

# The epigenetic logic of gene activation

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Centre de Regulació Genòmica, Universitat Pompeu Fabra



**SIMONS**  
**INSTITUTE**  
for the Theory of Computing

Programs | Summer 2022



Computational Innovation and Data-Driven  
Biology

*Jul. 5 – Aug. 5, 2022*

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Beatrice Borsari



Sílvia Pérez-Lluch



A preprint of this work is available on bioRxiv:  
<https://doi.org/10.1101/2020.11.20.391524>





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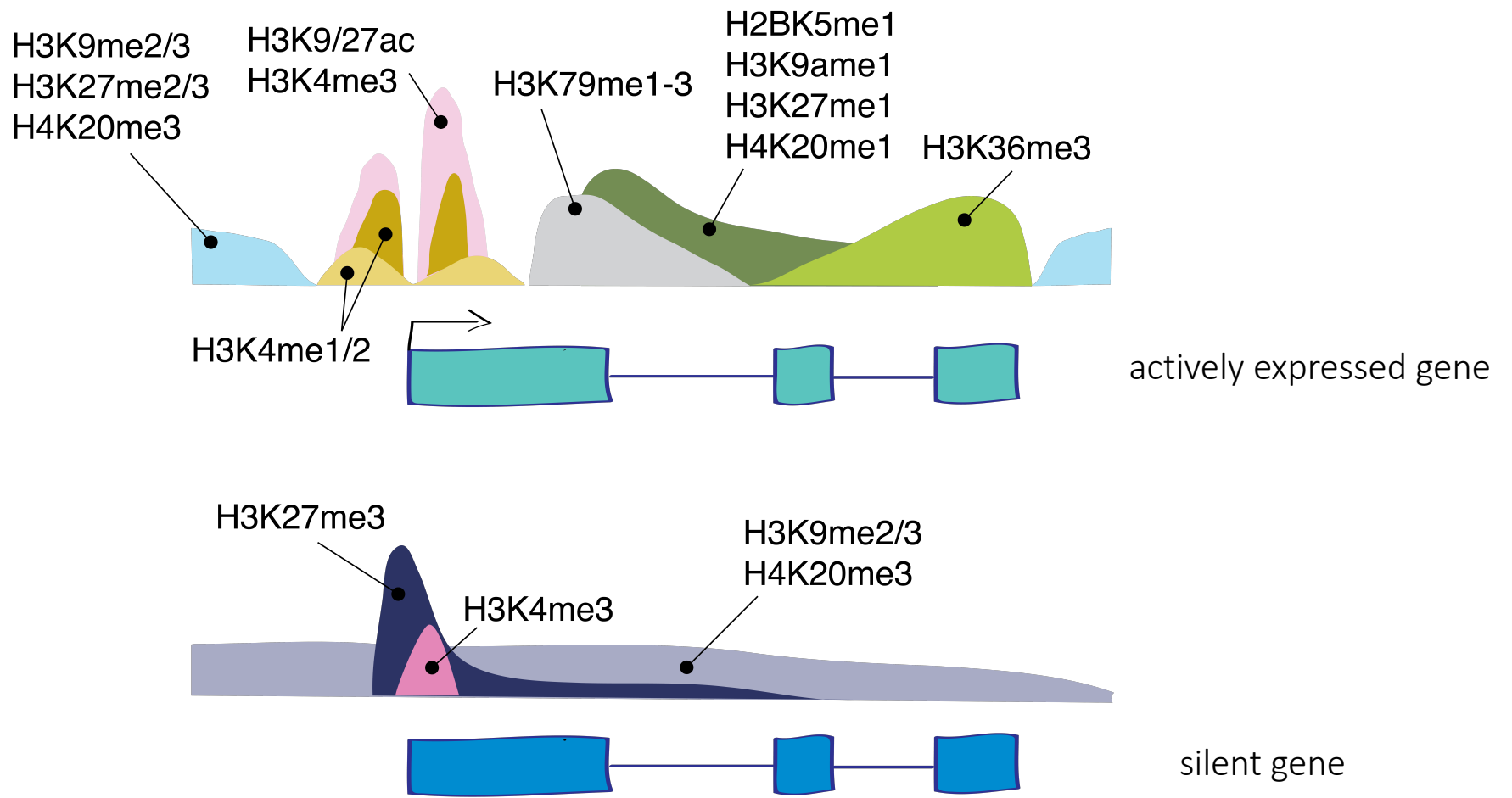
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# Histone code

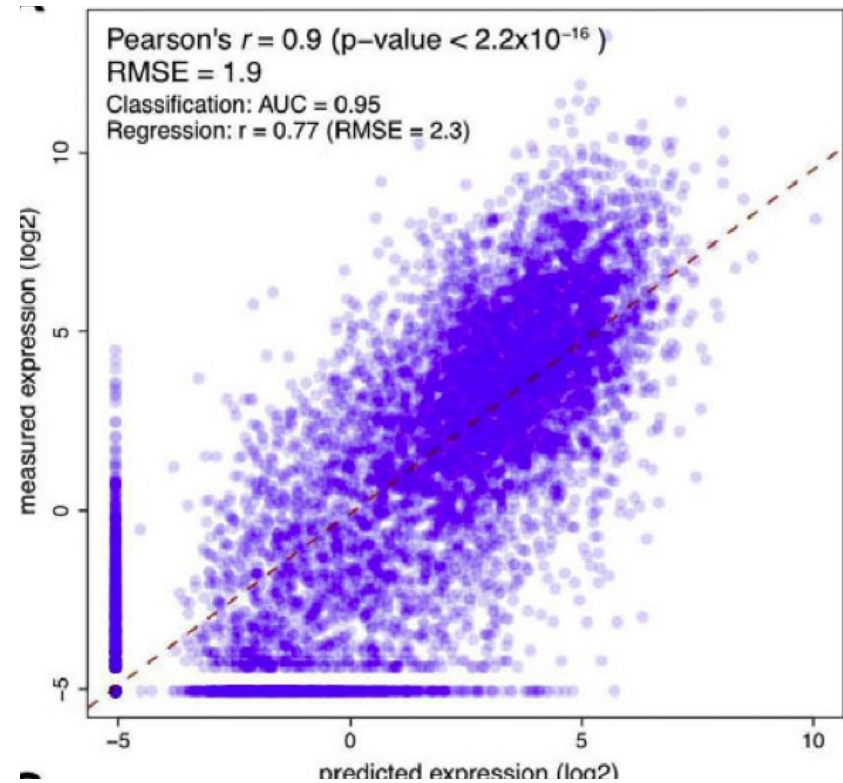
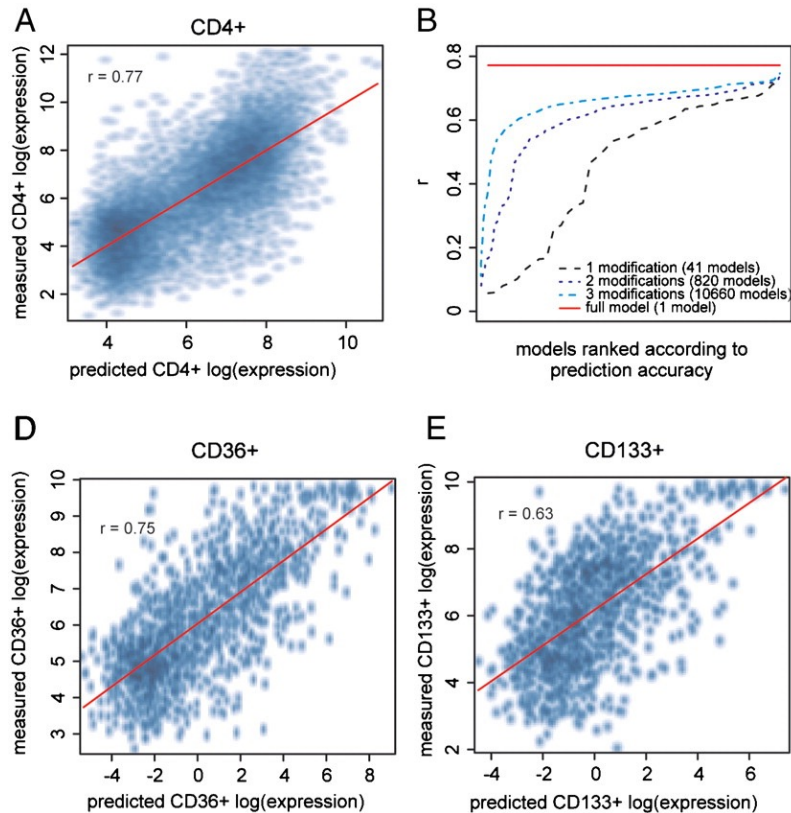
From Wikipedia, the free encyclopedia

The **histone code** is a [hypothesis](#) that the transcription of genetic information encoded in [DNA](#) is in part regulated by chemical modifications to [histone](#) proteins, primarily on their unstructured ends.



Adapted from *Barth and Imhof, 2010*

# Histone modification levels are predictive of gene expression levels



# nature genetics

VOLUME 47 NUMBER 10 OCTOBER 2015  
www.nature.com/naturegenetics

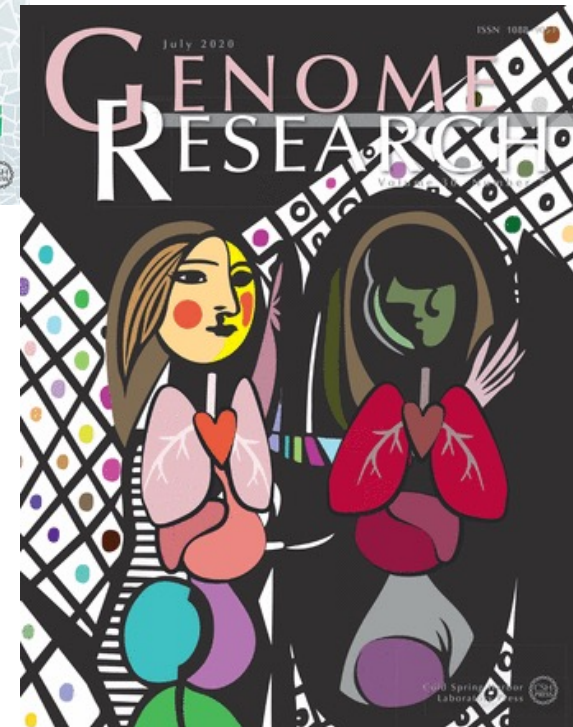
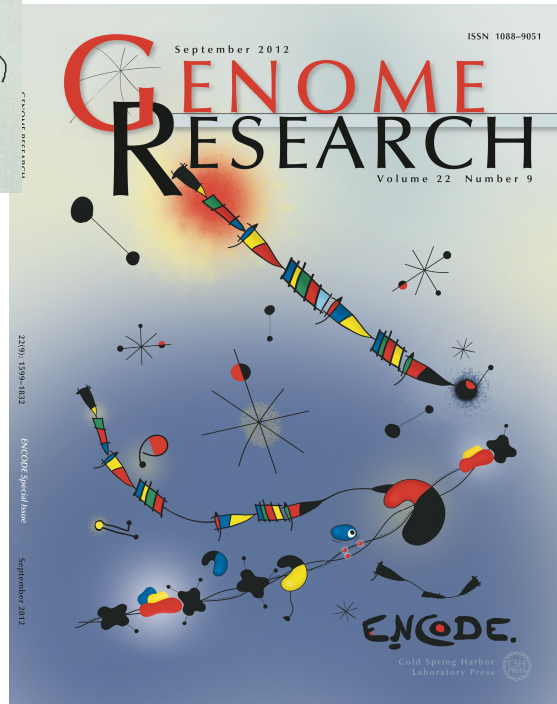
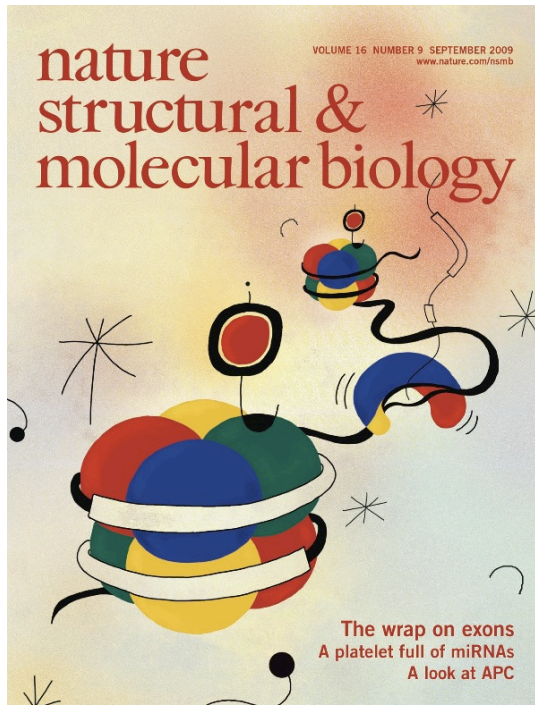
## Absence of canonical marks of active chromatin in developmentally regulated genes

Silvia Pérez-Lluch, Enrique Blanco, Hagen Tilgner, Joao Curado, Marina Ruiz-Romero, Montserrat Corominas & Roderic Guigó

