind to H3K9m3 by itself
- can bind to H3K9-specific histone methylases

full-length HP1 α



■ GAL4-HP1 α
■ GAL4 only

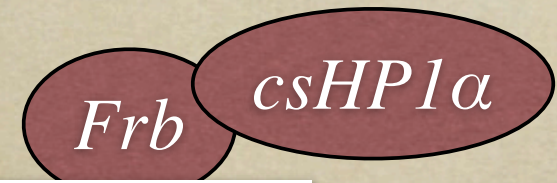
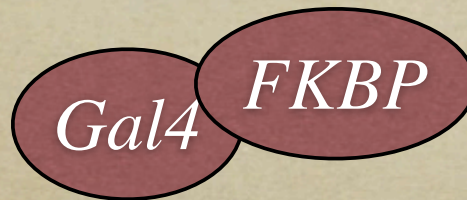
csHP1 α



■ GAL4-csHP1 α
■ GAL4 only

Inducible recruitment of HP1 to the Oct4 promoter

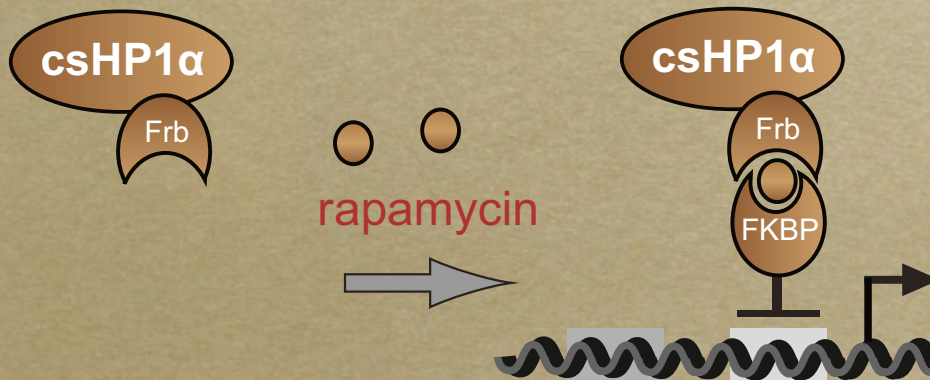
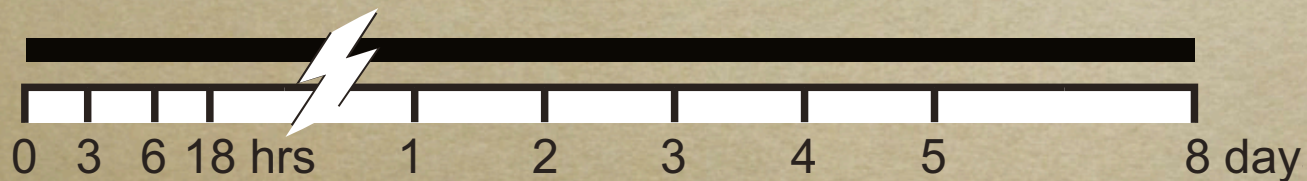
2 lentiviral transfections



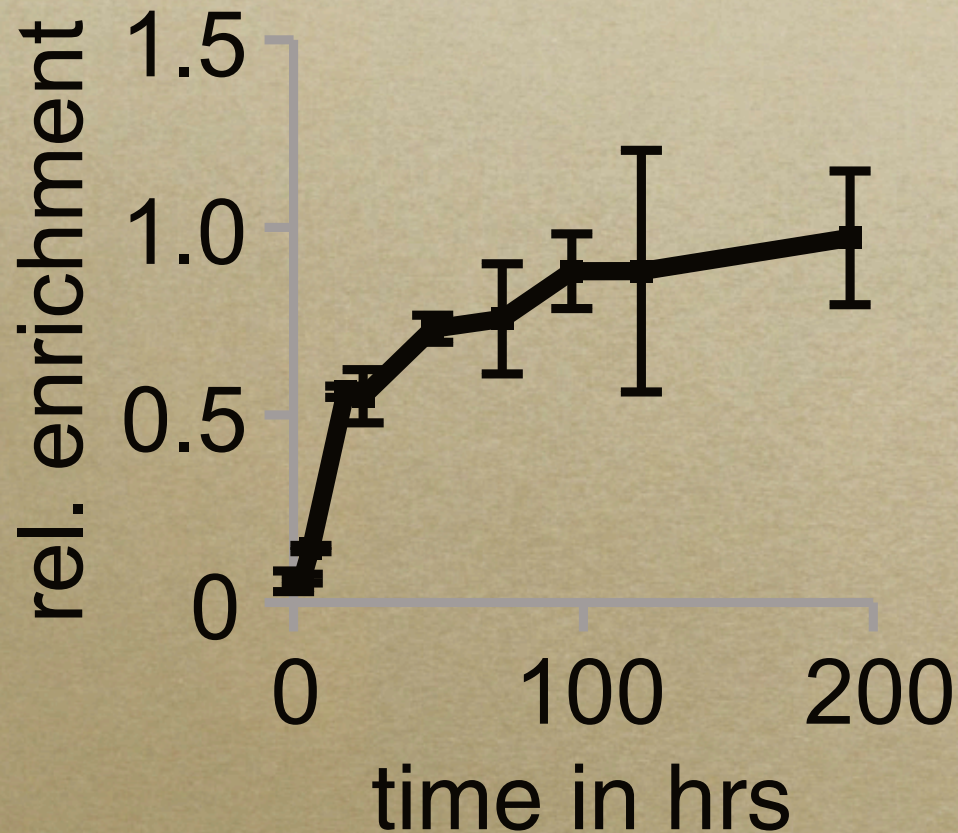
Tagged for ChIP

time line

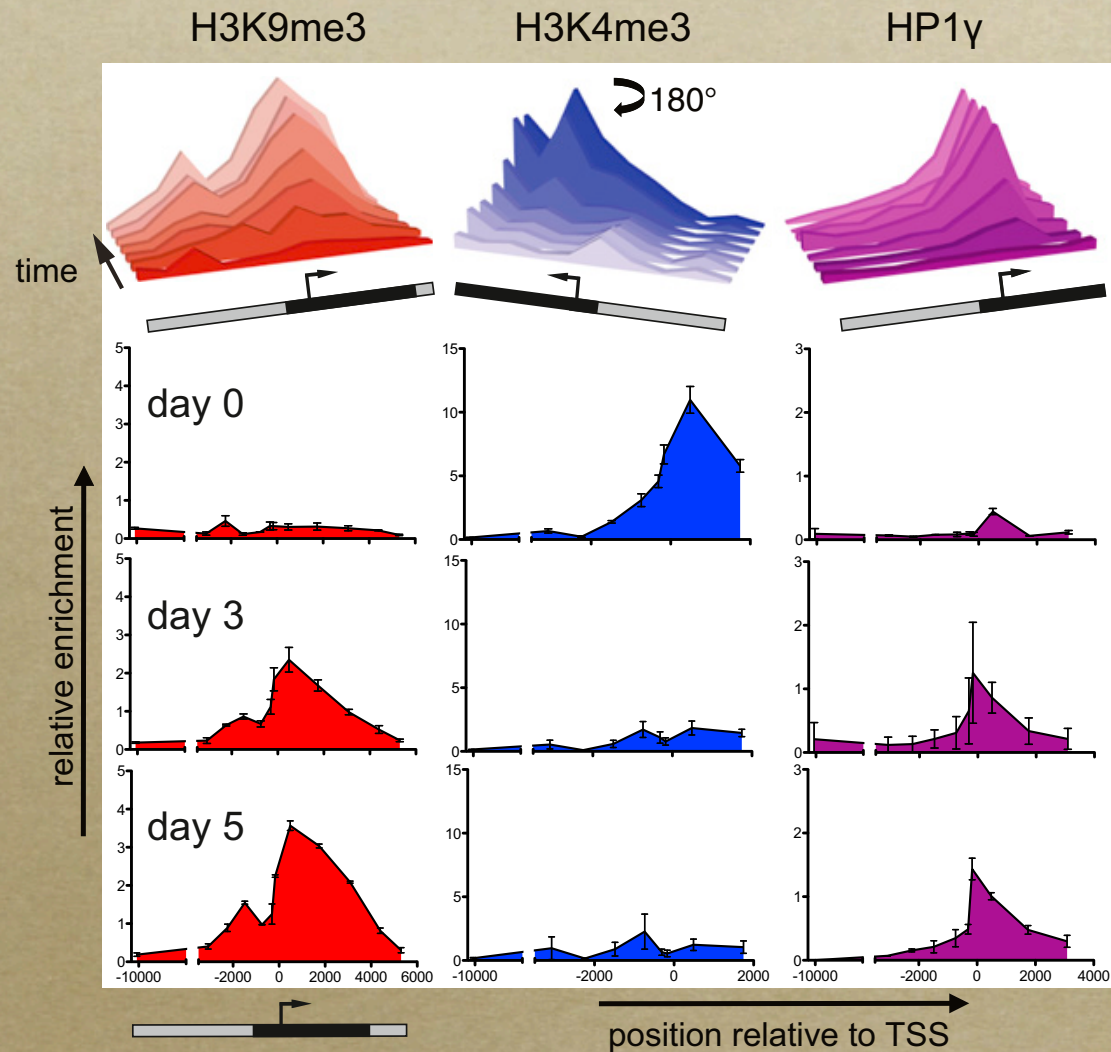
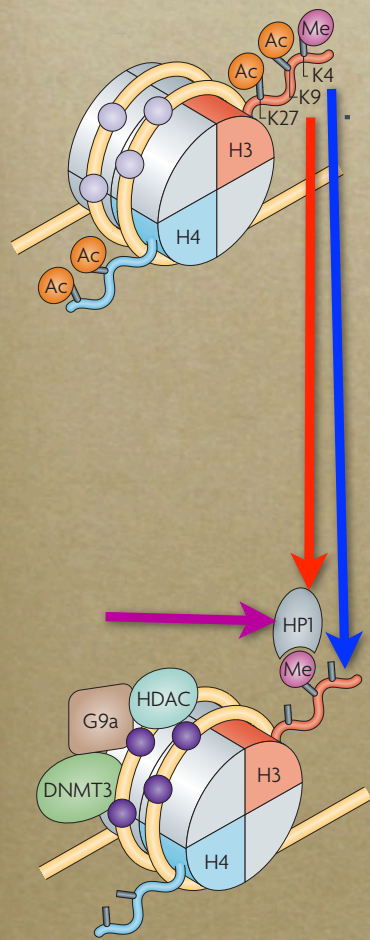
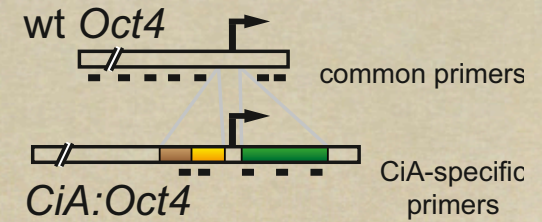
csHP1 (rap)