Cover by Luisa Lente inspired in Dali's *Butterfly Landscape* (*The Great Masturbator in Surrealist Landscape with DNA*)

Polycomb repression in 3D  
AAV2 in hepatocellular carcinoma  
Negligible missing heritability







# Transcription and Histone modifications

2011

**Histone modification: cause or cog?**

2012

**Transcription in the Absence of Histone H3.2 and H3K4 Methylation**

2015

Absence of canonical marks of active chromatin in developmentally regulated genes

2017

**Is H3K4me3 instructive for transcription activation?**

2017

**MII3 and MII4 Facilitate Enhancer RNA Synthesis and Transcription from Promoters Independently of H3K4 Monomethylation**

2020

Histone H3K27 acetylation is dispensable for enhancer activity in mouse embryonic stem cells

2020

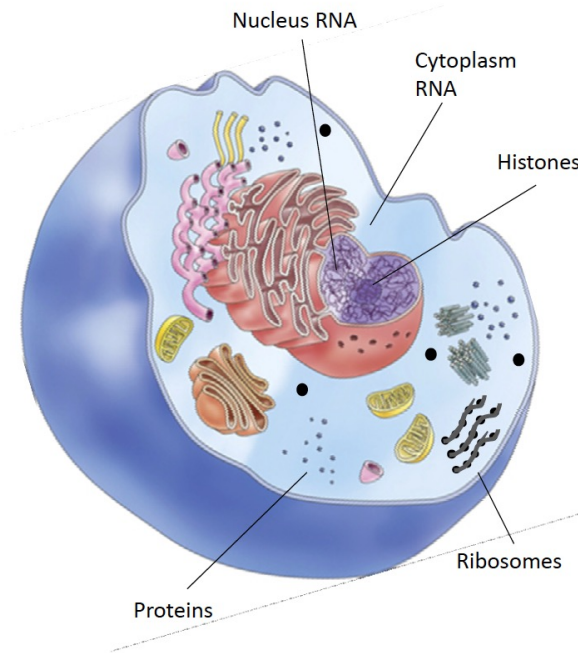
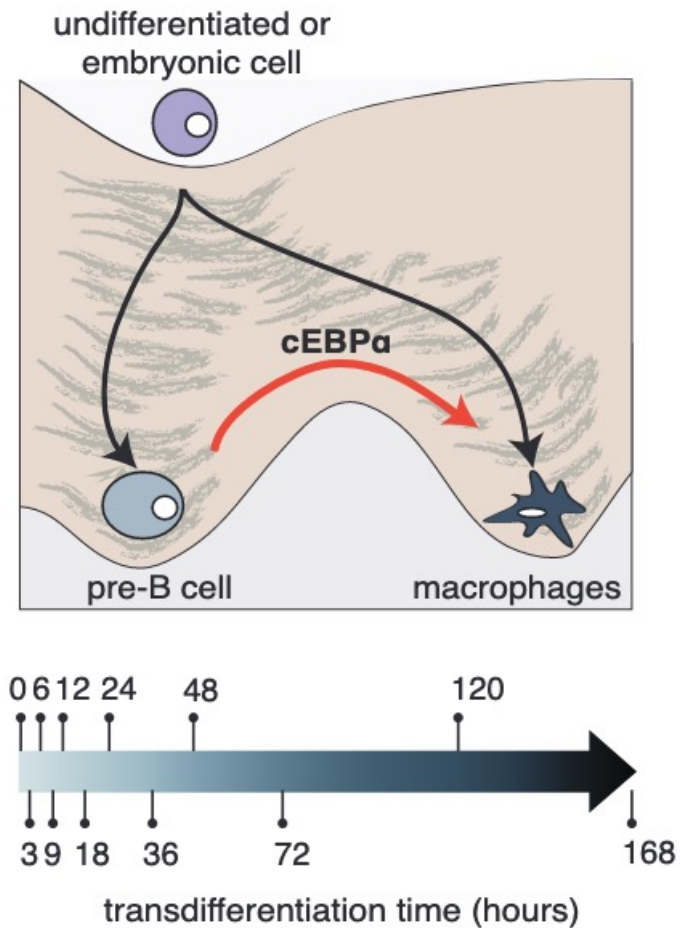
**Reevaluating the roles of histone-modifying enzymes and their associated chromatin modifications in transcriptional regulation**

# Is A the cause of B?

- If we disrupt **A** and **B** still occurs →  
A cannot be the cause B
- If **B** always occur before **A** →  
A cannot be the cause of B

# RNA-maps:

induced transdiferentiation from b-cells to macrophages



## RNAseq

- total RNA
  - Fractions:
    - ❖ Nucleus
    - ❖ Cytoplasm
- Stranded  
PolyA selection  
Paired end 75bp

## Ribosome profiling ChIPseq

- H3K4me1
  - H3K4me2
  - H3K4me3
  - H3K9ac
  - H3K9me3
  - H4K20me1
  - H3K27ac
  - H3K27me3
  - H3K36me3
- Single read 50pb

## Proteomics

Model by Thomas Graf, CRG

216 data sets

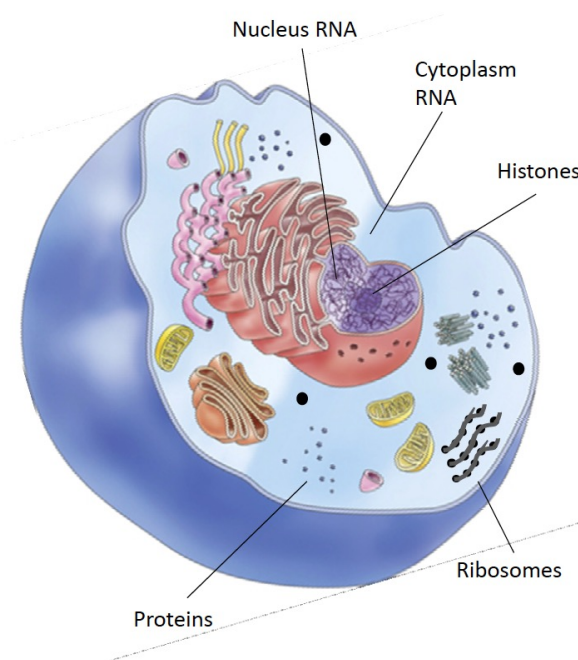
# RNA-maps:

induced transdiferentiation from b-cells to macrophages

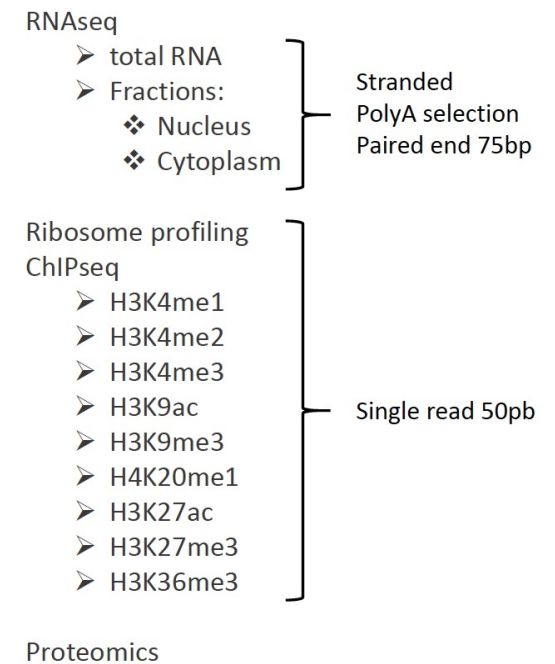


- A process that occurs with massive transcriptional changes
- Homogeneous cellular system
- Highly synchronized
- **Important!:** Within each biological replicate, we performed all assays in the same pool of transdifferentiating cells

<https://www.encodeproject.org/>



Model by Thomas Graf, CRG



216 data sets

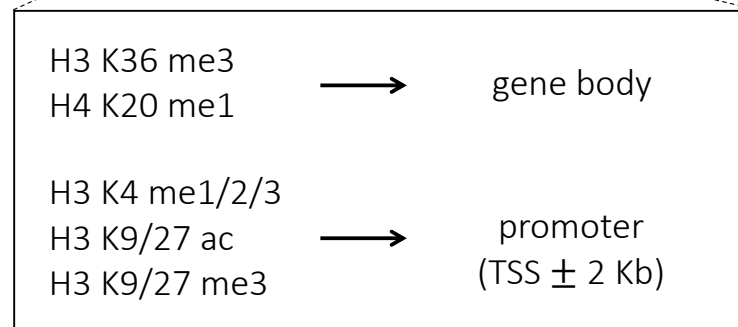
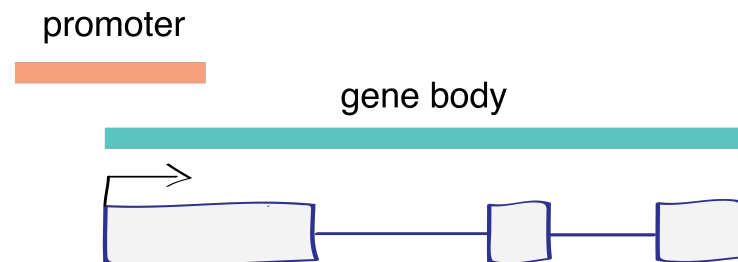
# Measuring marking of genes

gene expression matrix

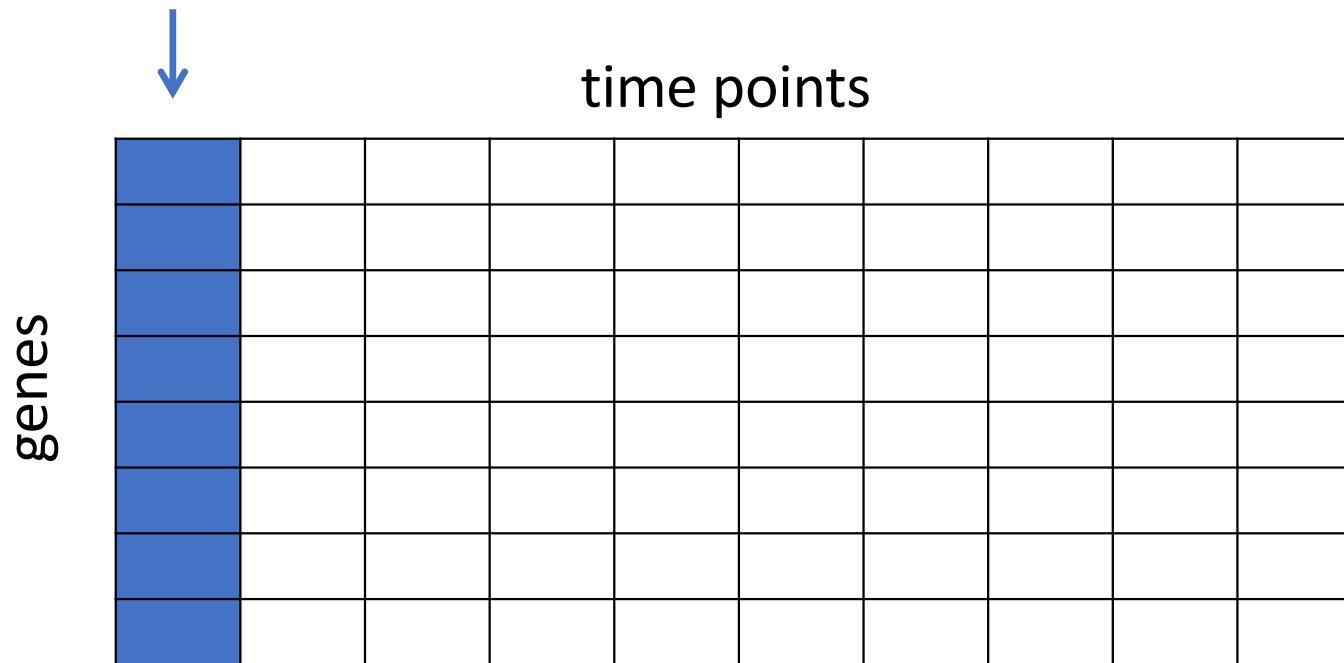
genes	time		
	0h	...	168h
$g_1$	TPM	...	TPM
$g_2$	TPM	...	TPM
$g_3$	TPM	...	TPM
...	...	...	...
$g_n$	TPM	...	TPM

histone mark matrix

genes	time		
	0h	...	168h
$g_1$	signal	...	signal
$g_2$	signal	...	signal
$g_3$	signal	...	signal
...	...	...	...
$g_n$	signal	...	signal

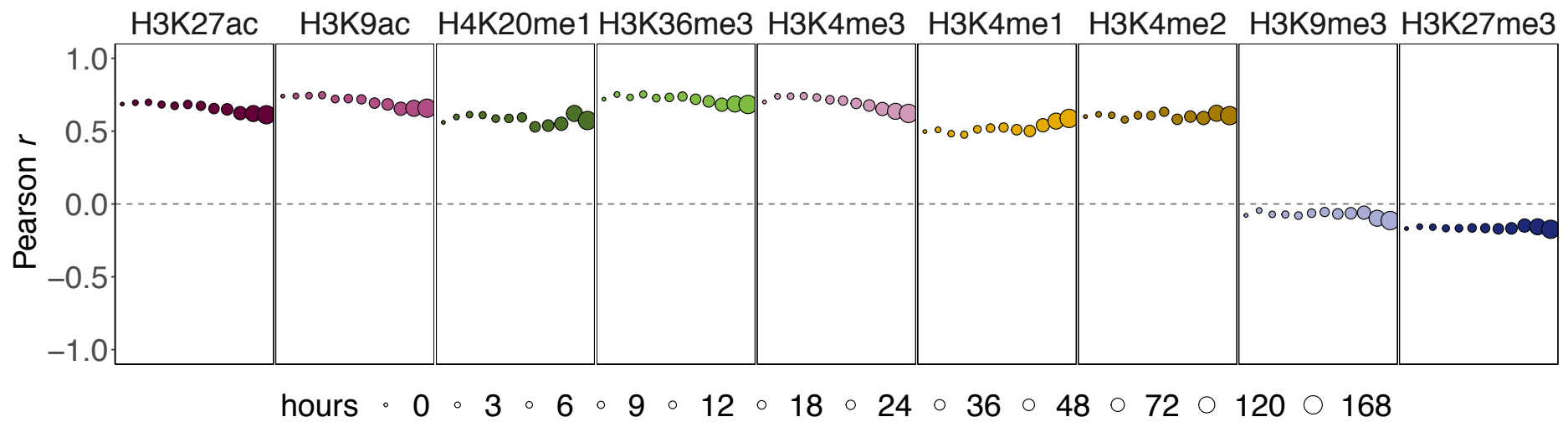


# Correlation between gene expression and histone modifications

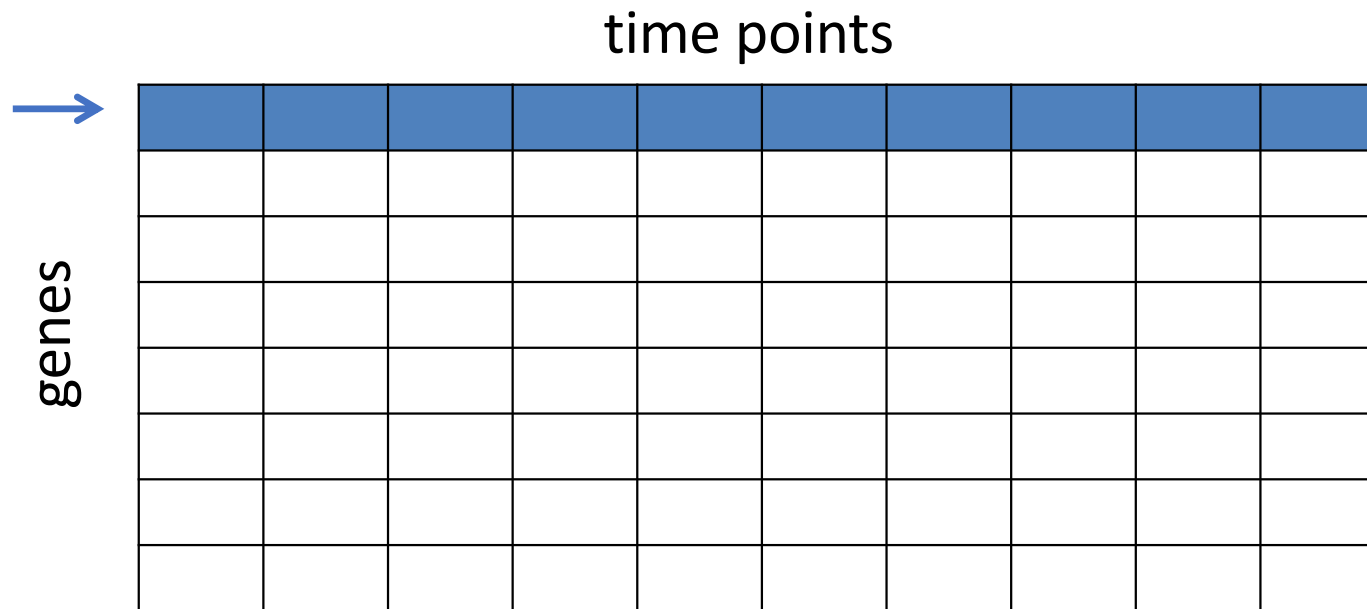


**Steady state correlations**

# Correlation between gene expression and histone modifications



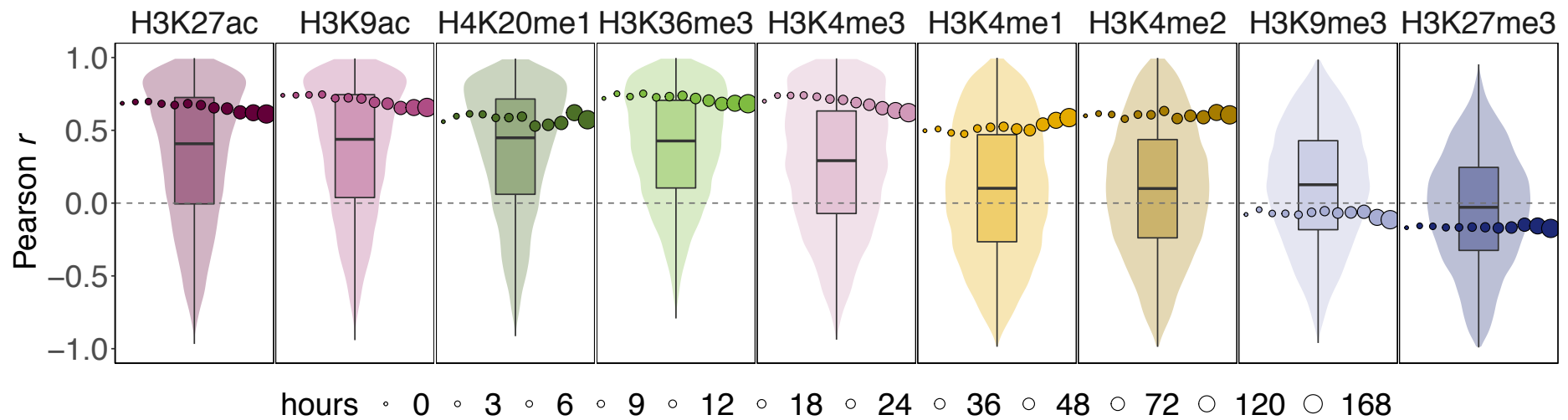
# Correlation between gene expression and histone modifications



**Time course correlations**



# Correlation between gene expression and histone modifications



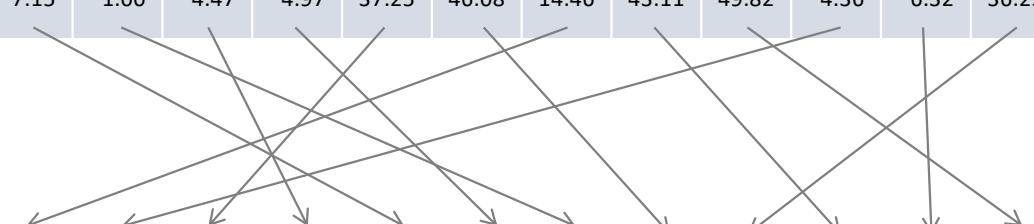
# Correlation between gene expression and histone modifications (randomization)

Gene X

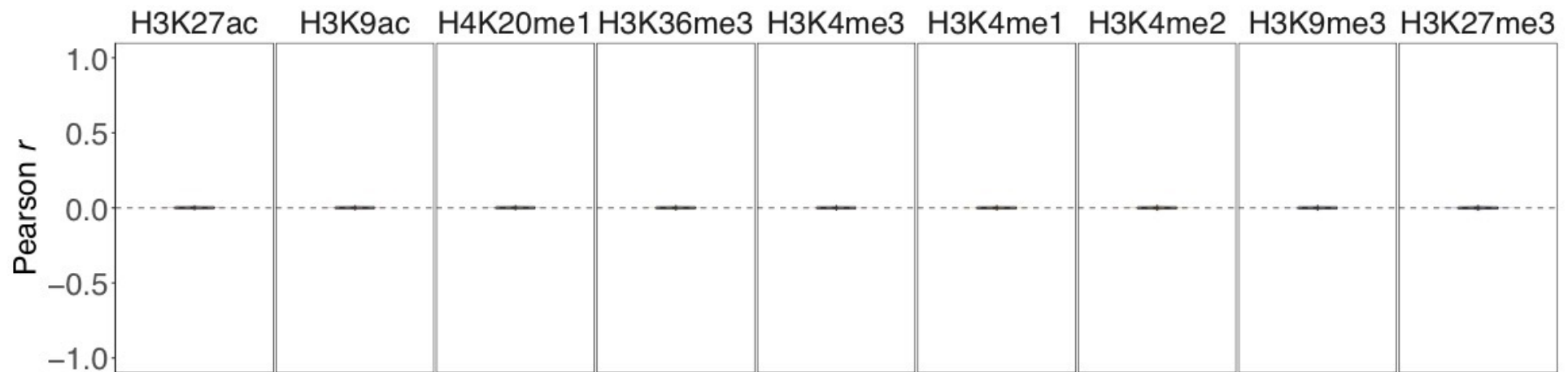
Time (hours)	0	3	6	9	12	18	24	36	48	72	120	168
Expression	109	2	4	11	98	483	1013	1562	2370	52	2273	1283
H3K4me3	7.15	1.00	4.47	4.97	37.25	46.08	14.46	43.11	49.82	4.36	6.32	36.25

randomization

H3K4me3	14.46	4.36	37.25	4.47	7.15	4.97	1.00	46.08	36.25	43.11	6.32	49.82
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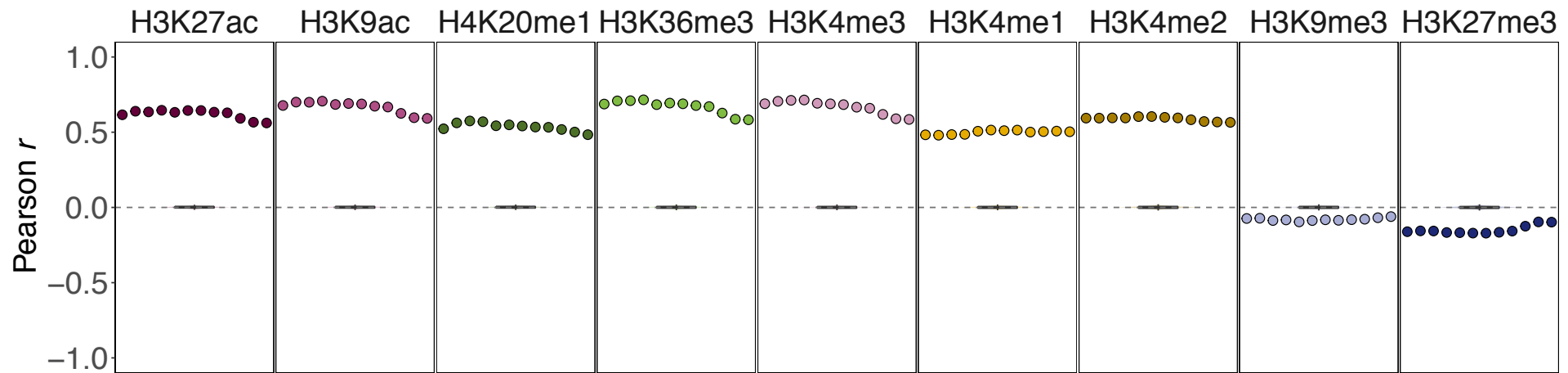


# Correlation between gene expression and histone modifications (after randomization)



**Time course correlations**

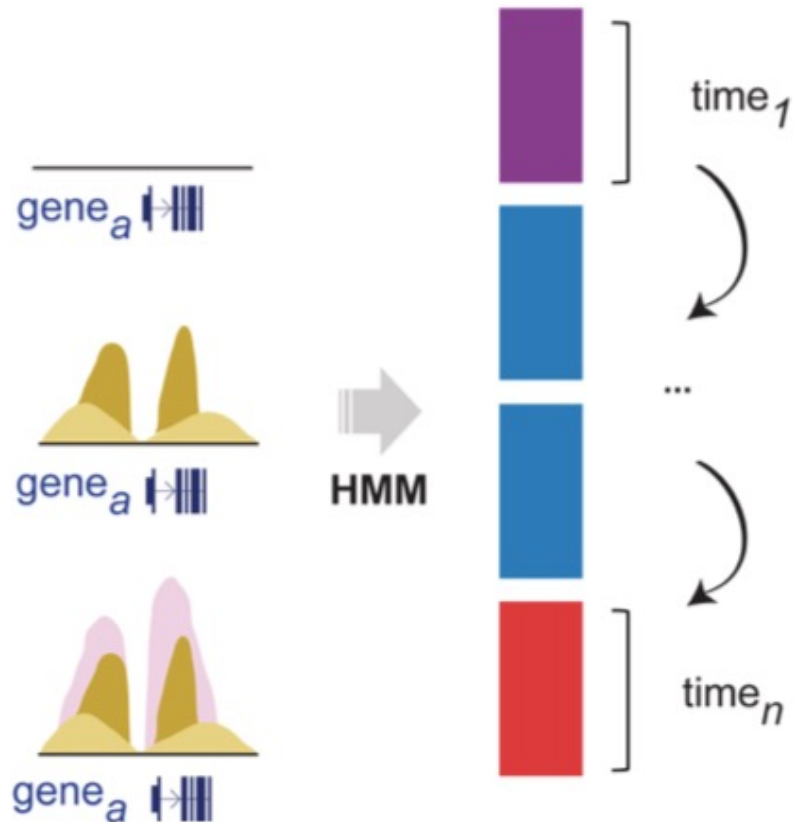
# Correlation between gene expression and histone modifications (after randomization)