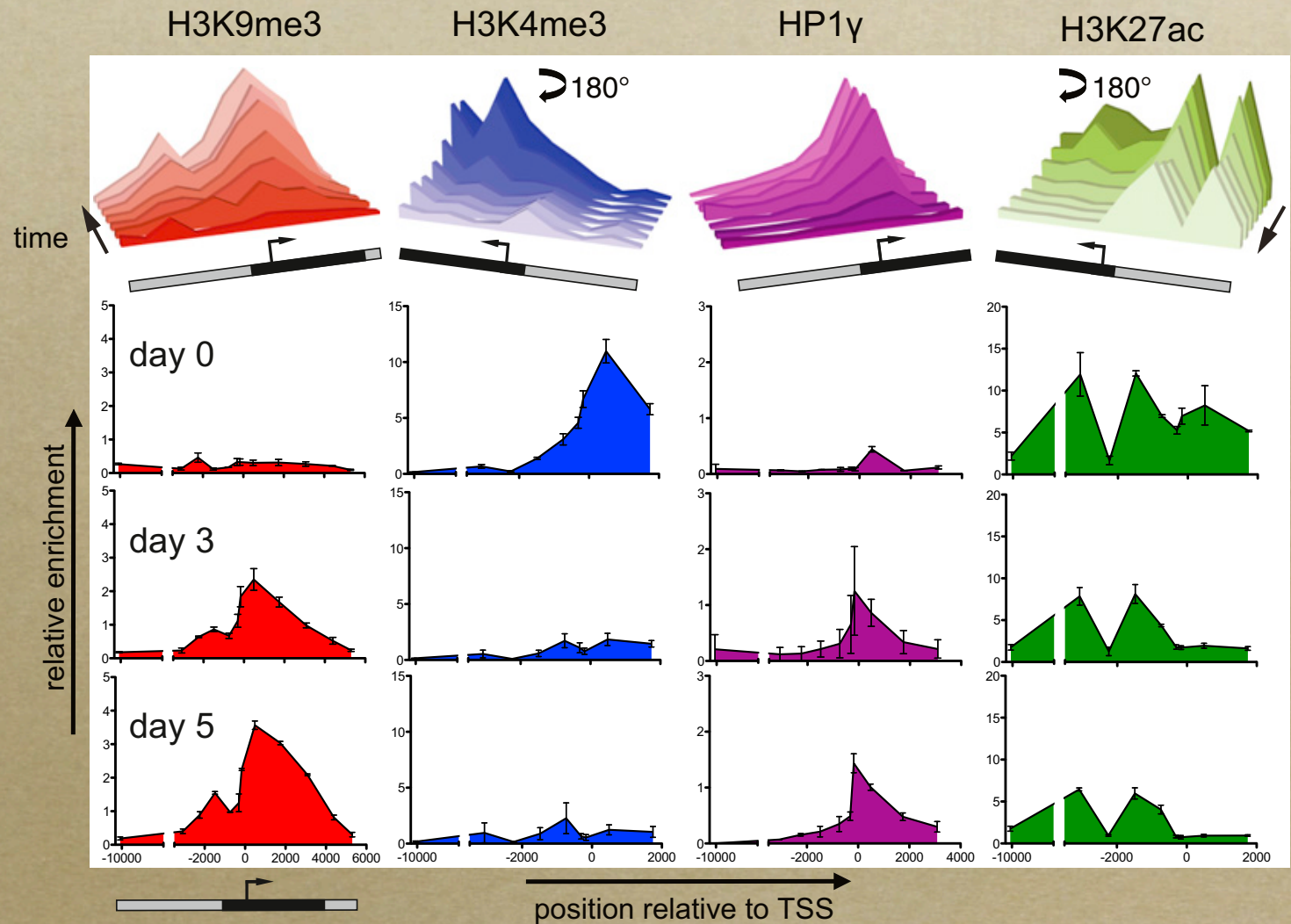
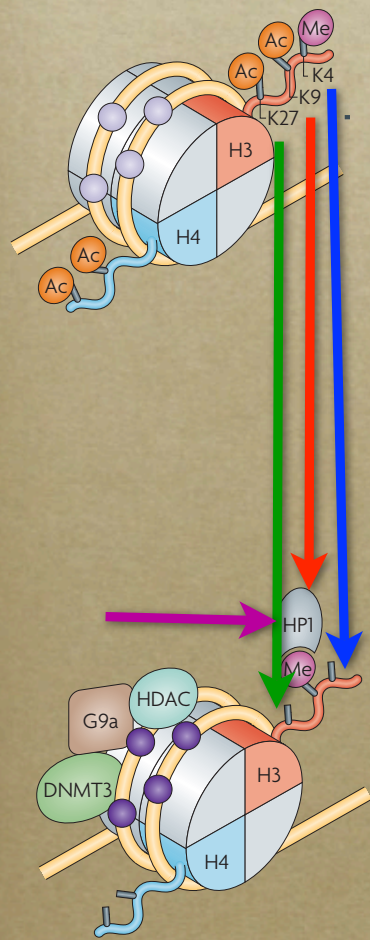
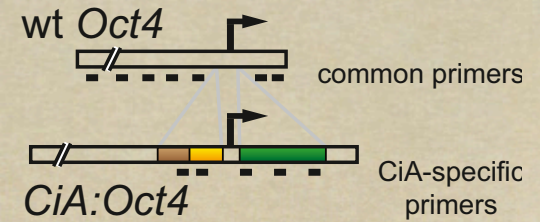
cstHPI α is recruited to the Oct4 locus by 6h and saturated by 24 hours



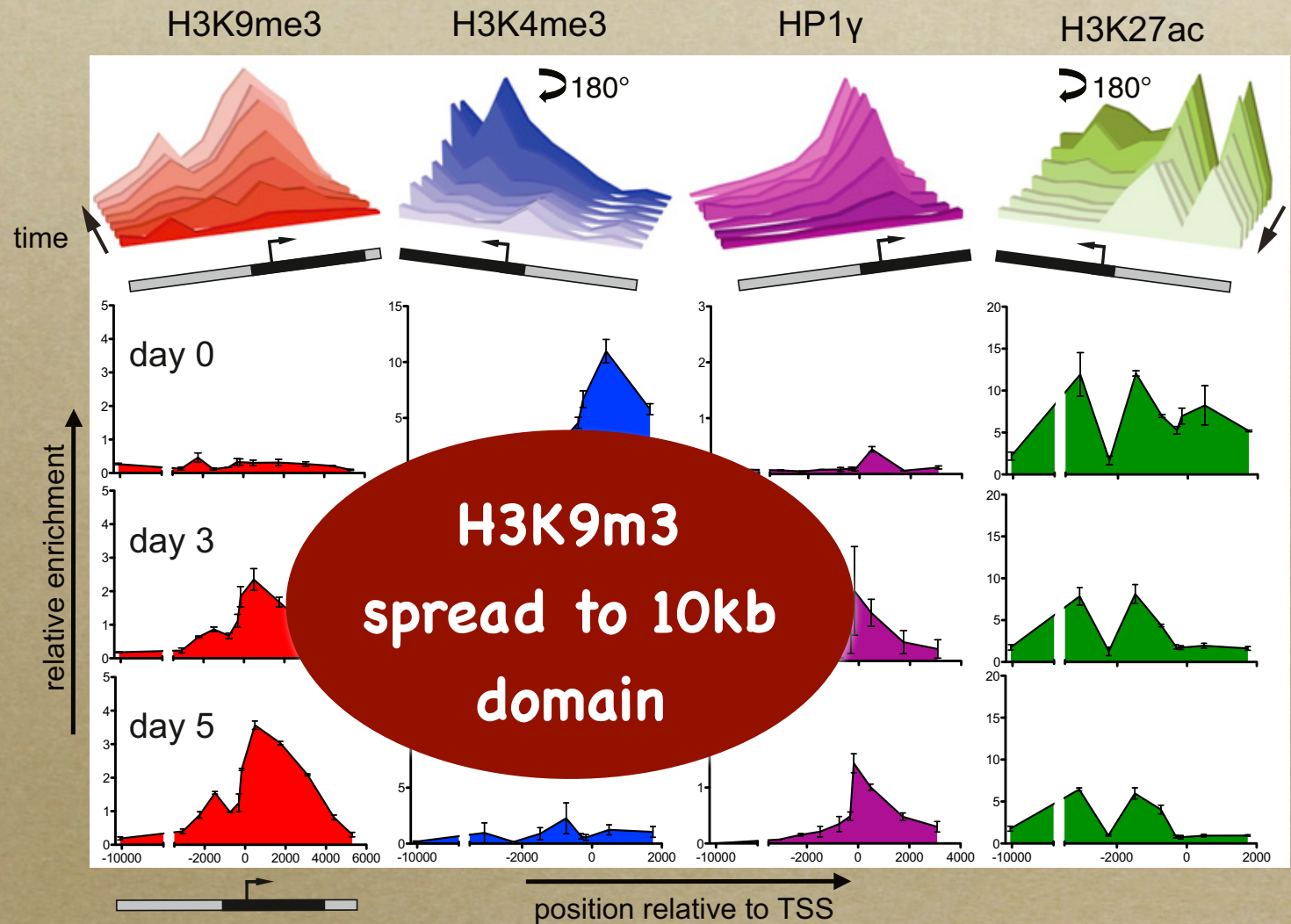
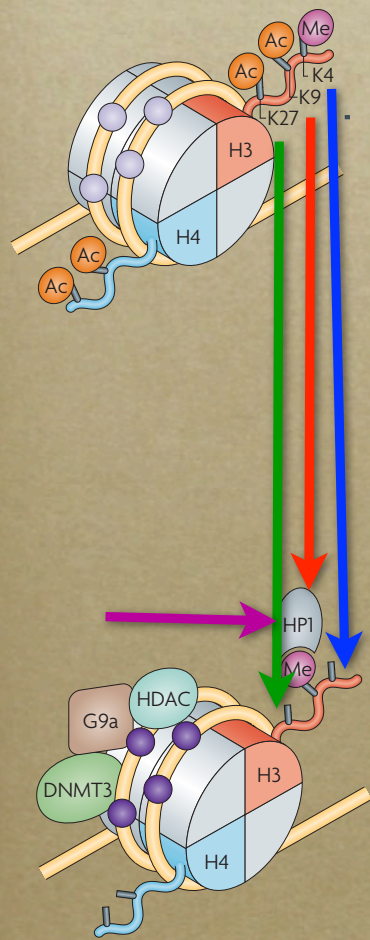
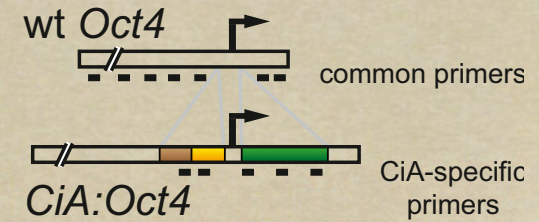
Establishment of a repressive histone code



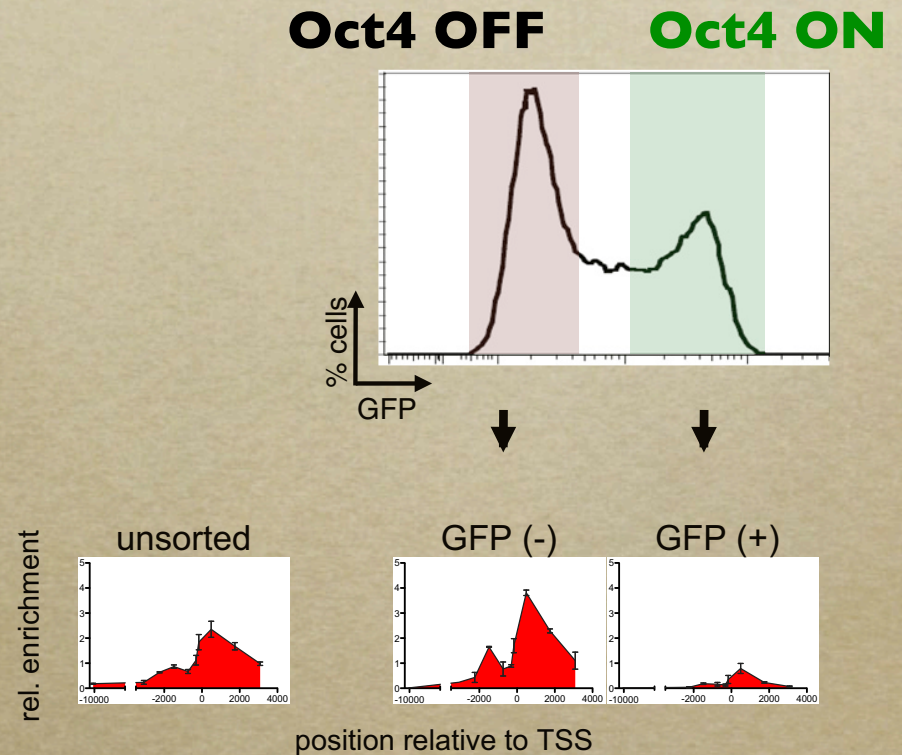
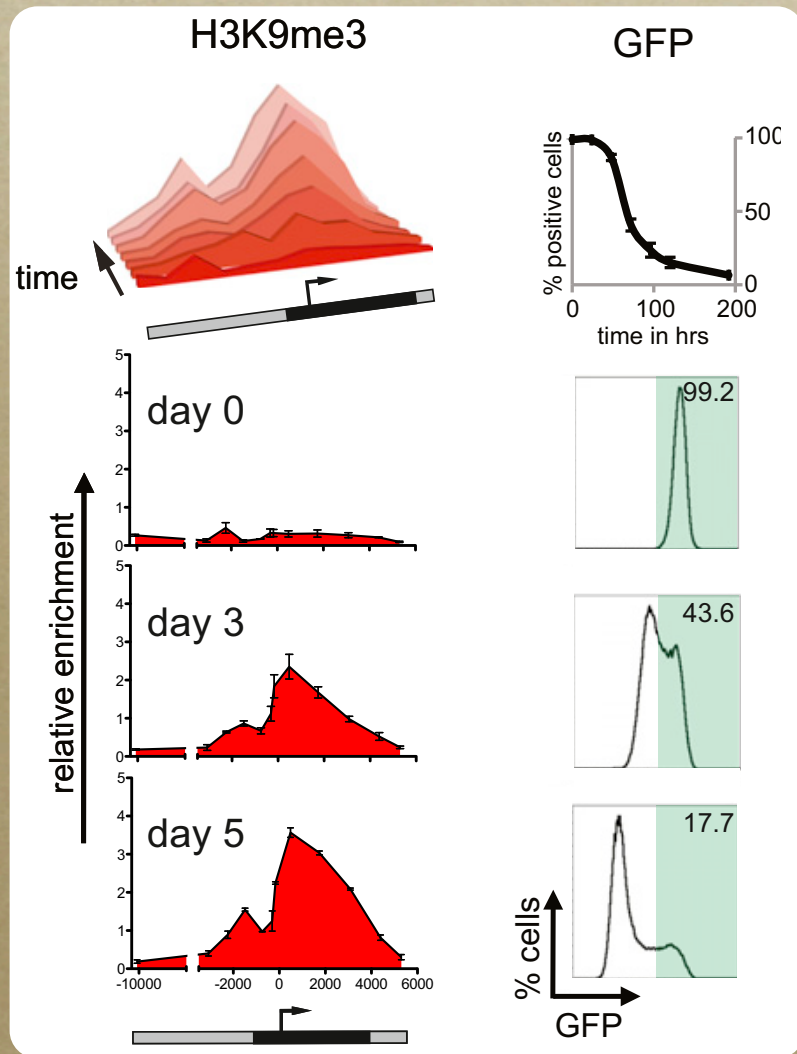
Establishment of a repressive histone code