**Steady state correlations**

# temporal HMMs

epigenome of gene<sub>a</sub> at time-points 1 ... n



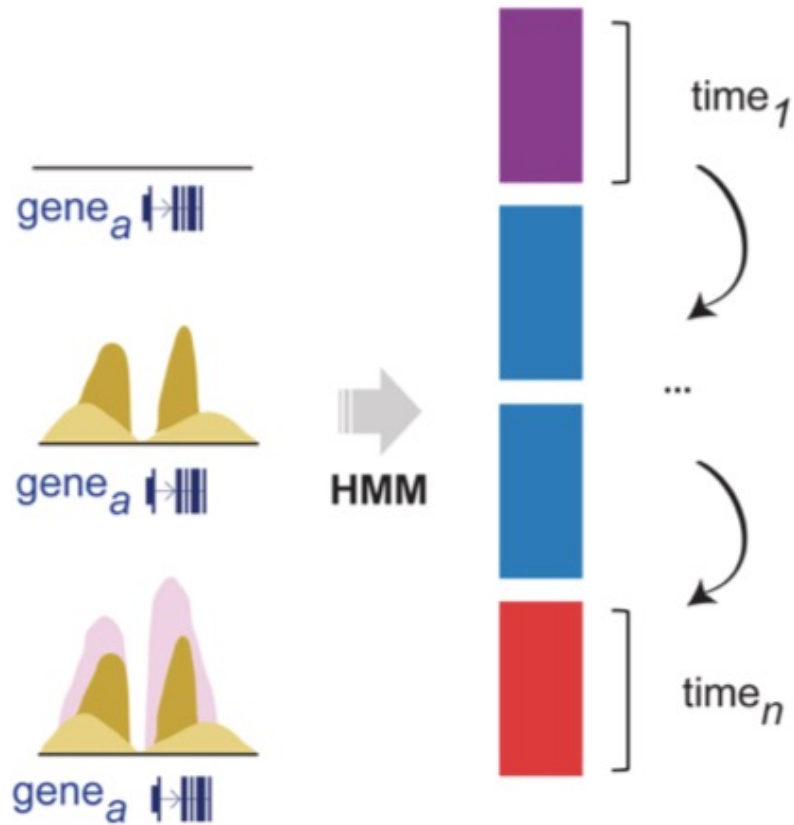
segmentation of time sequence  
(for a given gene)

Each gene at each time point is defined by a combination of histone modifications (**a state**).

The HMM identifies the set of states in which genes can be found and models the transition between states

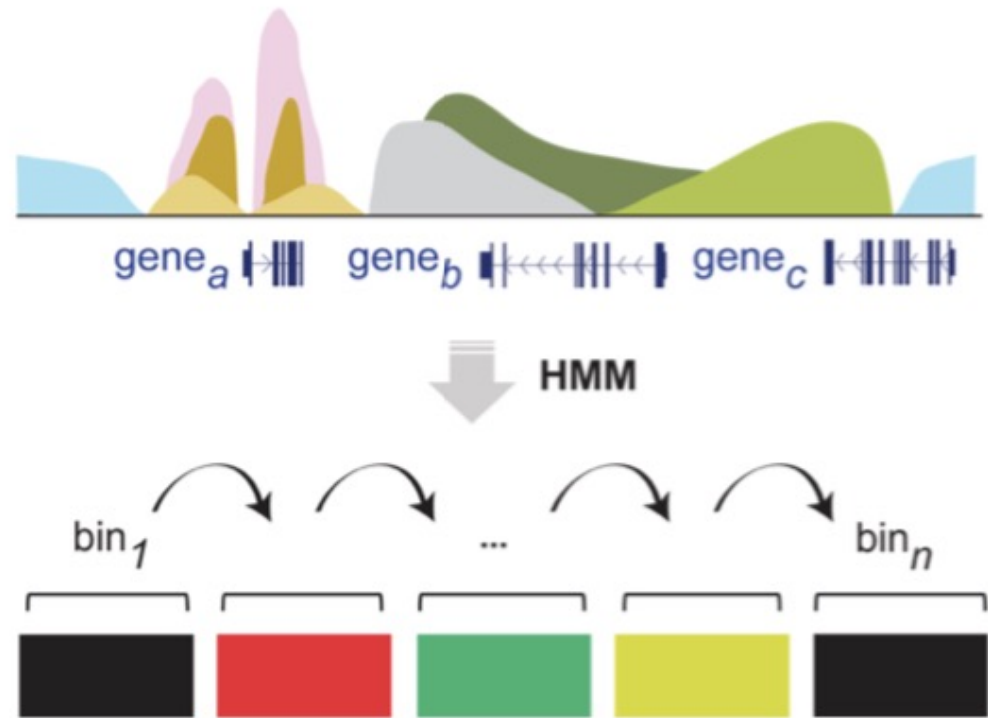
# temporal HMMs

epigenome of gene<sub>a</sub> at time-points 1 ... n



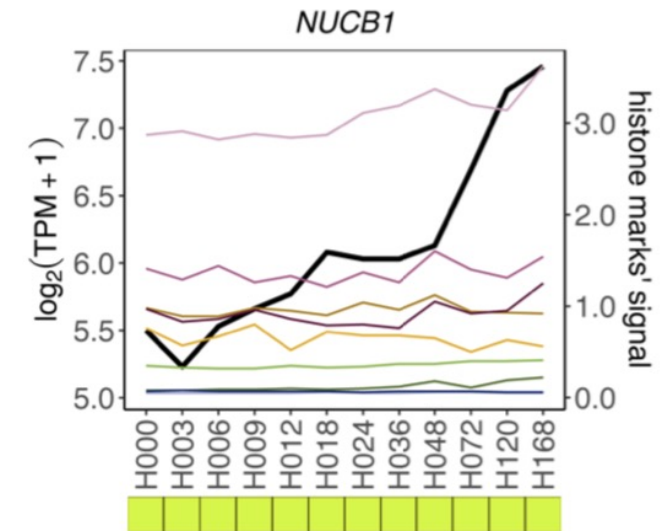
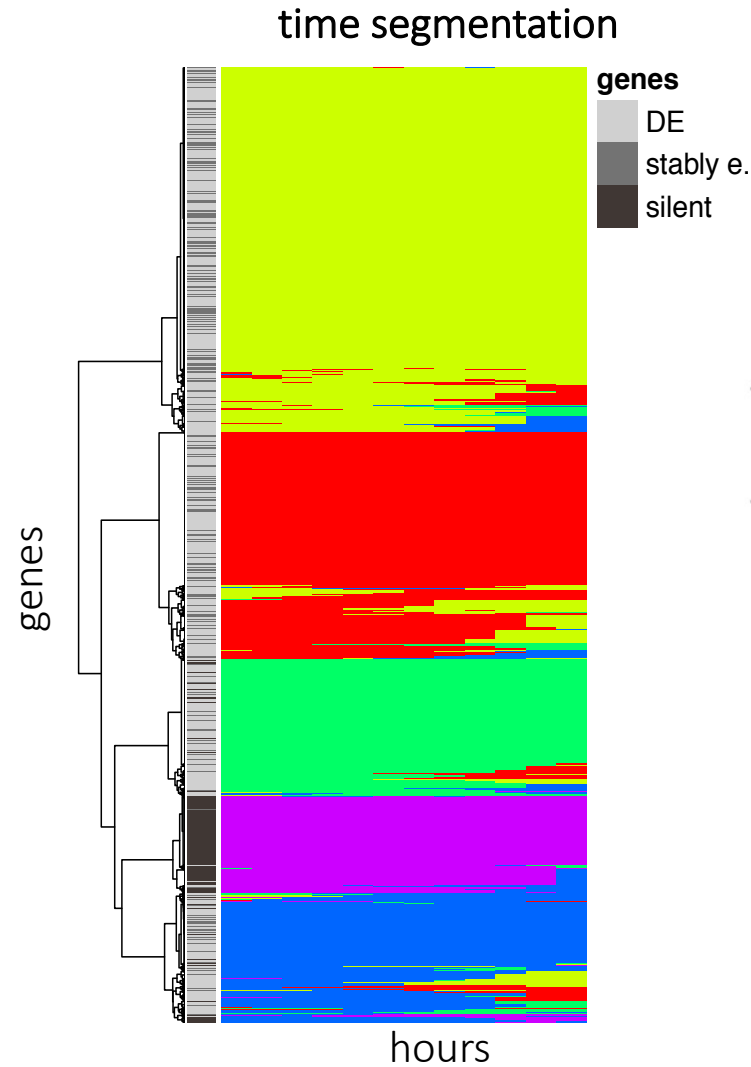
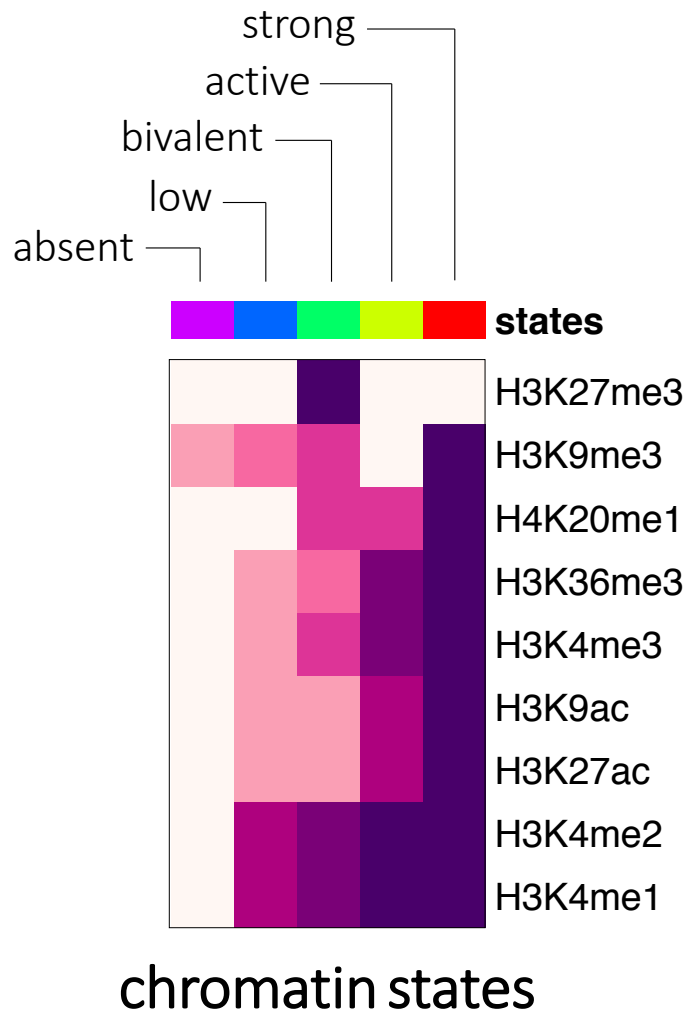
segmentation of time sequence  
(for a given gene)