Establishment of a repressive histone code

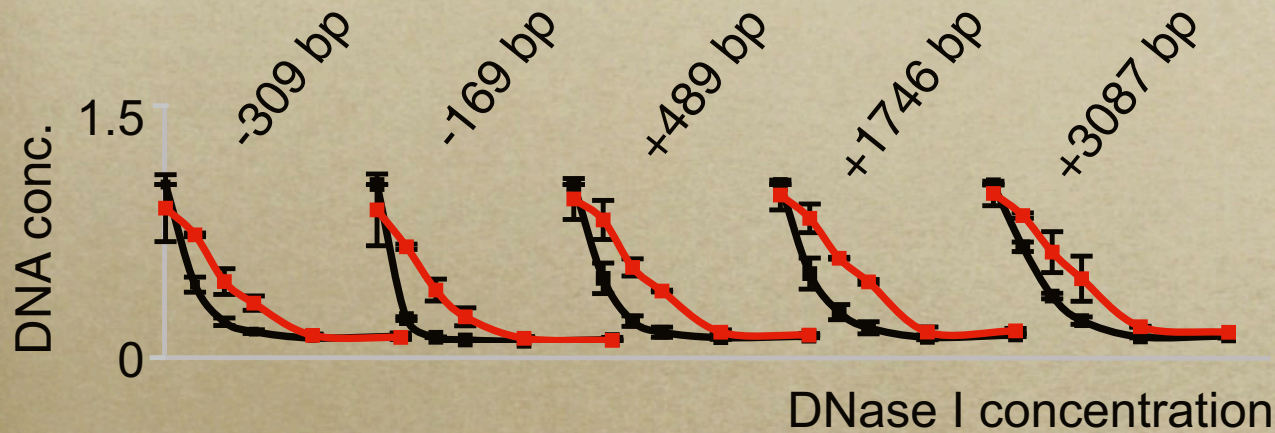


In single cells, the Oct4 promoter is a bistable switch

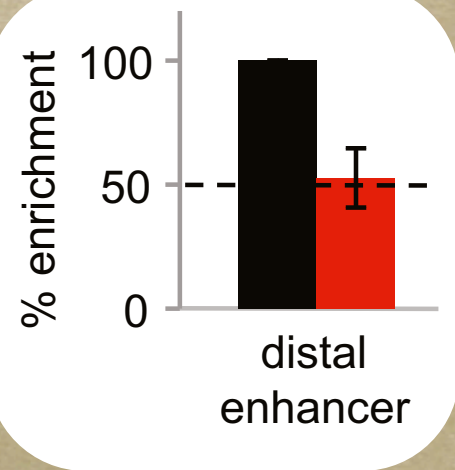
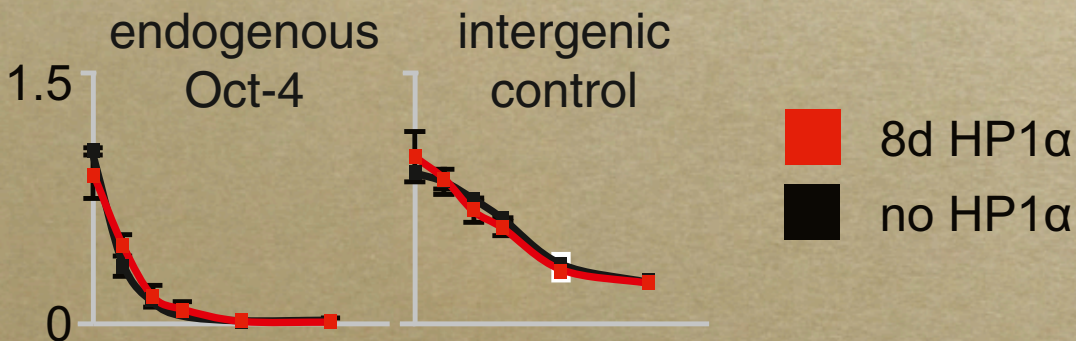


All-or-none repression
Stochastic initiation

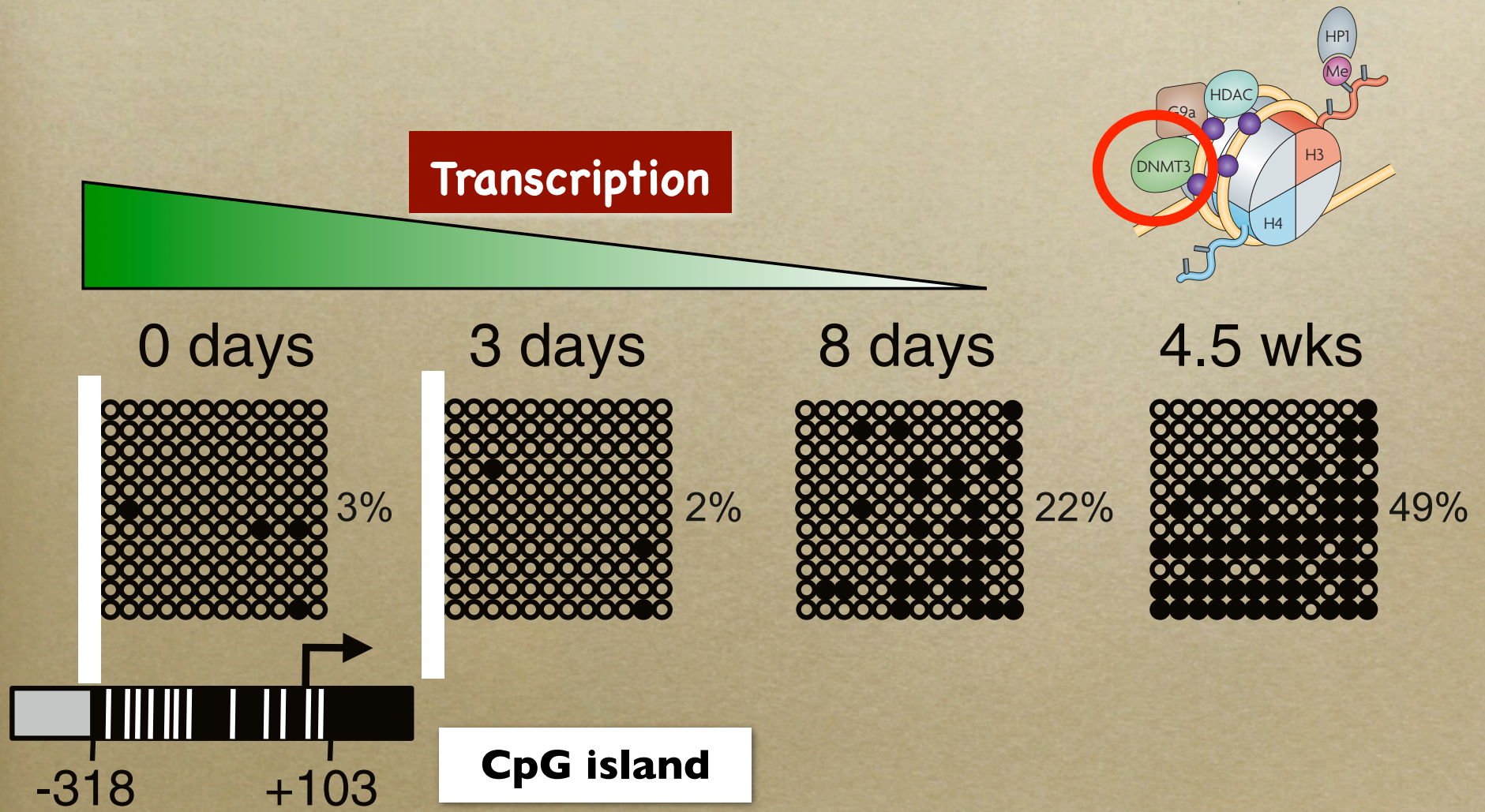
Chromatin at the Oct4 locus compacts with HP1 recruitment



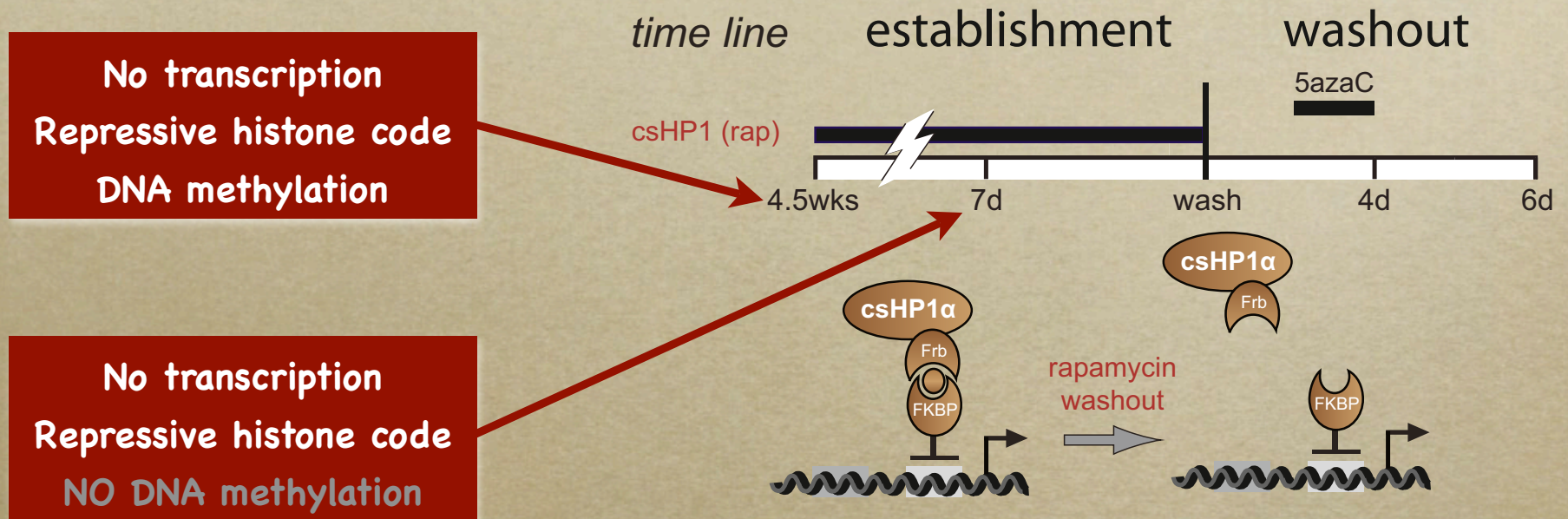
2kb upstream



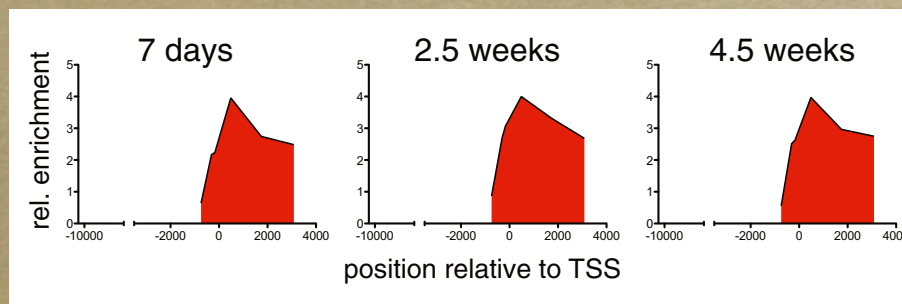
DNA methylation of the Oct4 locus follows histone-mediated silencing (slowly...)



Switch flipped. Can we let go and expect it to stay flipped?



H3K9m3

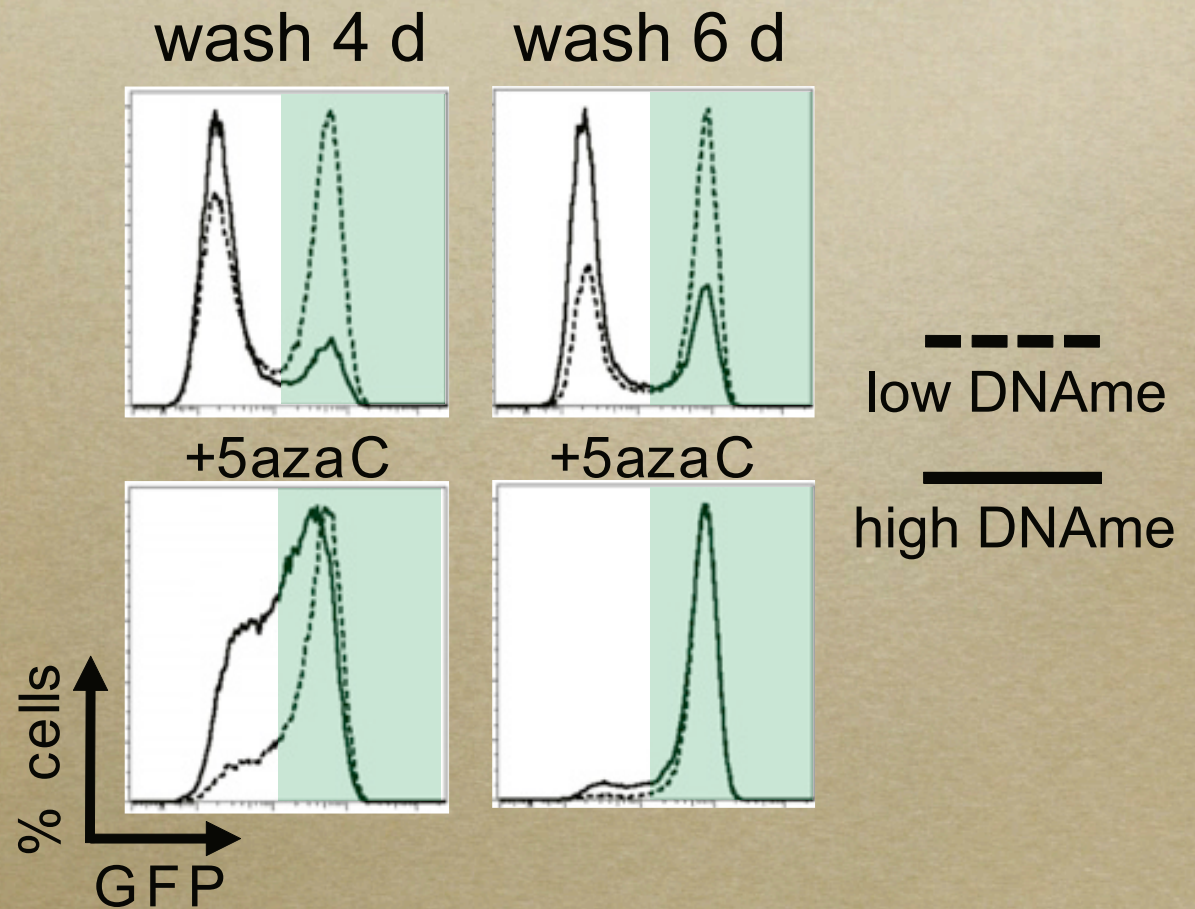


DNA methylation stabilizes the OFF state of the Oct4 promoter

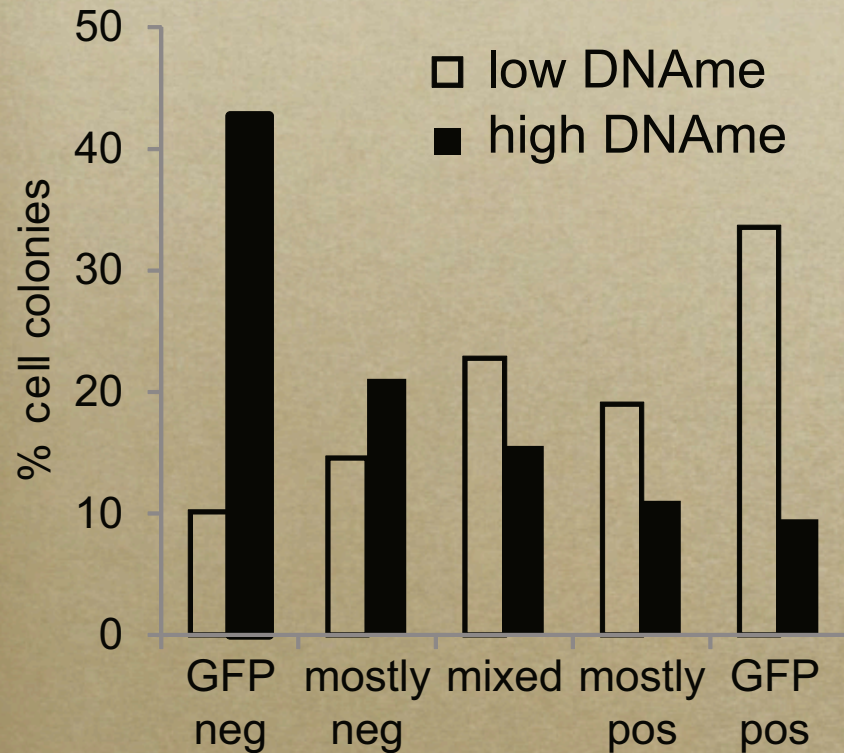
FACS sort
GFP-negative
cells

washout

FACS analysis



DNA methylation enhances heritable transmission of the OFF state



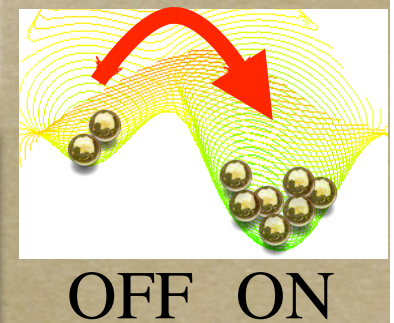
DNA methylation

- heritable OFF state - up to a point
- rare stochastic switching

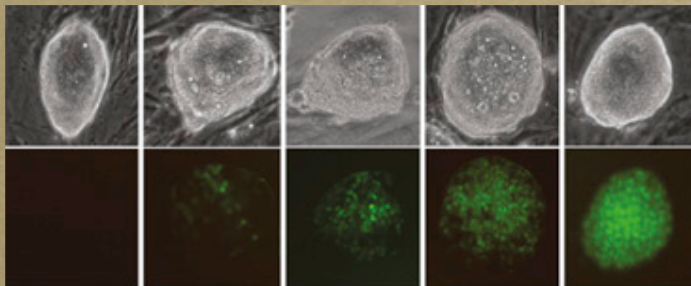


Histone-mediated repression only

- somewhat heritable OFF state
- frequent stochastic switching



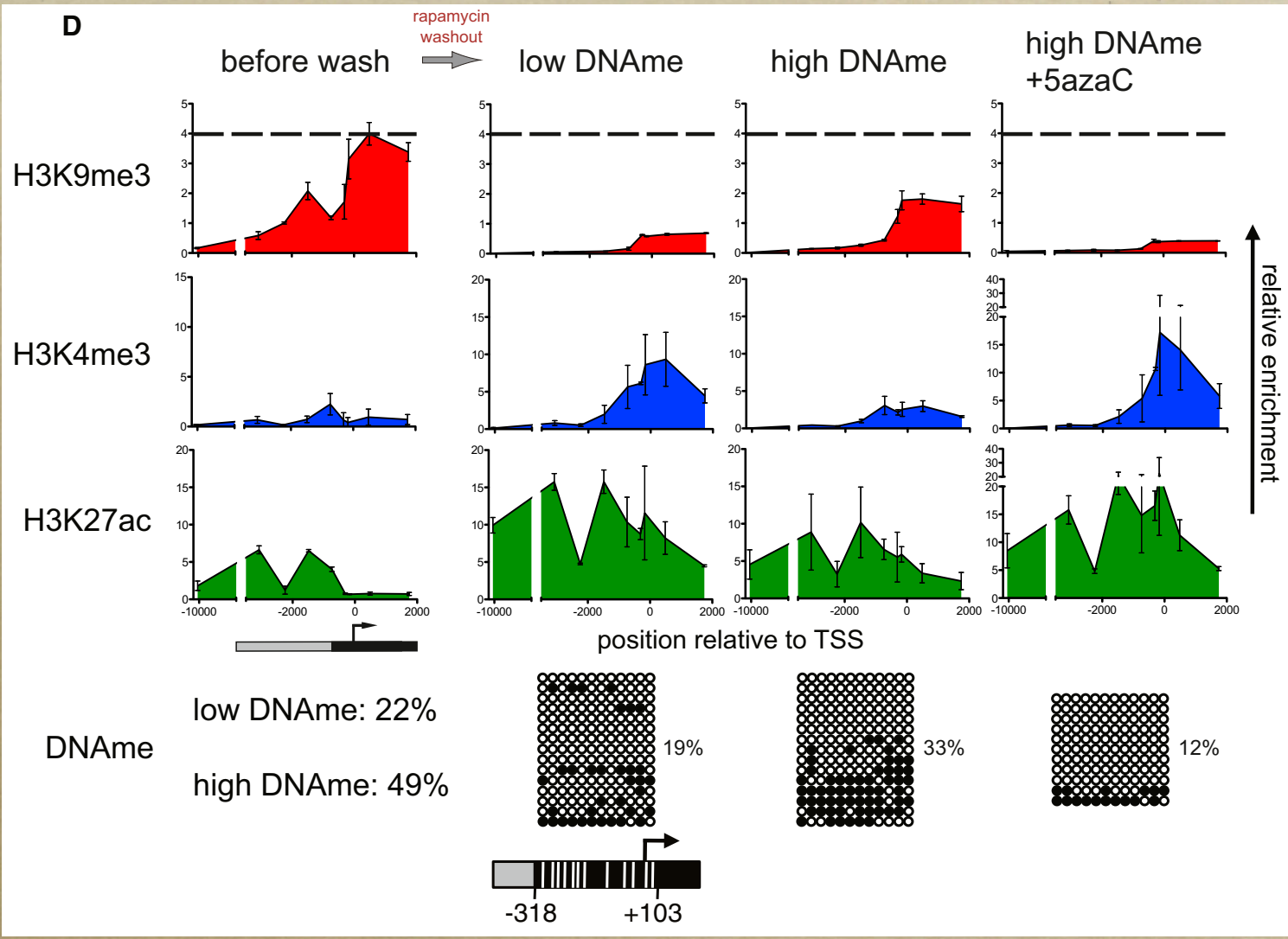
example colonies



Phase

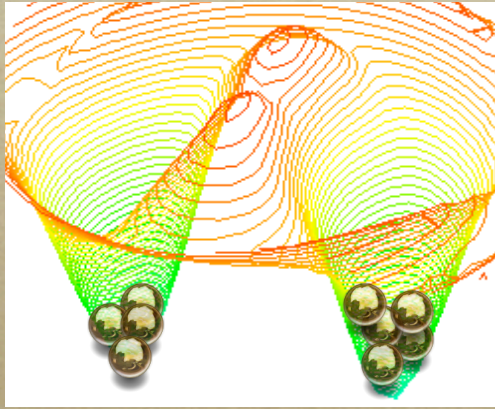
GFP

DNA methylation helps maintain repressive histone code following washout



ES cell summary:

ON \rightarrow OFF \rightarrow ON



OFF

ON

cellular environment in ES cells

ON

csHP1 α

Bistable regime

csHP1 α

OFF

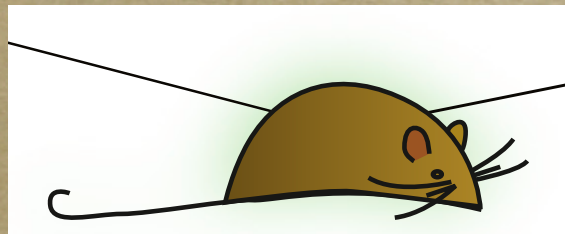
OFF

OFF

How about OFF → ON → OFF?