whole epigenome at time-point n

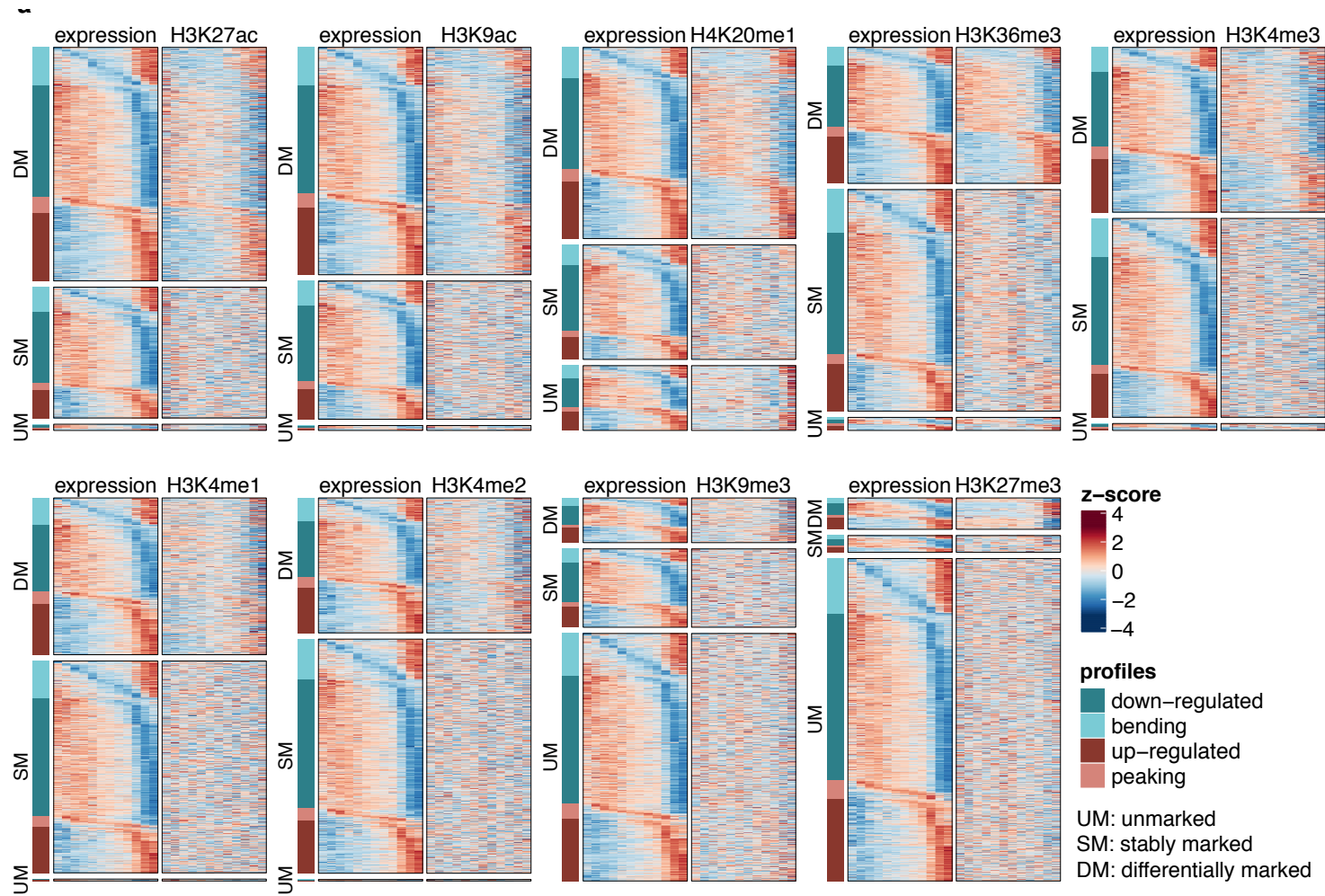


segmentation of genome sequence  
(for a given time-point)

# A limited number of major chromatin states

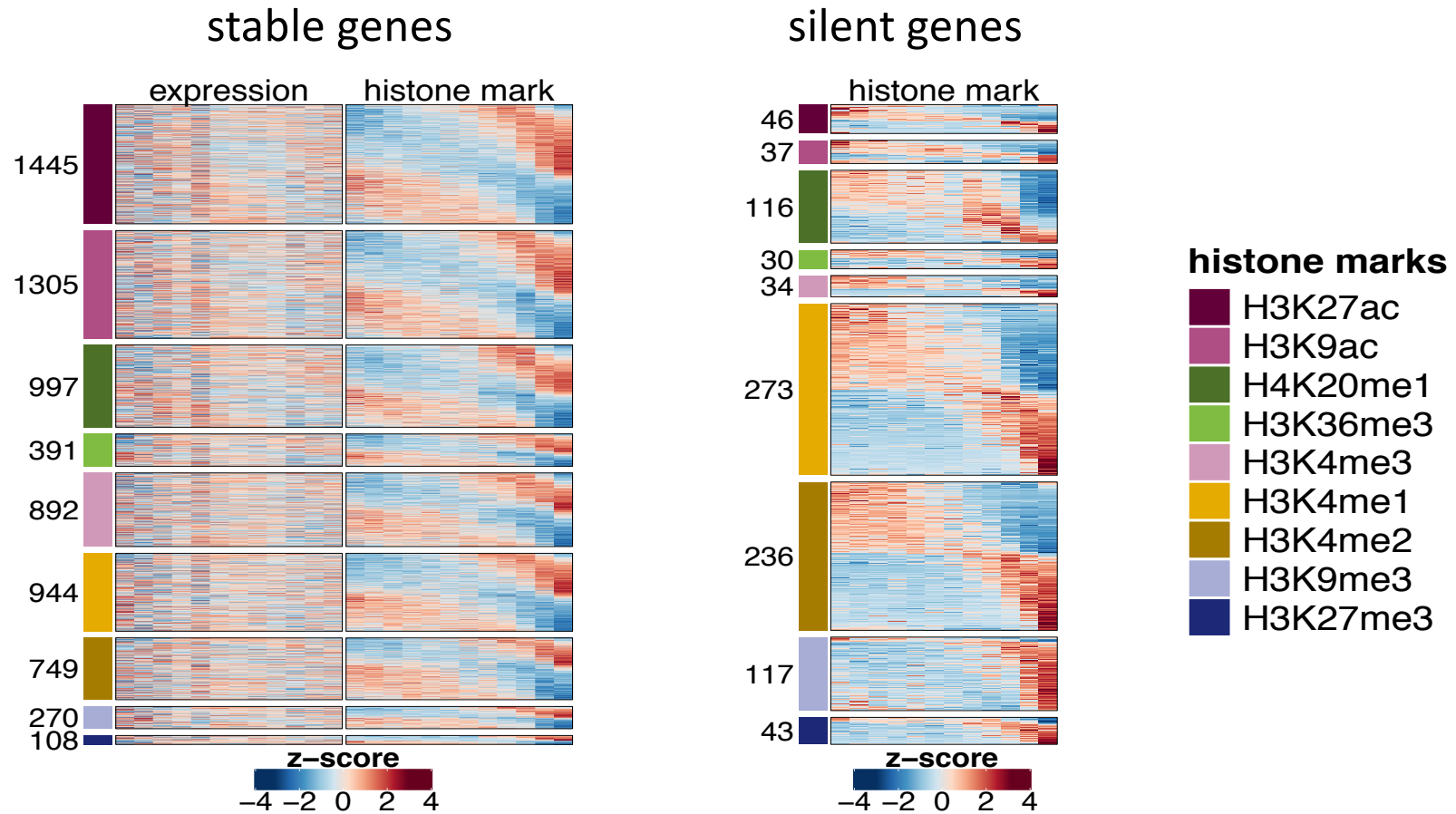


# Many differentially expressed genes are constantly marked

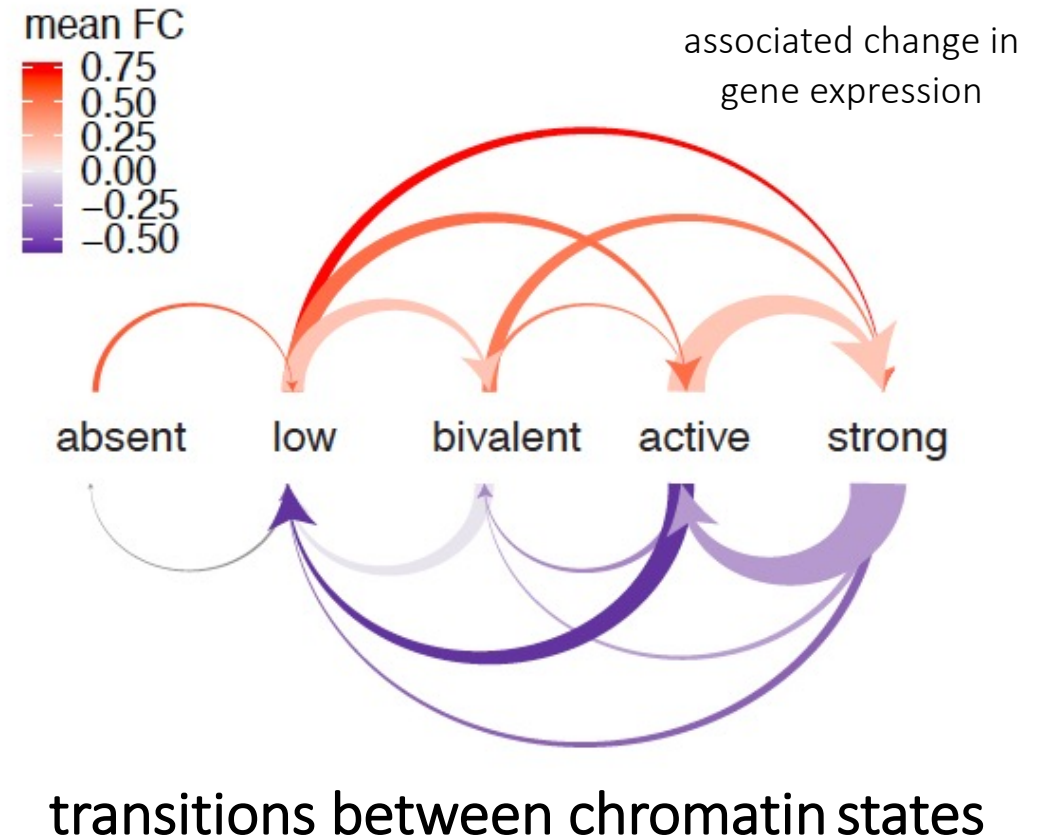
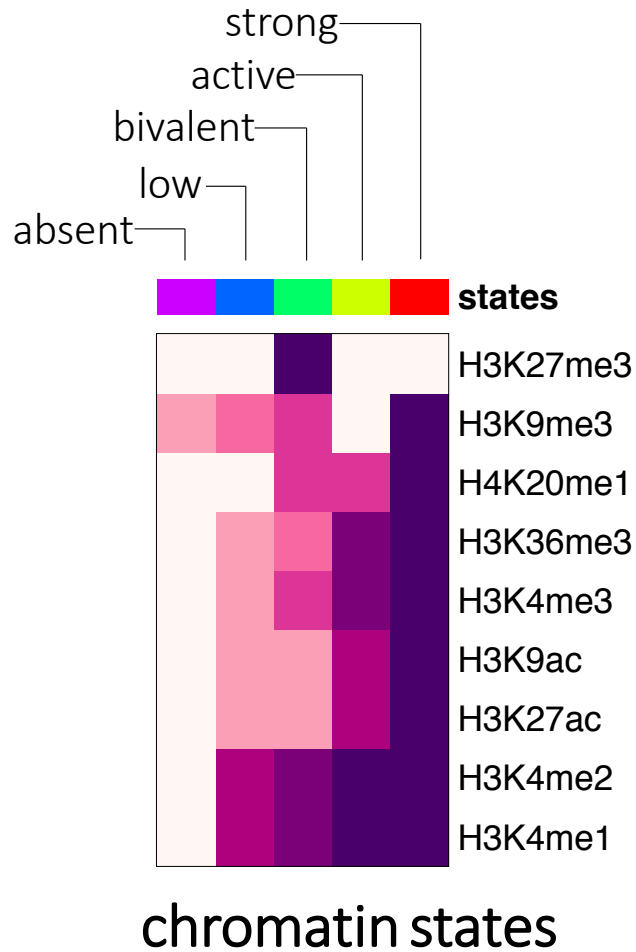




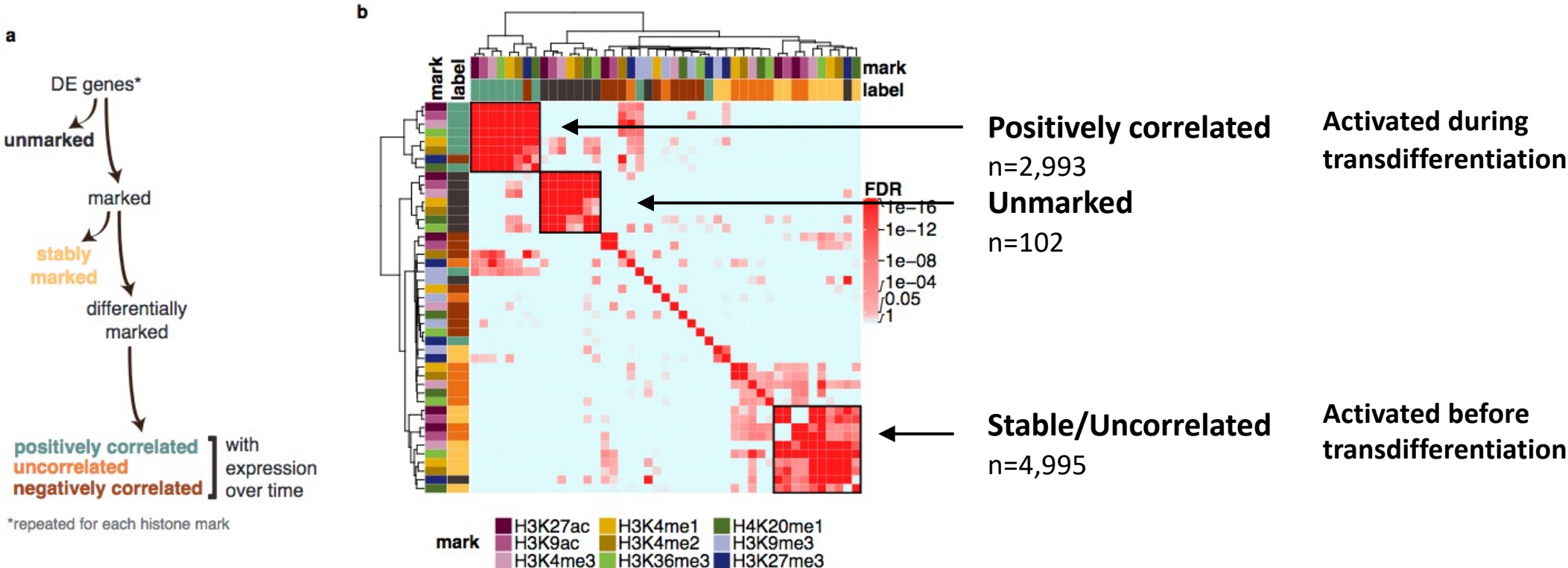
# Stable or silent genes may be differentially marked