Can transcription factors alter epigenetics of the Oct4 promoter?

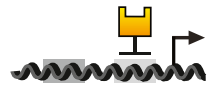
CiA:Oct4
EC cells



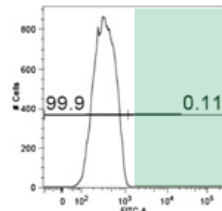
CiA:Oct4
MEFs

In MEFs, Oct4 is silenced by repressive chromatin

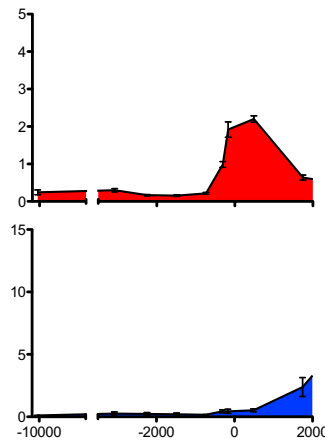
CiA:Oct4 in MEFs



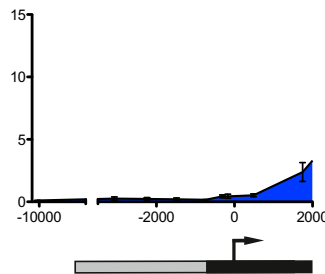
GFP



H3K9me3

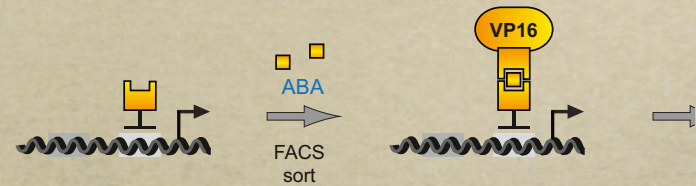


H3K4me3

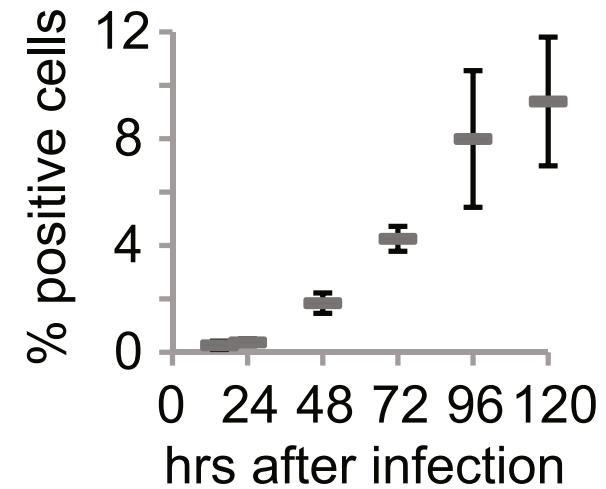


CiA:Oct4 in MEFs

reactivated CiA:Oct4

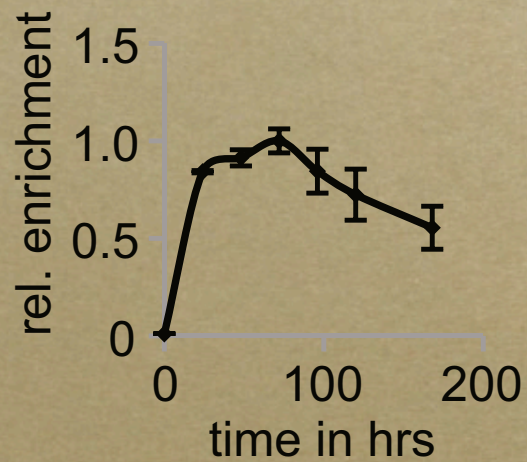
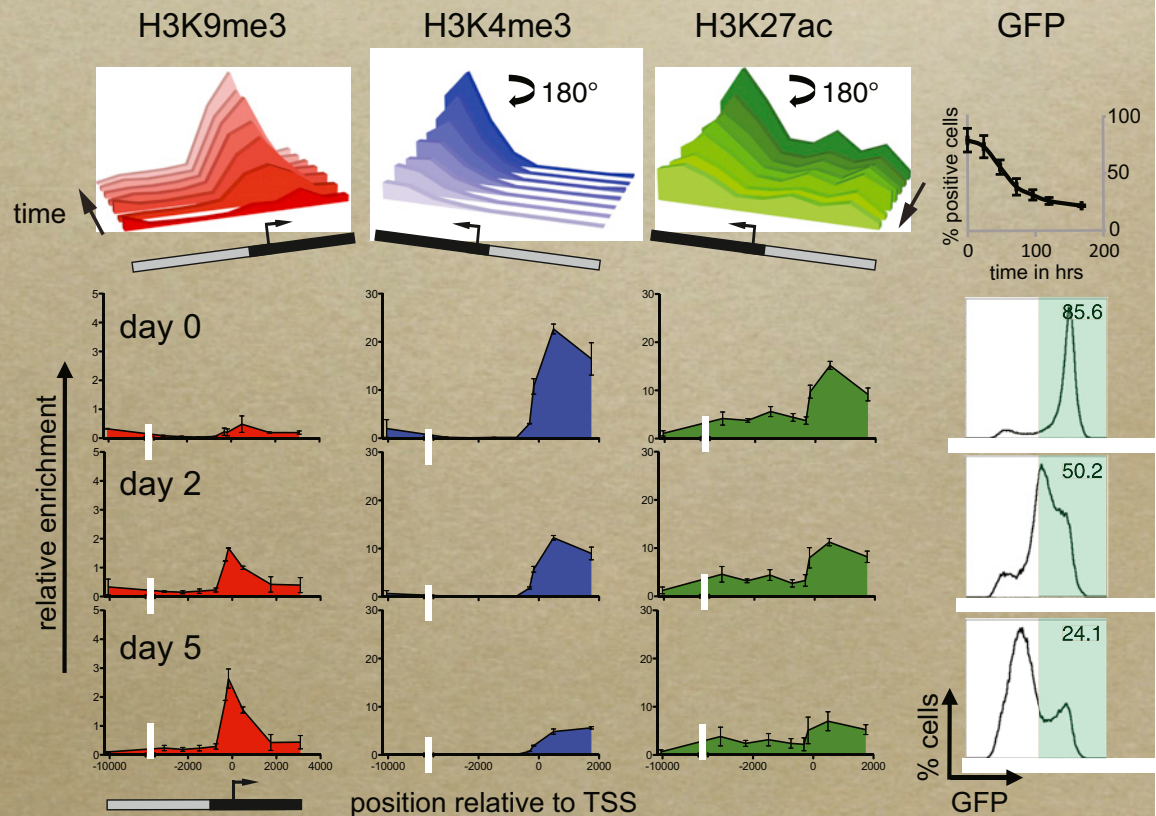
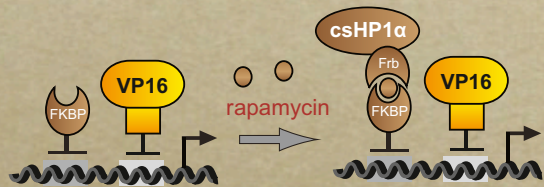
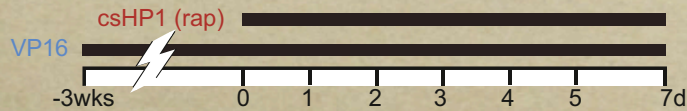


A



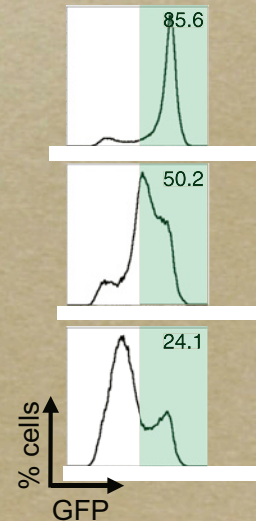
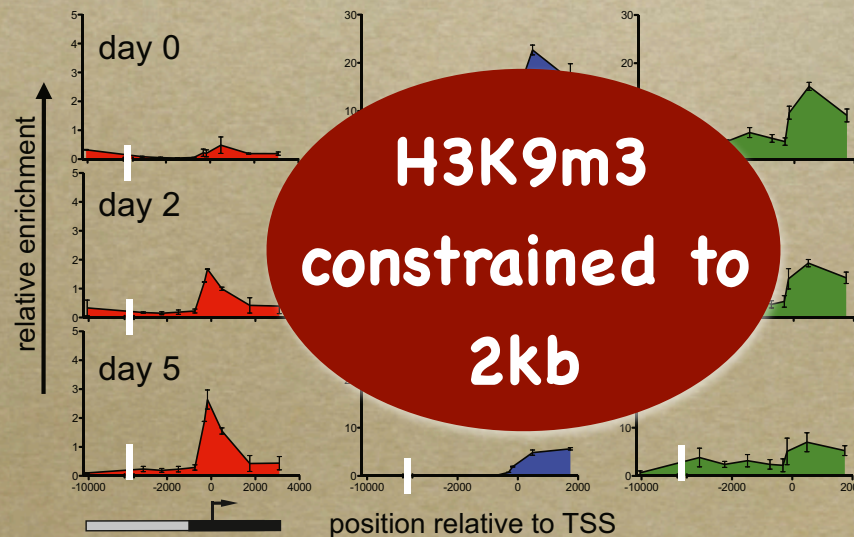
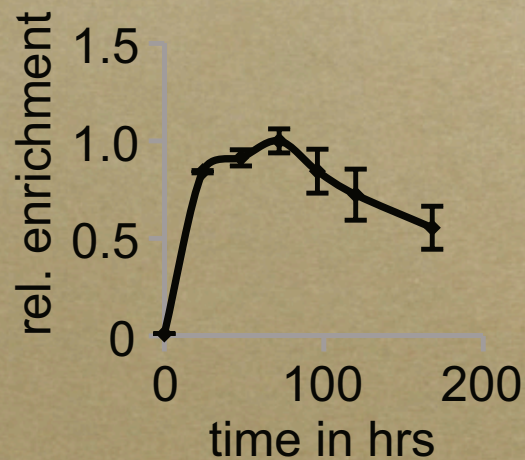
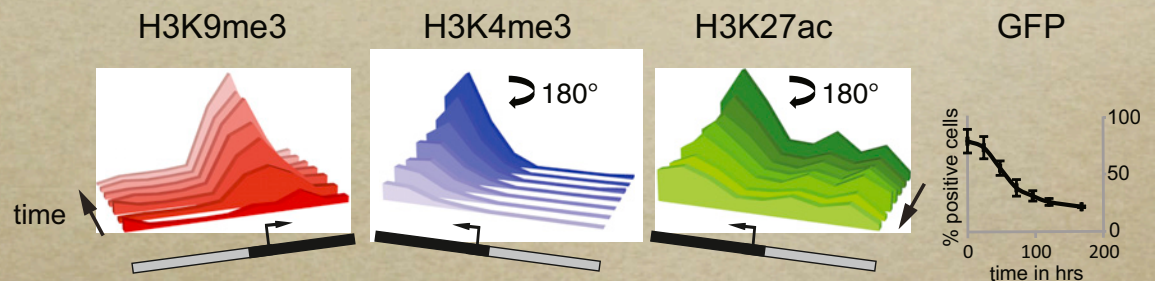
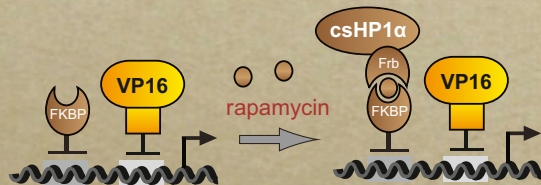
TF binding does not block targeted HP1 recruitment