# When changes in expression and chromatin occur they tend to be consistent

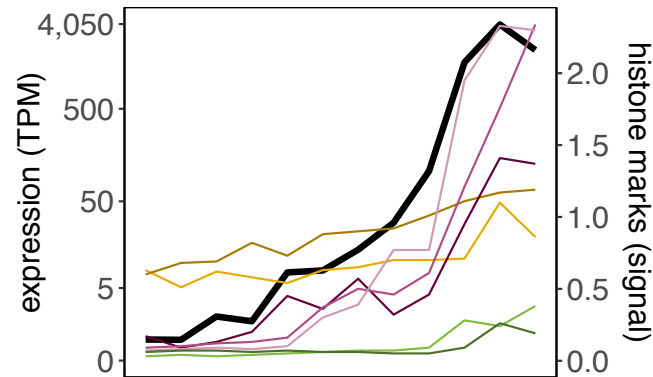
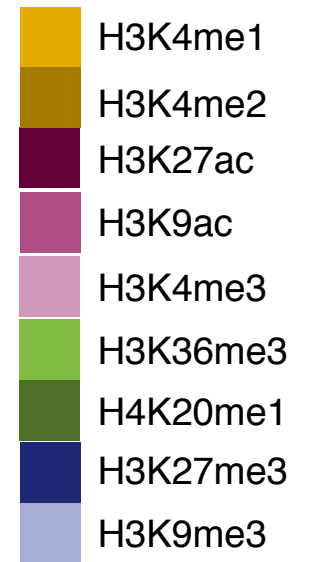
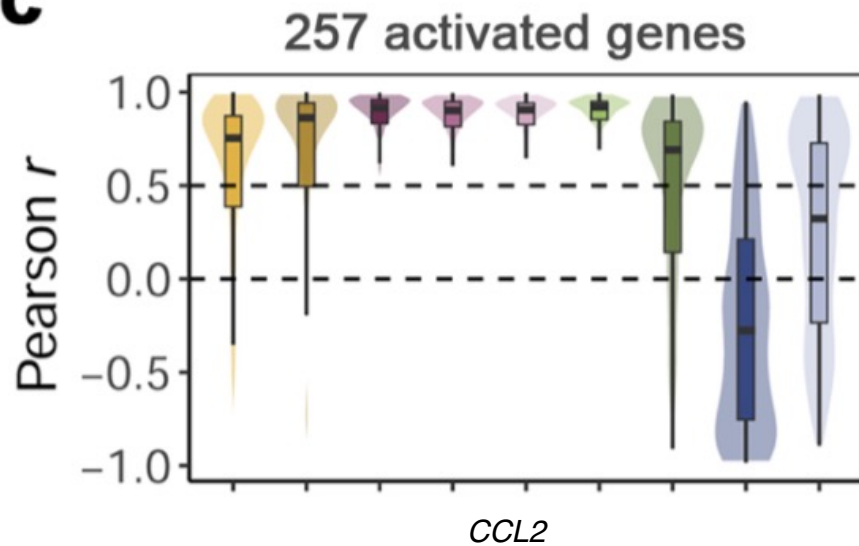


# Three main modes of association between gene expression and chromatin marking



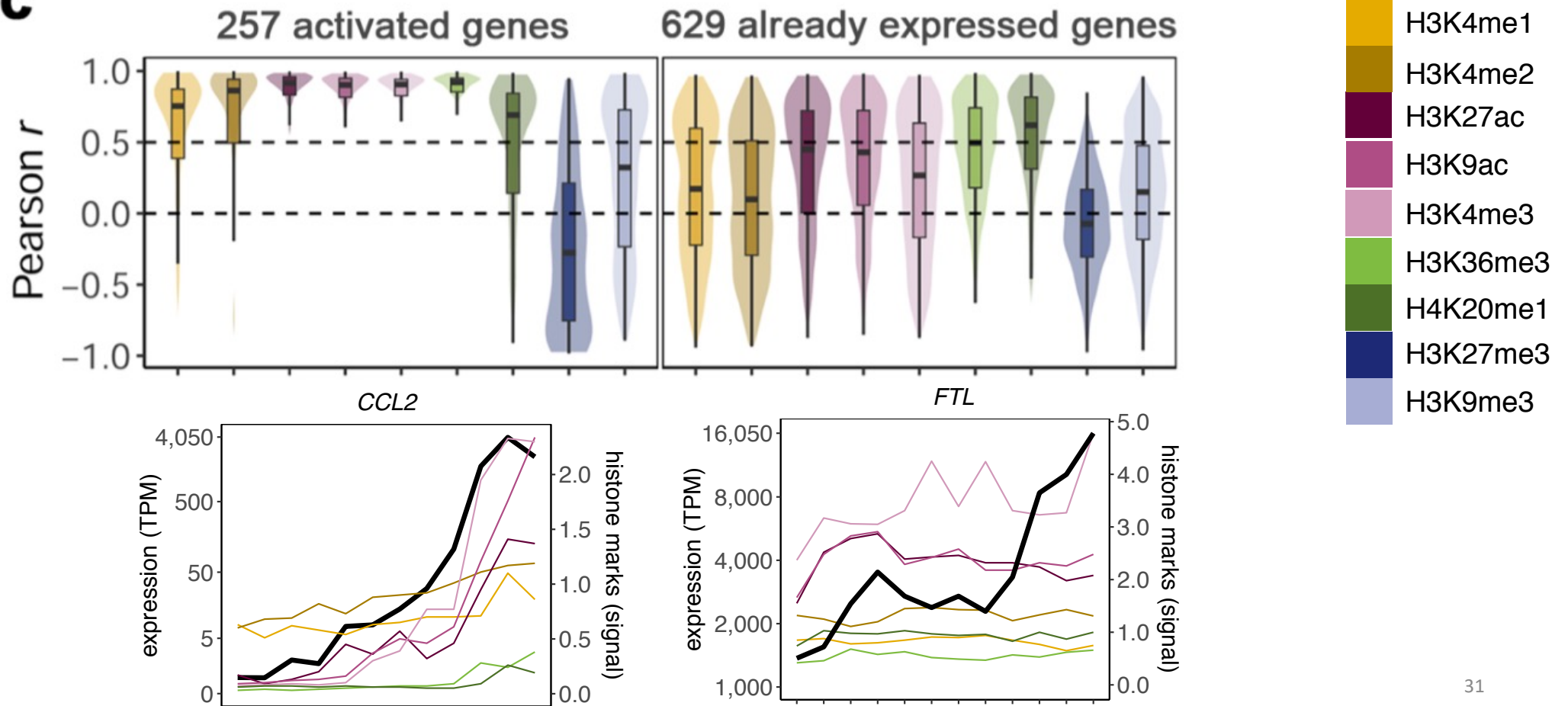
# Correlation between gene expression and chromatin marking is particularly strong at the time of gene activation