time line

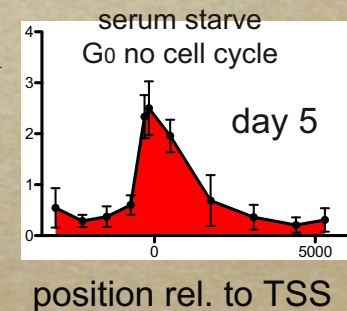
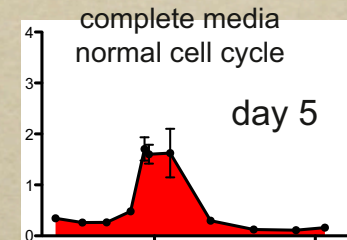
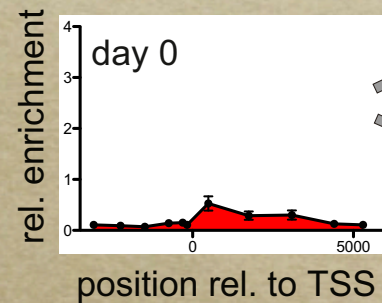
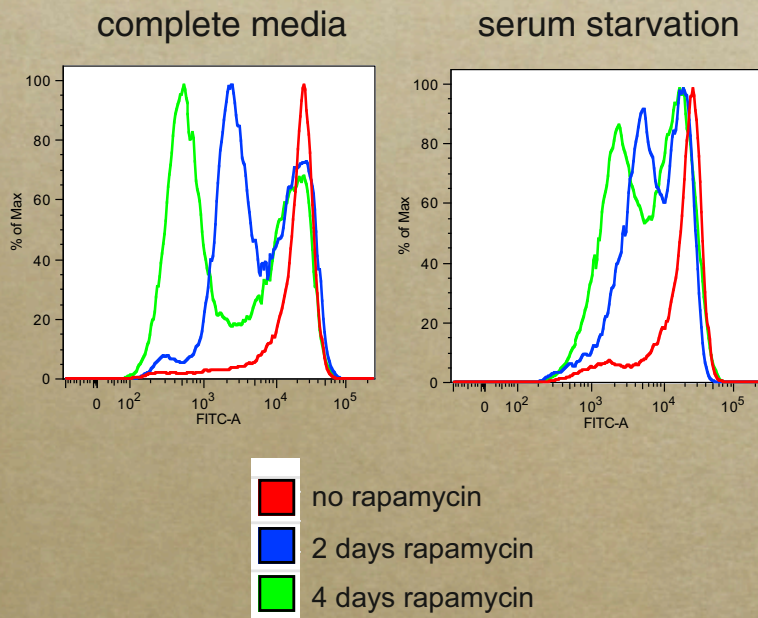


TF binding does not block targeted HP1 recruitment

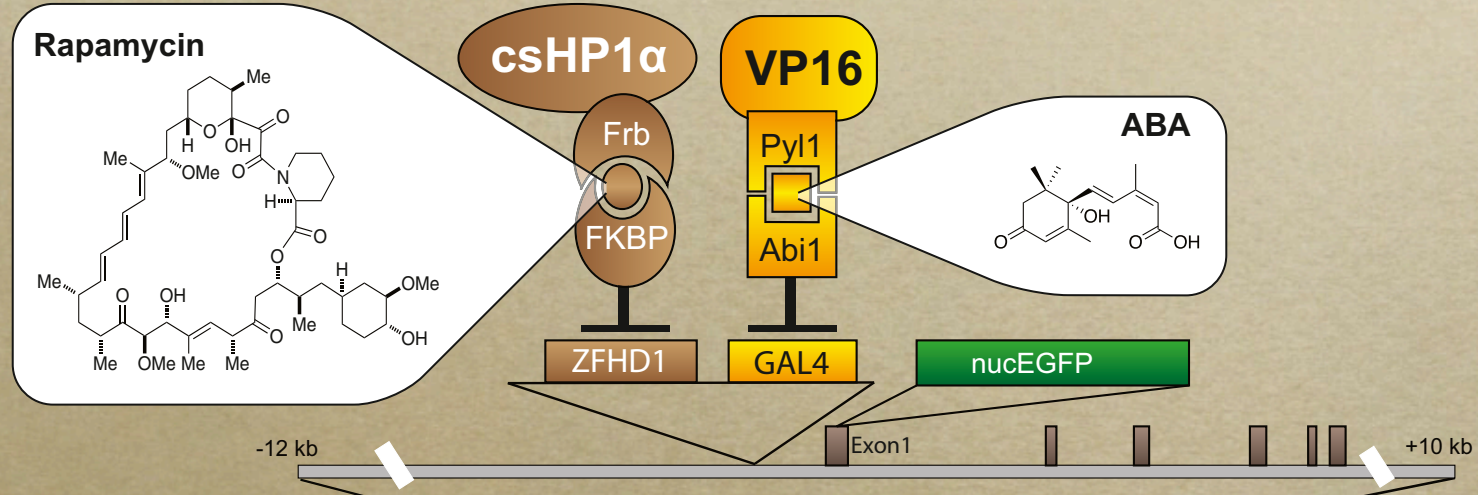
time line



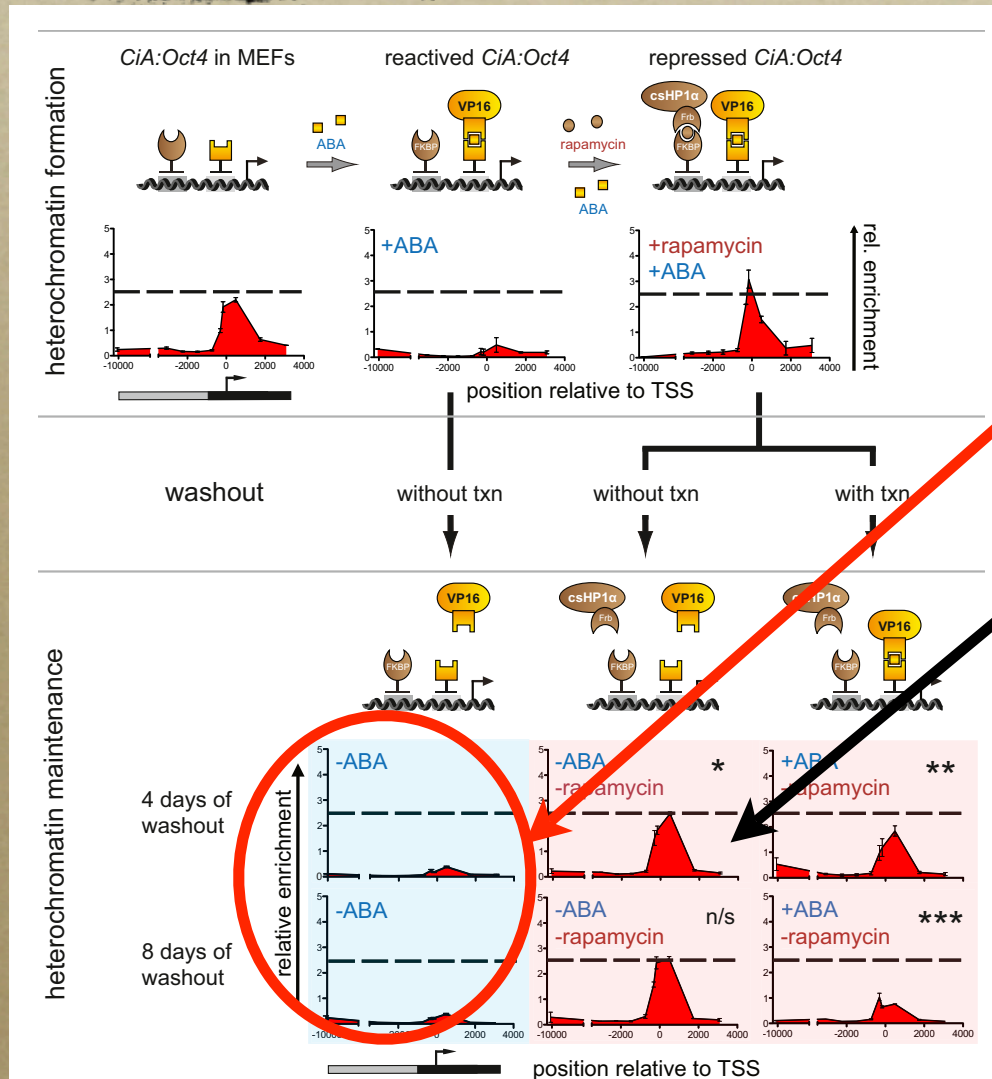
Replication-dependent histone exchange is not required for Oct4 silencing



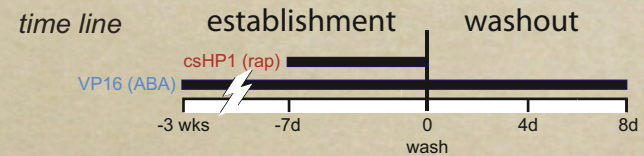
Combinatorial recruitment system



Bistability of the transcription switch



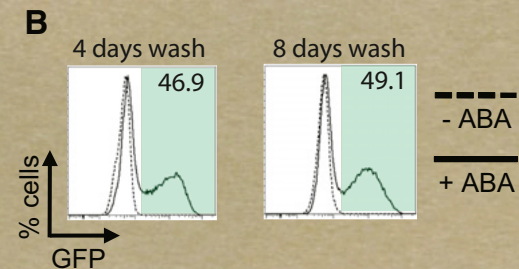
A



• a transcription pulse can switch cells to a stable **ON** state!

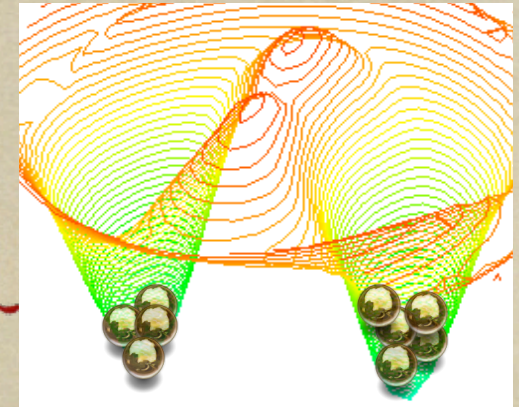
• once repressive chromatin is established, it stays **OFF** in the absence of transcription

• keeping TF tethered turns a fraction of cells back on



MEF summary:

OFF → ON → OFF



OFF

ON

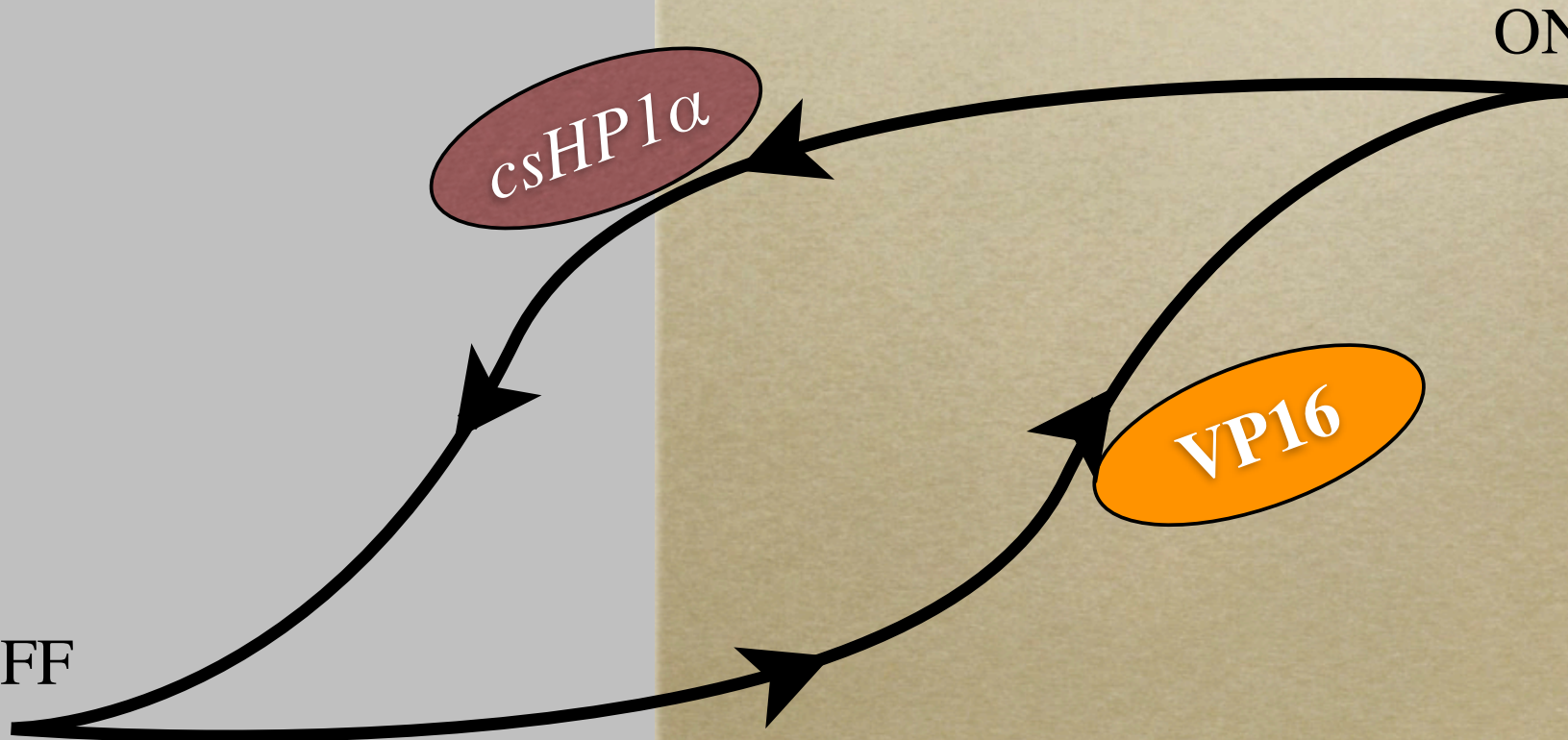
cellular environment in
MEF cells

ON

csHP1 α

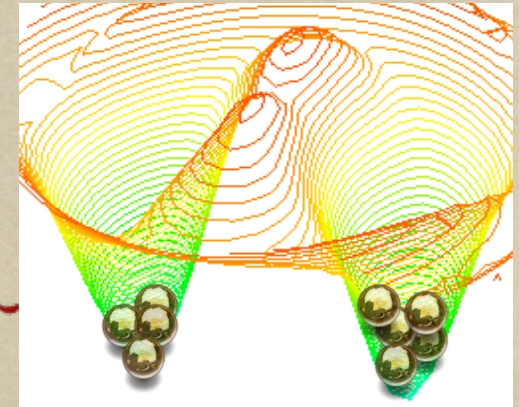
VP16

OFF



MEF summary:

OFF → ON → OFF



OFF

ON

cellular environment in
MEF cells

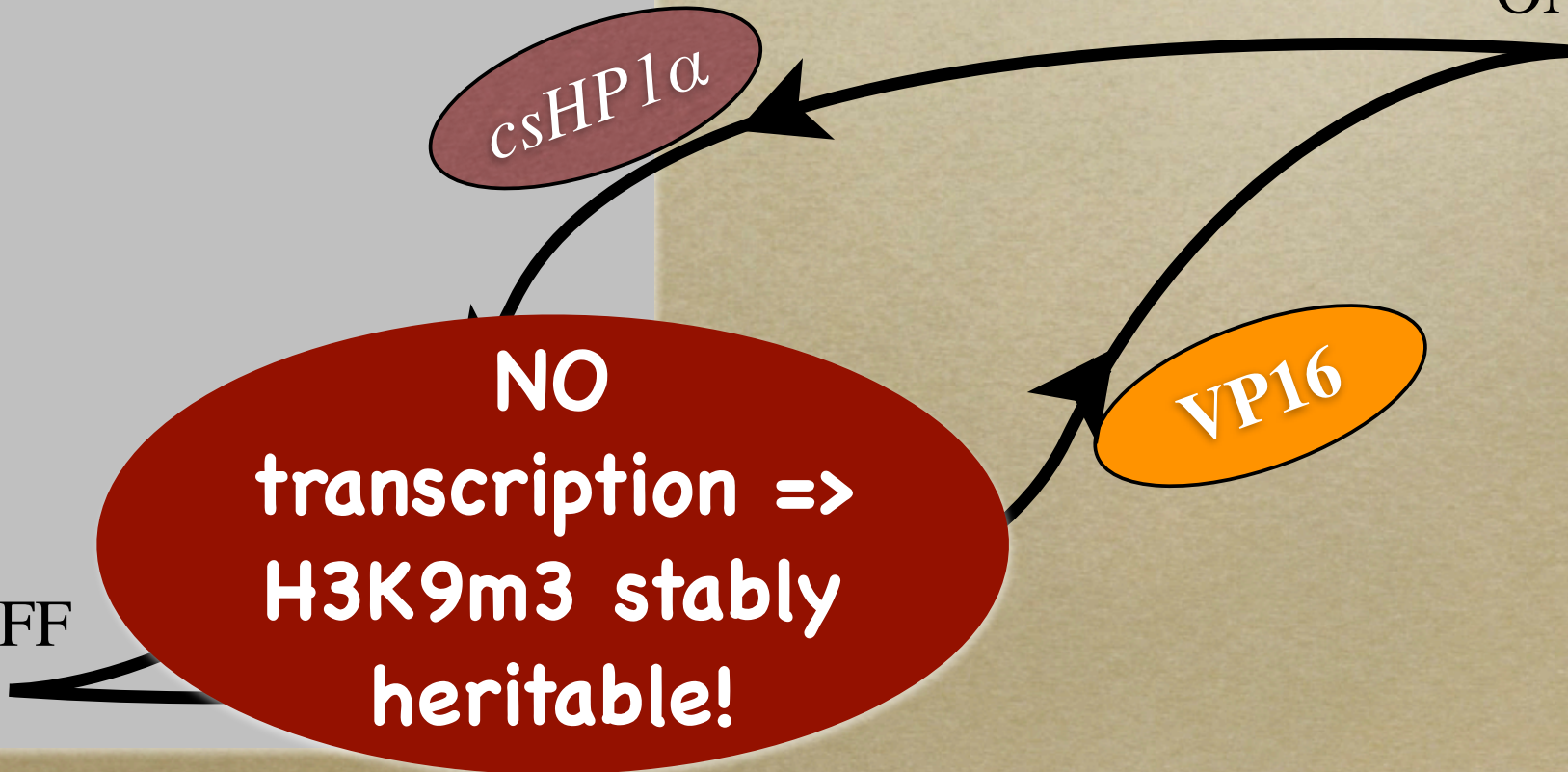
ON

csHP1 α

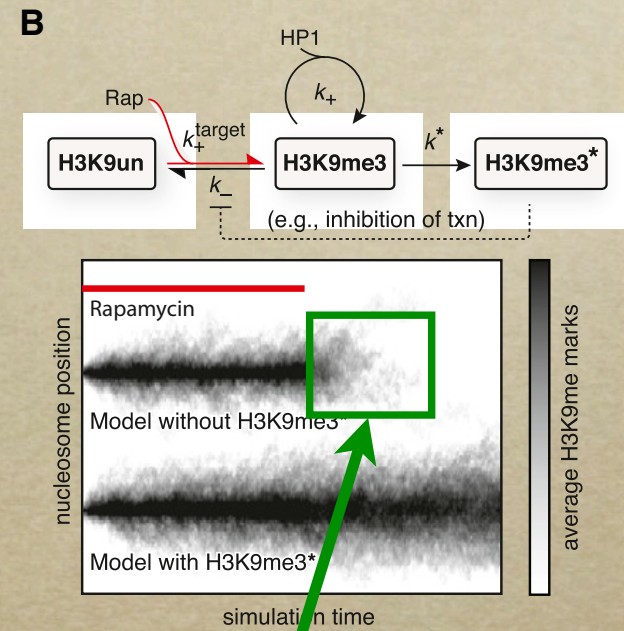
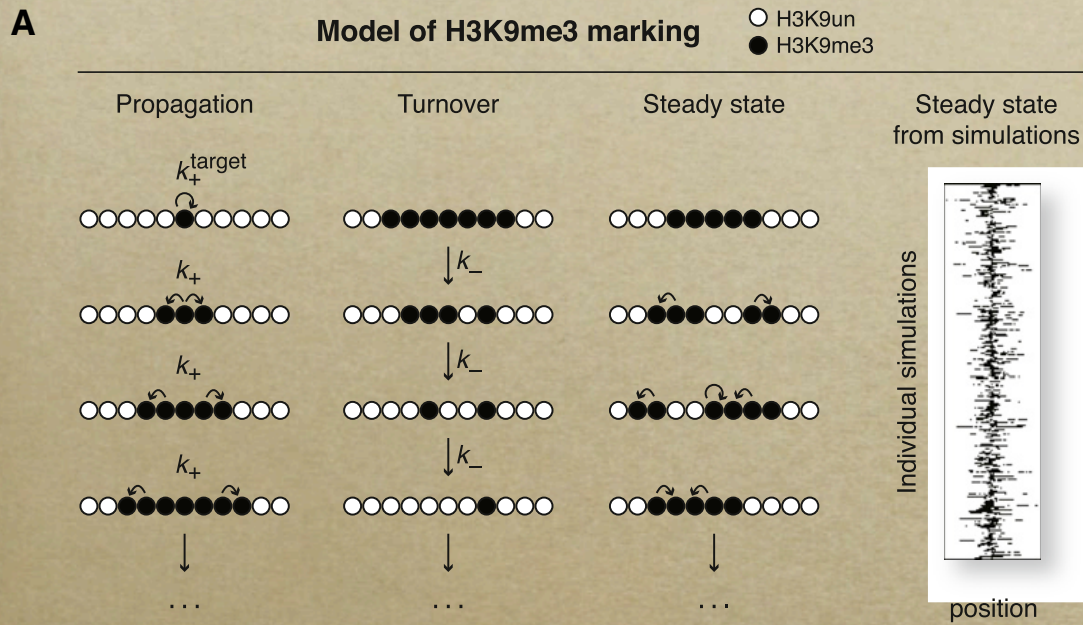
NO
transcription =>
H3K9m3 stably
heritable!

VP16

OFF



"Copy enzymes": linear, stochastic H3K9me3 propagation

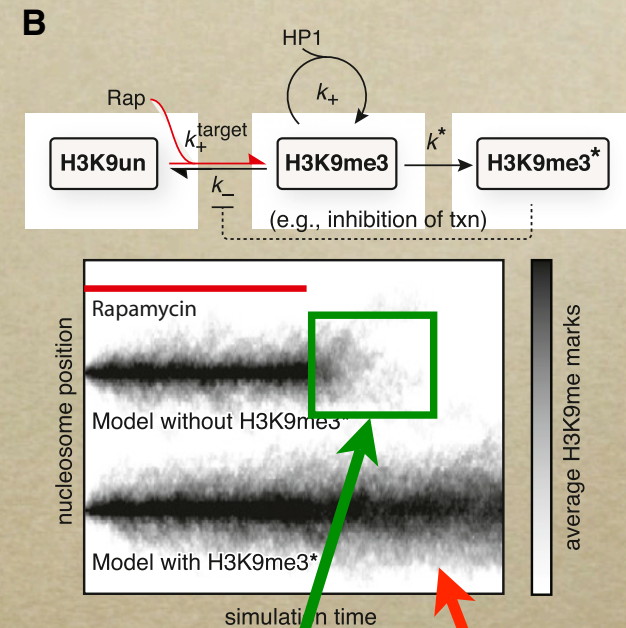
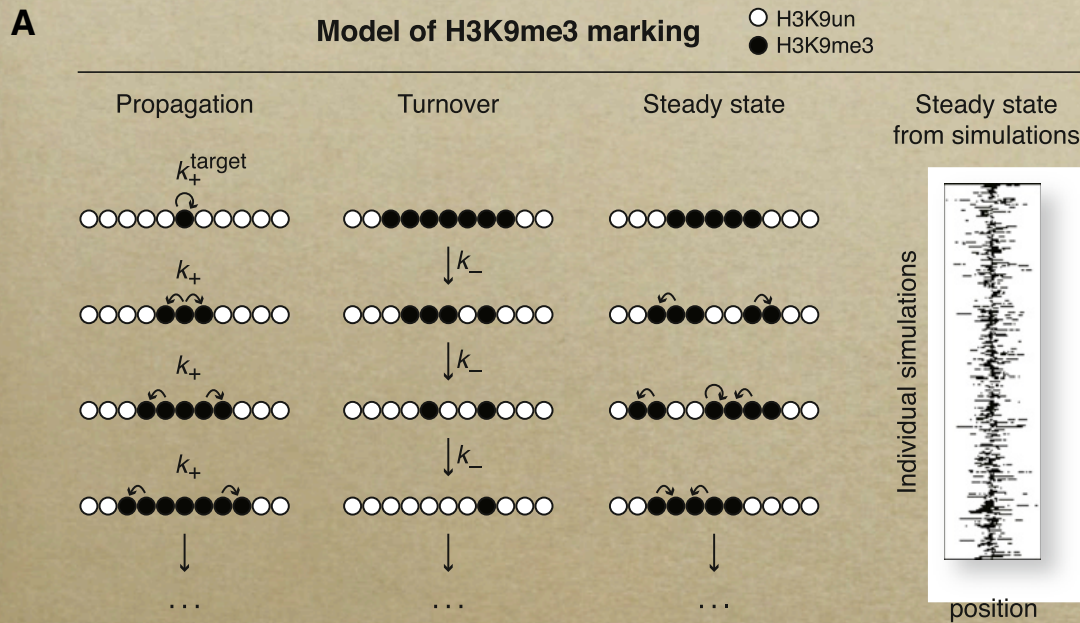


peak at nucleation site

soft borders

• rapid collapse when nucleation site inactive

"Copy enzymes": linear, stochastic H3K9me3 propagation



peak at nucleation site

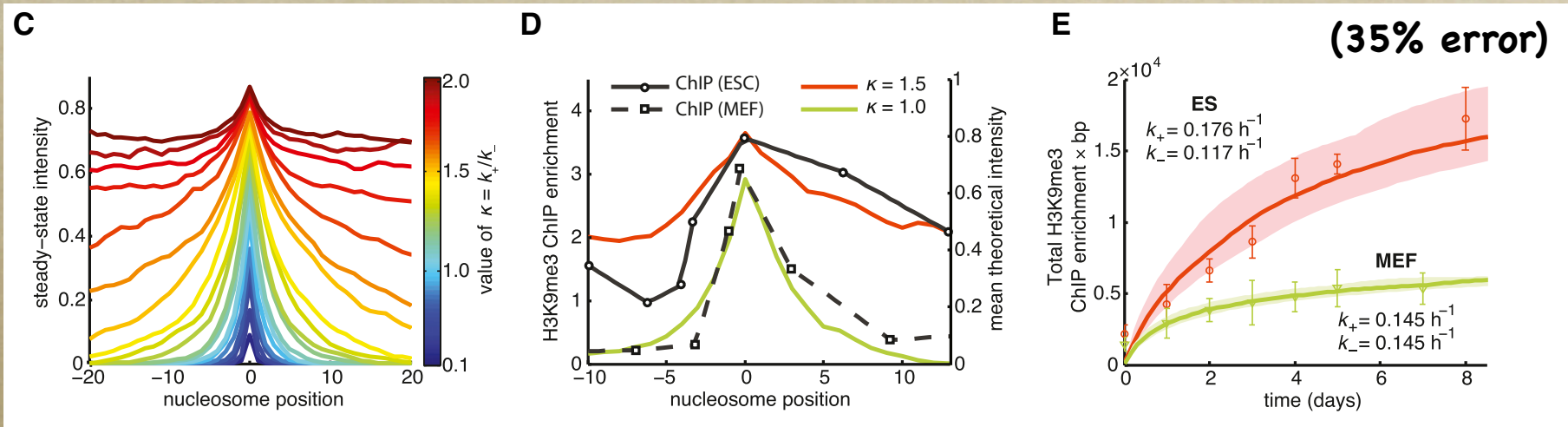
soft borders

• rapid collapse when nucleation site inactive

• stabilized H3K9m3 => stable OFF state

- lack of transcriptional activators
- DNA methylation
- chromatin compaction of the locus

Model + experiment => rate of H3K9me3 spreading and turnover



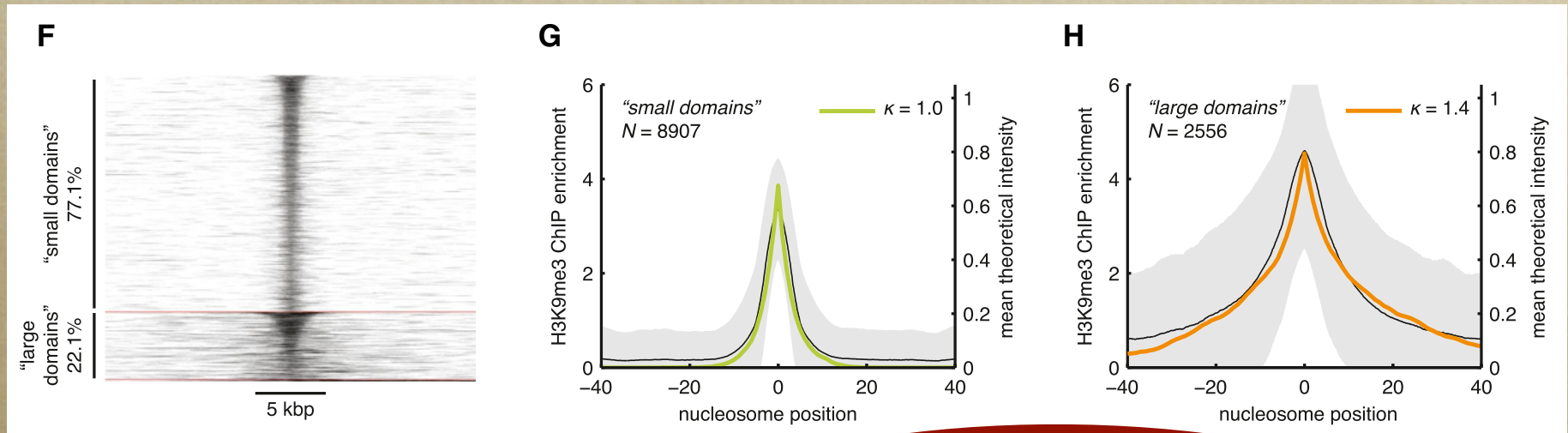
$$k_{\text{bounded}} = k_+/k_- < 1.5$$

ES: spreading is faster,
turnover is slower

- 10kB domain in ES
- 2kB domain in MEFs

propagation rate to neighboring nucleosome
~ every 5.7 hr in ES cells
~ every 6.9 hr in MEFs.

Kinetic Model Predicts Shapes of Genomic H3K9me3 Domains



**H3K9me3 ChIP-seq,
mouse ES cells**

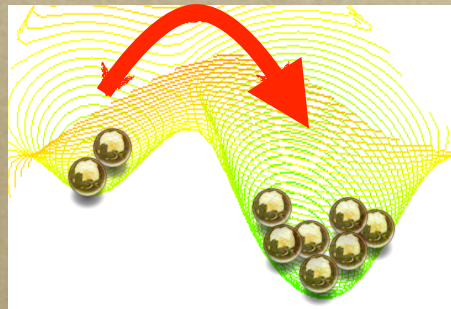
**2 distinct rates
account for 99.2% H3K9m3
domains**

Conclusions

- *a nucleation point for HP1 binding can turn off a gene via altering the histone code, in spite of TFs to drive transcription*
- *DNA methylation or absence of transcription stabilizes the OFF state*
- *strong tethering of a transcription factors can turn locus ON, overcoming repressive chromatin (as long as nucleation is weak)*

Conclusions (in pictures)

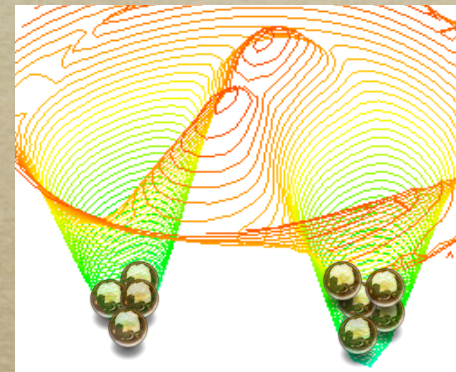
Histone-code
alone is bistable



OFF ON

- OFF: stably heritable in the **absence** of transcriptional activators
- OFF: heritable in the presence of TFs in the cell, but frequent stochastic ON-flip
- ON: stably heritable in the presence of **active transcription** and lack of HPI nucleation

Histone-code +
DNA methylation
strongly bistable



OFF ON

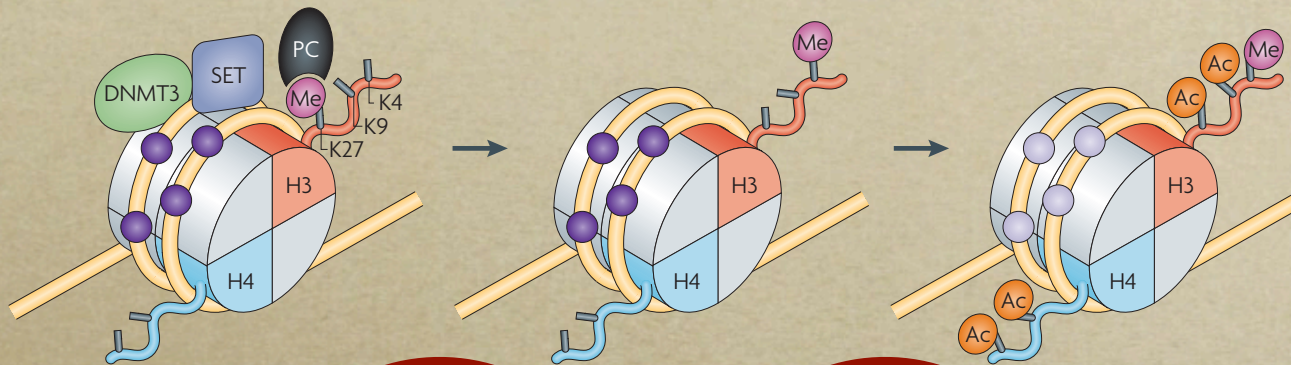
- OFF: stably heritable, with rare stochastic ON-flip

Strengths

- **I never learned this much epigenetics from anywhere else**
 - not from reviews
 - not from conference
- **Proves heritable bistability of histone code**
 - attention to the role of stochastic events, dependent on barrier height
- **Simple, conceptual kinetic model -> broad applicability: H3K9m3 marks are copied to neighbors**

Drawbacks?

Help!



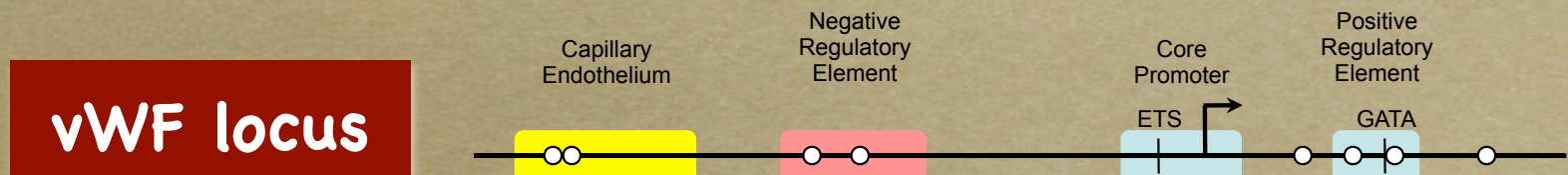
first
event?

first
event?

- Q: what determines the endogenous sites of H3K9m3 nucleation?

Outlook

- **How does the histone - DNA methylation (double) switch work in EC genes induced/repressed by signals**
 - Mardsen: DNA methylation stays off, histone code is flipped ON/OFF by biological signals
 - Aird lab :vWF promoter - DNA methylation (and histone) undergoes stochastic flips
- **Do the DNA methylation-related results of this paper only apply to CpG islands?**



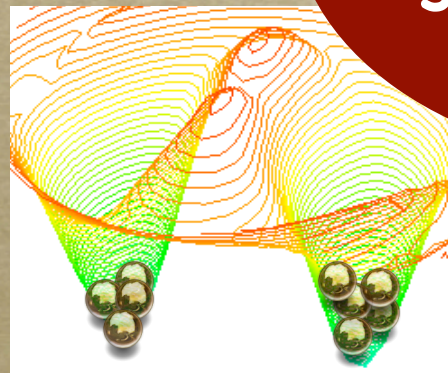
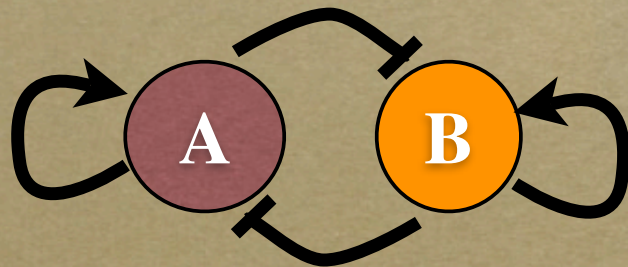
What is epigenetics?

Original definition:

- * mechanisms by which different cellular phenotypes are clonally heritable, without altering the genetic code

What types of mechanism?

- * DNA methylation
- * nucleosomal histones
- * regulatory circuits with positive feedback



OFF ON

Any
mechanism able to
generate strong
bistability

Thank you!

Dynamics and Memory of Heterochromatin in Living Cells

N.A. Hathaway, O. Bell, C. Hodges,

E.L. Miller, D.S. Neel, G.R. Crabtree

Cell 149, 1447–1460, 2012



Journal Club, 2014

Erzsébet Ravasz Regan