**c**

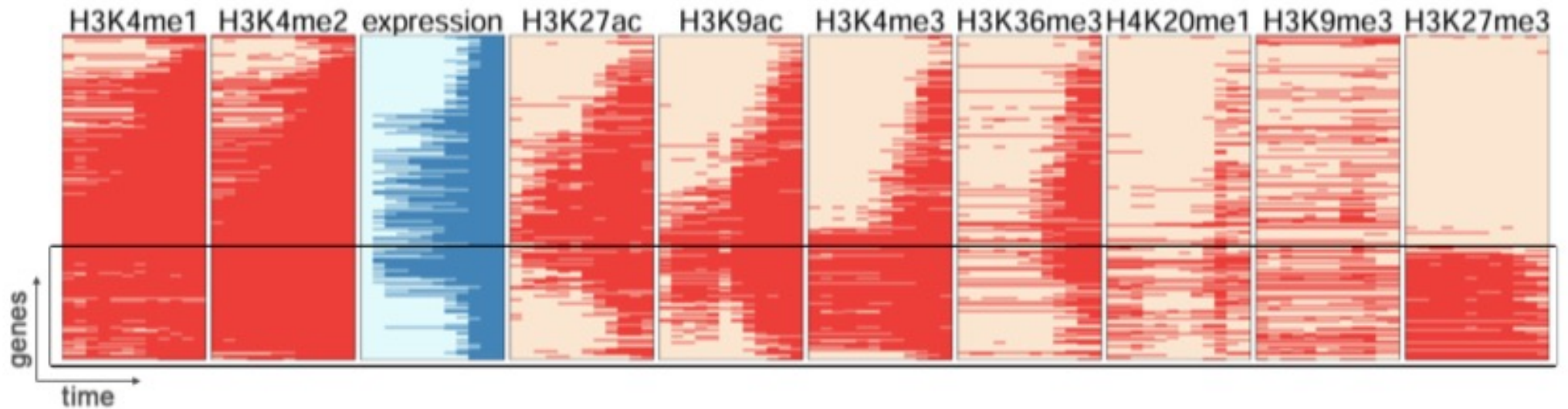


# Correlation between gene expression and chromatin marking is particularly strong at the time of gene activation

**c**



# Deciphering the order of chromatin mark deposition

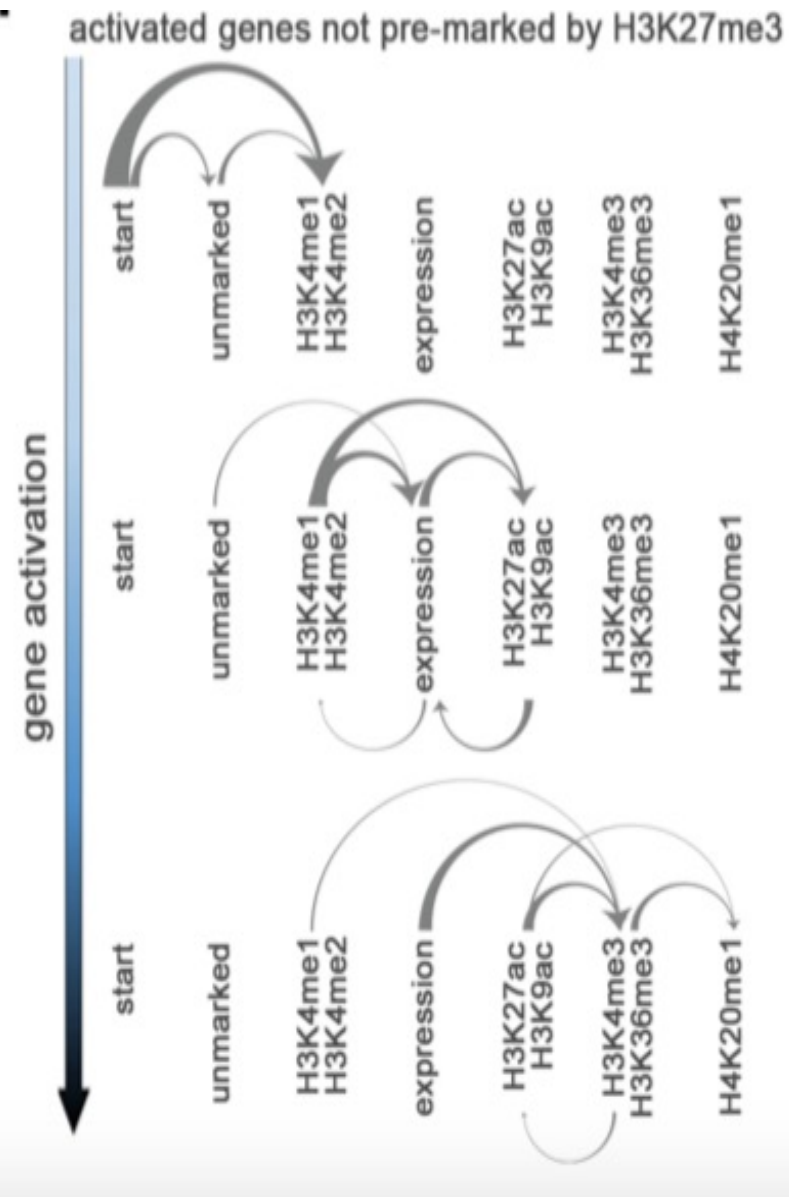


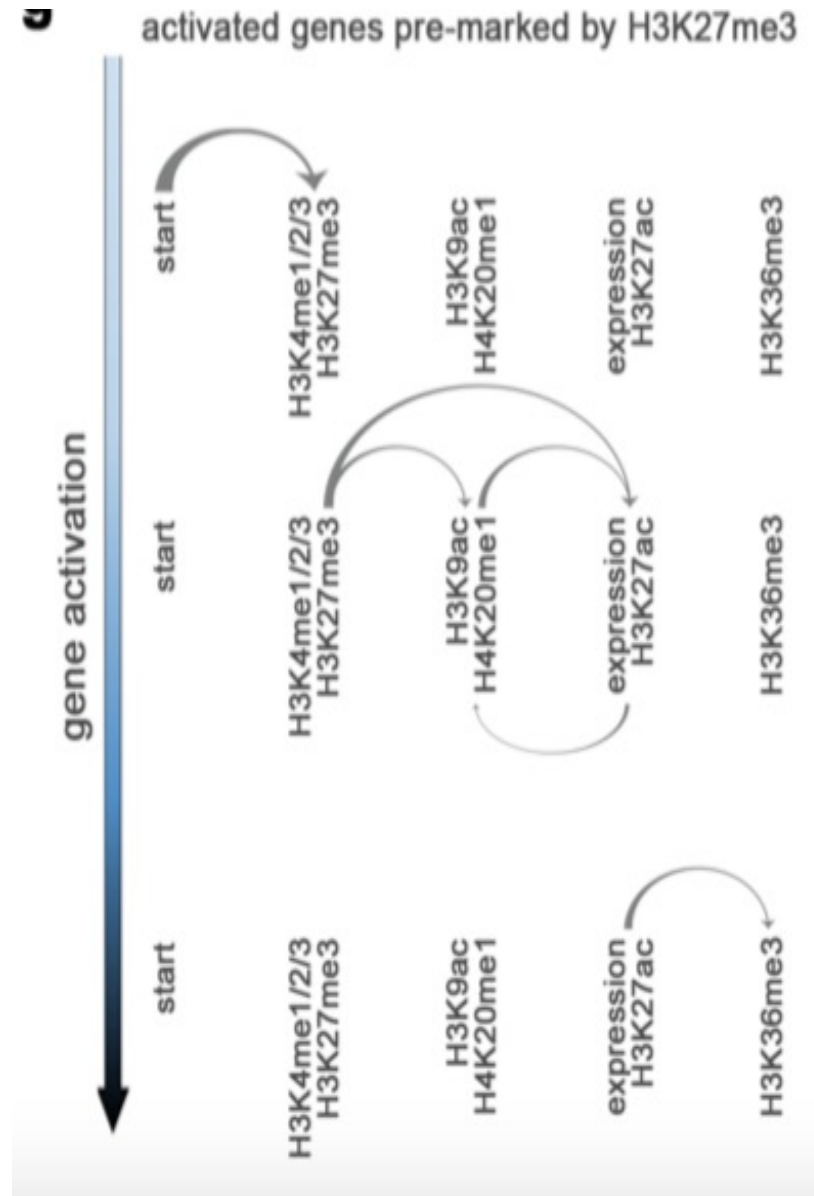
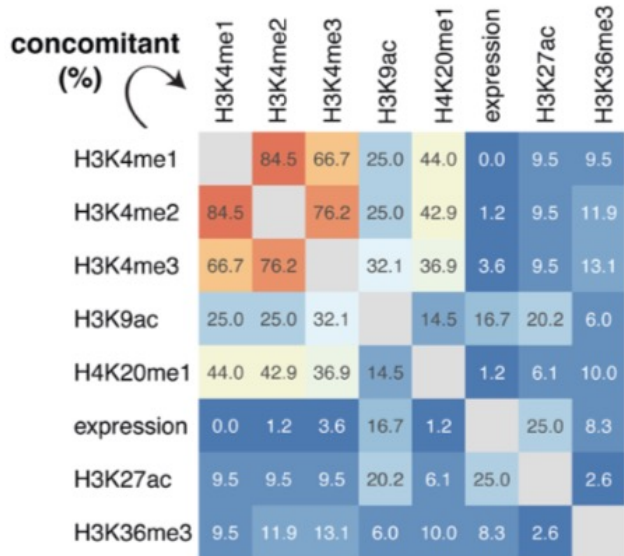
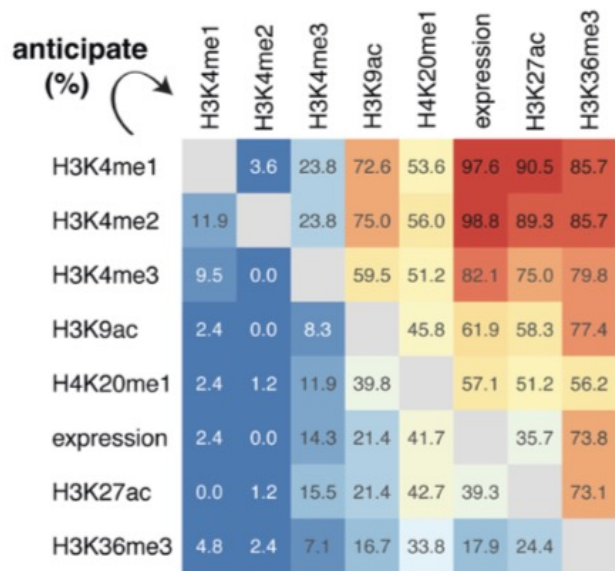
**anticipate (%)**

	H3K4me1	H3K4me2	expression	H3K27ac	H3K9ac	H3K4me3	H3K36me3	H4K20me1
H3K4me1		18.7	83.8	71.9	82.5	86.0	85.5	95.9
H3K4me2	5.3		83.2	69.6	83.0	86.0	85.5	95.3
expression	8.7	9.8		35.3	56.1	79.8	77.5	91.3
H3K27ac	4.1	6.4	46.2		59.7	72.3	69.5	87.2
H3K9ac	1.2	0.0	31.8	9.4		52.2	56.7	86.5
H3K4me3	1.8	0.0	15.0	11.3	15.7		38.4	80.3
H3K36me3	4.1	4.7	17.3	18.3	23.2	28.3		80.1
H4K20me1	0.6	0.6	6.9	4.5	5.8	7.5	6.0	

**concomitant (%)**

	H3K4me1	H3K4me2	expression	H3K27ac	H3K9ac	H3K4me3	H3K36me3	H4K20me1
H3K4me1		76.0	7.5	24.0	16.4	12.3	10.5	3.5
H3K4me2	76.0		6.9	24.0	17.0	14.0	9.9	4.1
expression	7.5	6.9		18.5	12.1	5.2	5.2	1.7
H3K27ac	24.0	24.0	18.5		30.8	16.4	12.2	8.3
H3K9ac	16.4	17.0	12.1	30.8		32.1	20.1	7.7
H3K4me3	12.3	14.0	5.2	16.4	32.1		33.3	12.2
H3K36me3	10.5	9.9	5.2	12.2	20.1	33.3		13.9
H4K20me1	3.5	4.1	1.7	8.3	7.7	12.2	13.9	





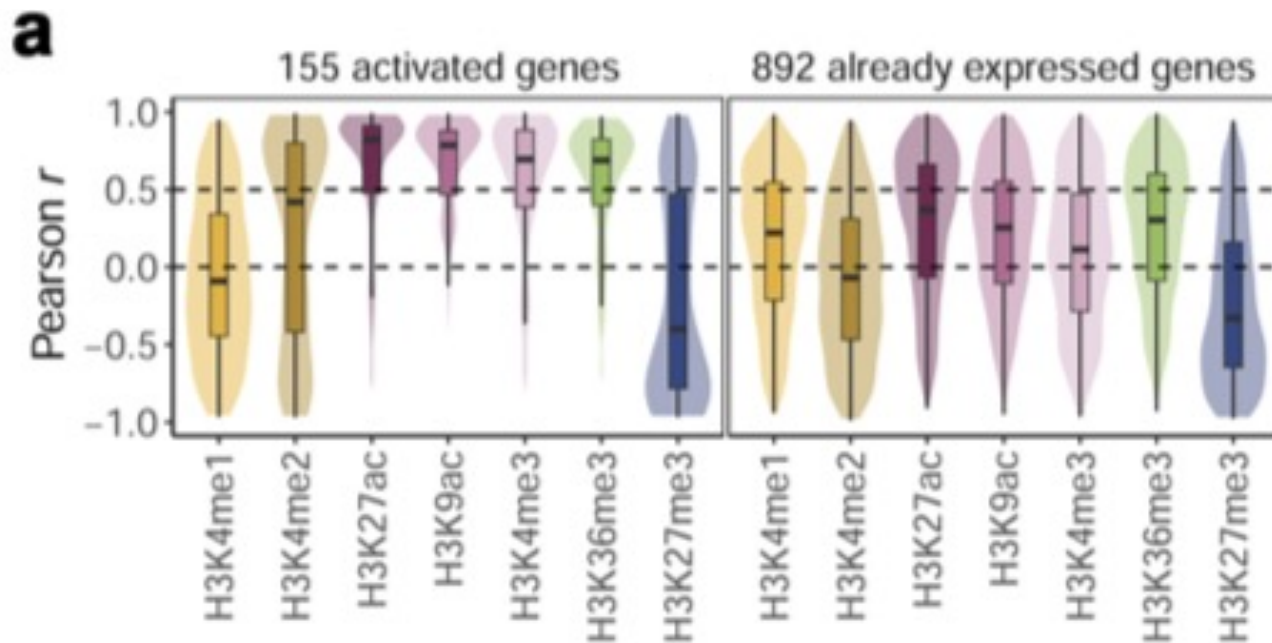


# Mouse developmental models recapitulate chromatin dynamics at gene activation

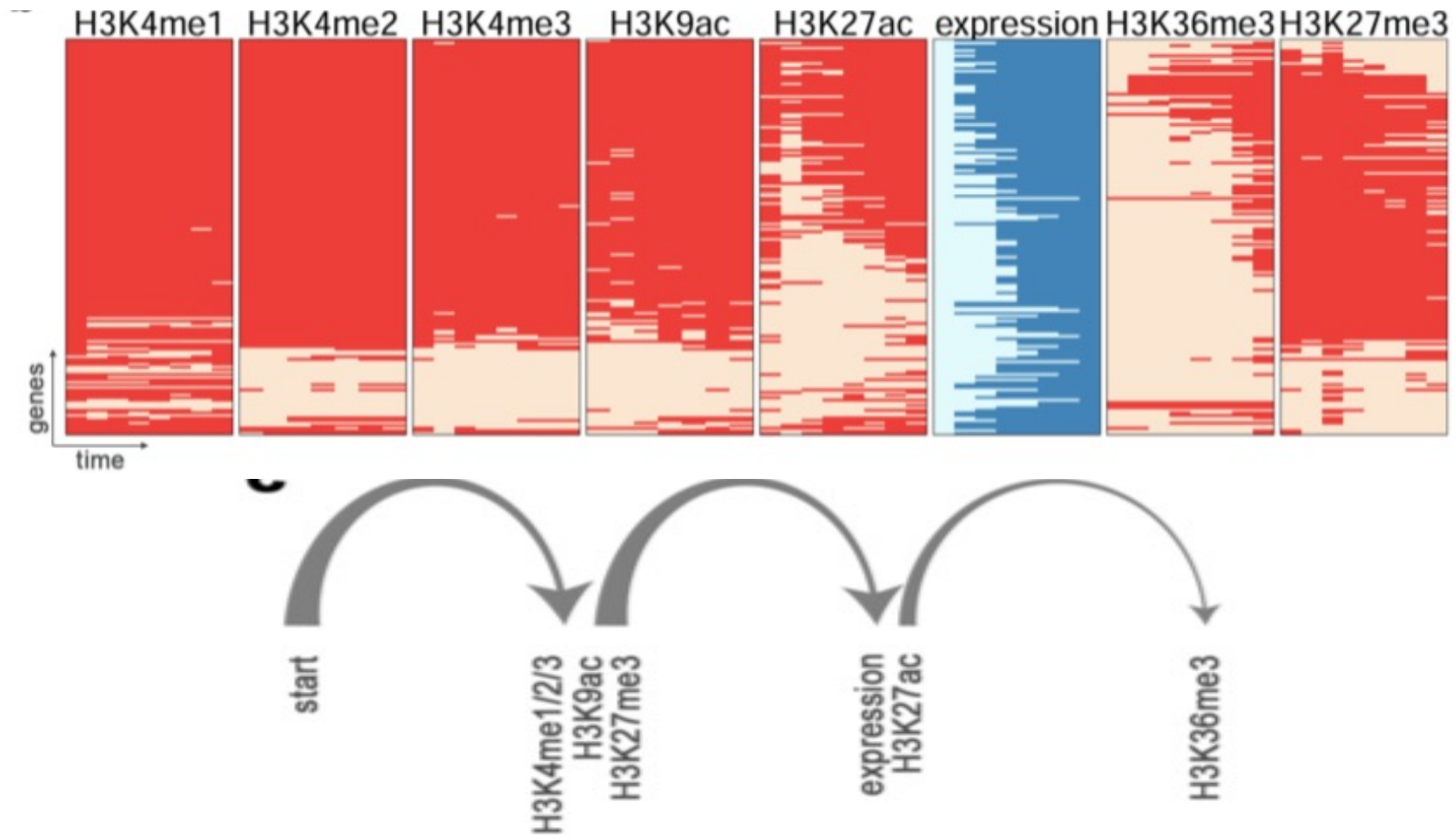
Gorkin, D. U. *et al. Nature* **583**, 744–751 (2020).

He, P. *et al. Nature* **583**, 760–767 (2020).

Seven histone modifications; Eight time points



# Mouse developmental models recapitulate chromatin dynamics at gene activation

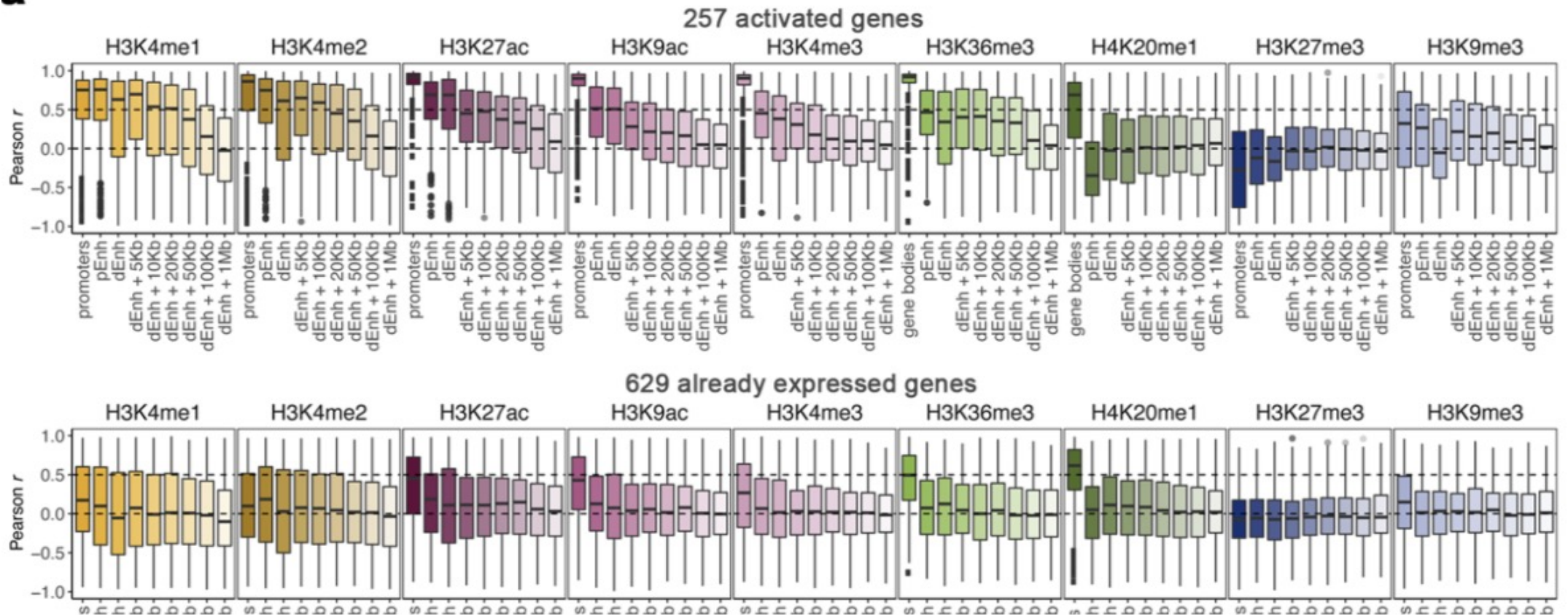


# Chromatin marking at enhancers

- **Putative proximal enhancers**  
from -5 Kb to -2 Kb upstream the first TSS of each gene
- **Distal enhancers. Activation By Contact (ABC)**  
enhancer-promoter pairs  
between 5 Kb and 1 Mb away from the gene's TSSs  
identified in any B-cell and/or macrophage lineages

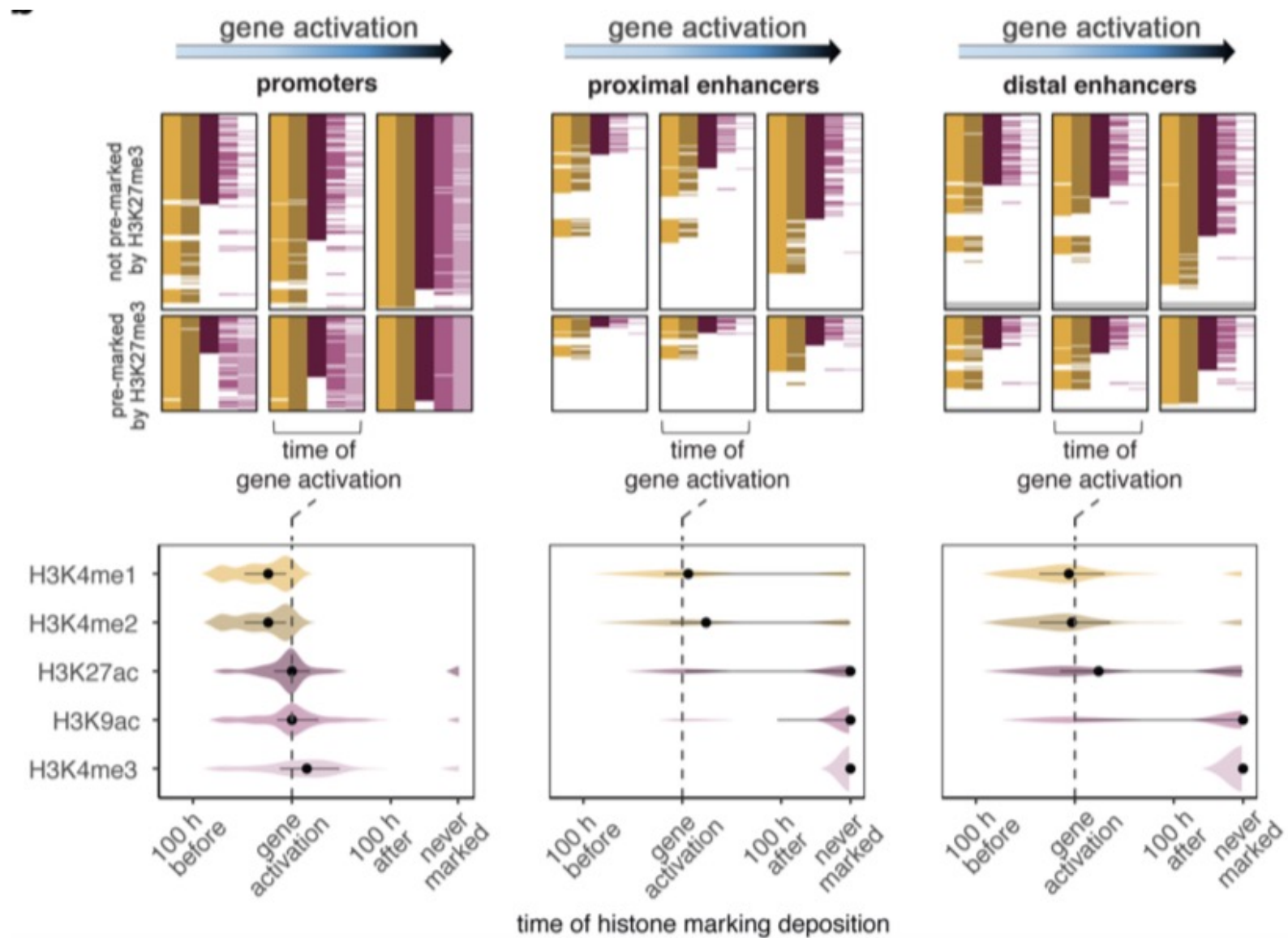
positive correlation between expression and active chromatin marking at enhancers at the time of activation strong effect of a broad epigenetic environment

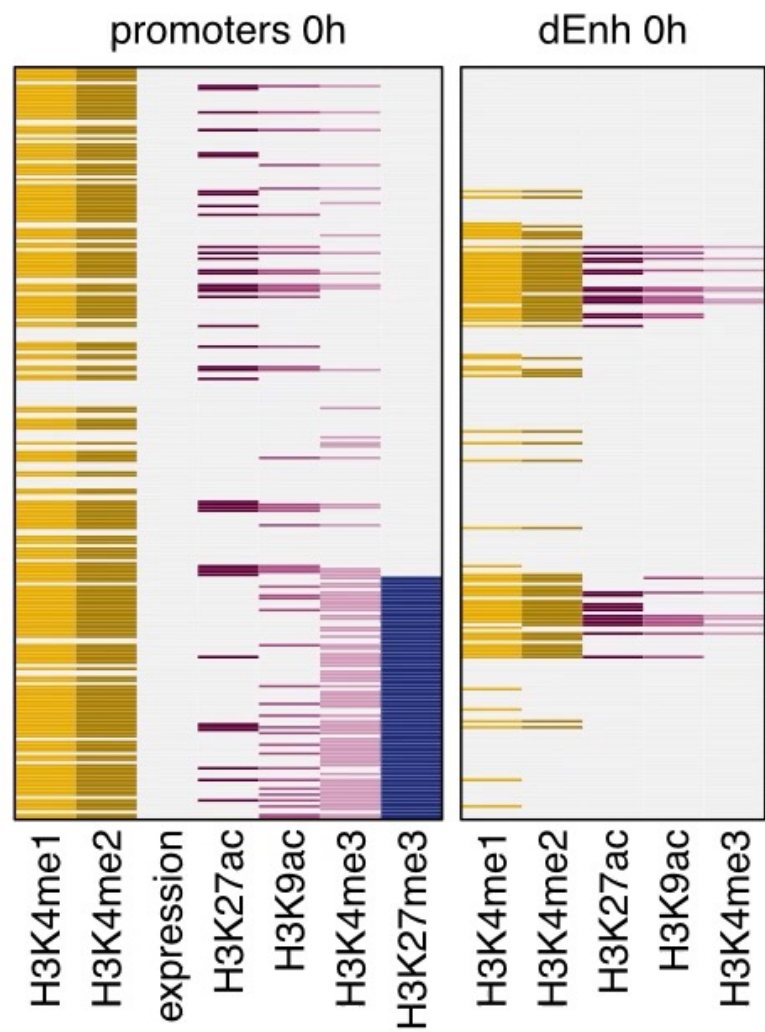
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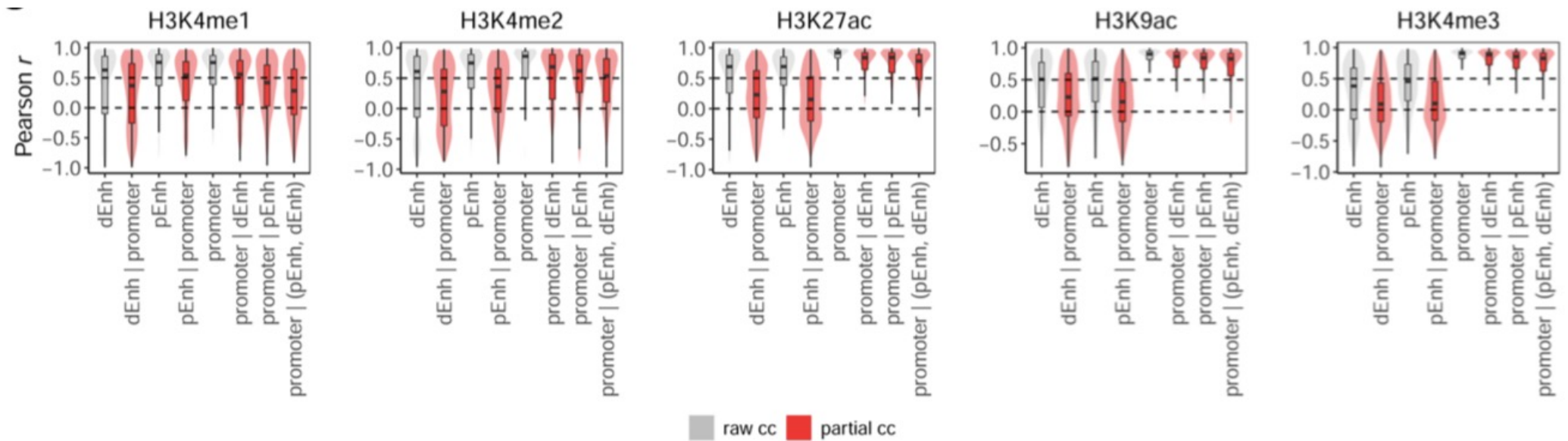
Chromatin marking at enhancers follows marking at promoters

257 genes specifically activated during transdifferentiation





# Association between marking at enhancers and expression is mostly mediated by marking at promoters



# Conclusions

- **The overall association between gene expression and chromatin marking is weaker than previously reported**
  - In some cases (H3K9me3) runs in the opposite direction than described
  - Many changes in gene expression occur without associated changes in chromatin marking (and vice versa)
- **There are a limited number of major chromatin states**
  - genes tend to remain in the same state during transdifferentiation, irrespective of changes in gene expression
- **There is strong association between histone modifications and gene expression only at the time of initial activation**
  - Further changes in gene expression, even larger than those occurring at gene activation, are essentially uncoupled from changes in histone modifications.
- **At the time of gene activation, there is a preferential order of marking**
  - H3K4me1 and H3K4me2 **precede** gene activation
  - H3K27ac and H3k9ac **occur with** gene activation
  - H3K4me3, H3k36me3 and H4k20me1 **follow** gene activation
- **Marking at enhancers generally follows marking at promoters, and it is not directly associated with gene expression**



# Acknowledgements

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Bruna R. Correa

Rory Johnson

Sílvia Pérez-Lluch

Beatrice Borsari



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