Bioinformatic approaches to regulatory genomics and epigenomics

376-1347-00L | week 10

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Plan for today

- Theory: from repression to activation and back
- Clustering on genomic signals
- Region-based GO enrichment analysis

There are degrees of accessibility (activation/repression)

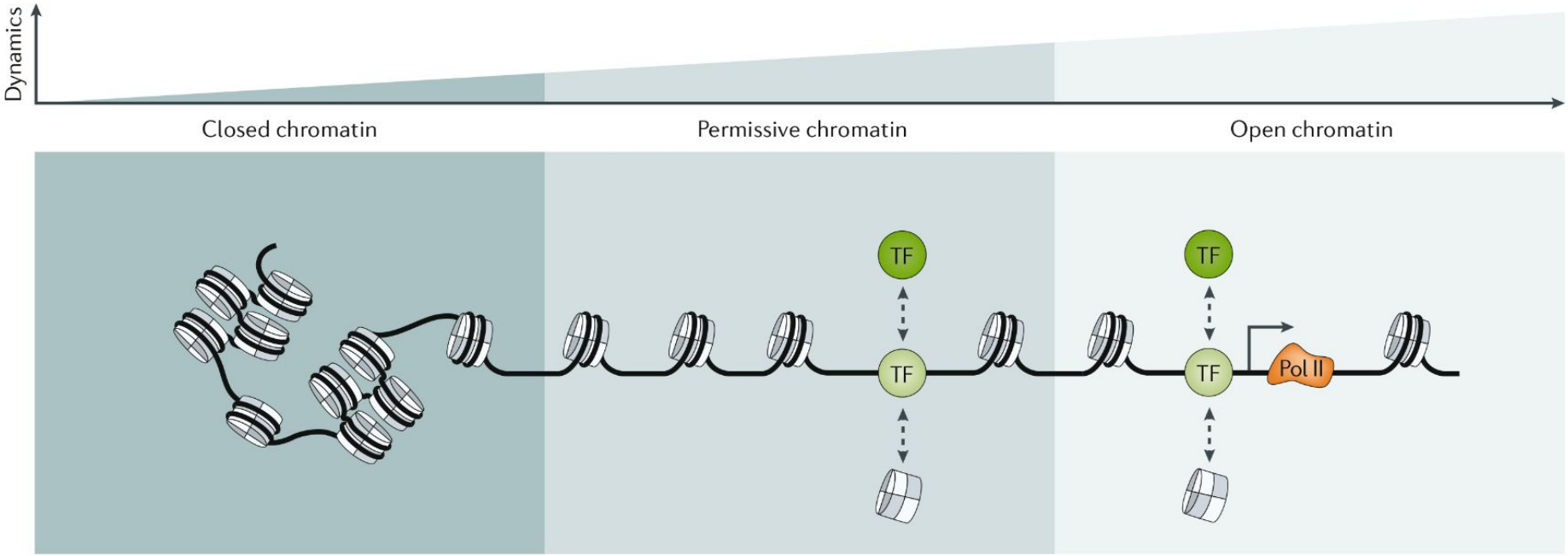
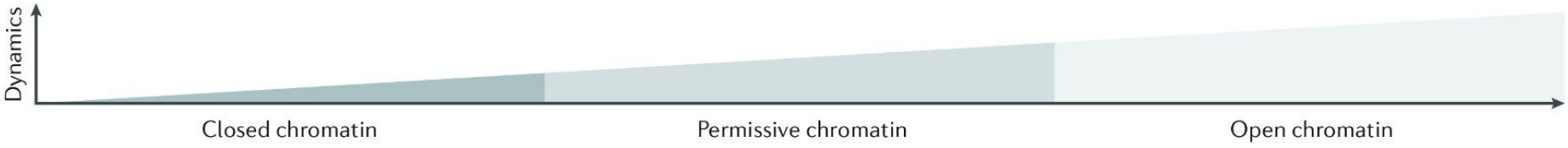


Fig. 1 | **A continuum of accessibility states broadly reflects the distribution of chromatin dynamics across the genome.** In contrast to closed chromatin, permissive chromatin is sufficiently dynamic for transcription factors to initiate sequence-specific accessibility remodelling and establish an open chromatin conformation (illustrated here for an active gene locus). Pol II, RNA polymerase II; TF, transcription factor.

(Klemm, Shipony and Greenleaf, 2019)

There are degrees of accessibility (activation/repression)



Heterochromatin (stable repression)

Very low accessibility

HP1 binding

DNA methylation

H3K9 methylation

?

Poised/silenced

Low accessibility

H3K27 methylation

Active elements

Very high accessibility

RNApol2 binding

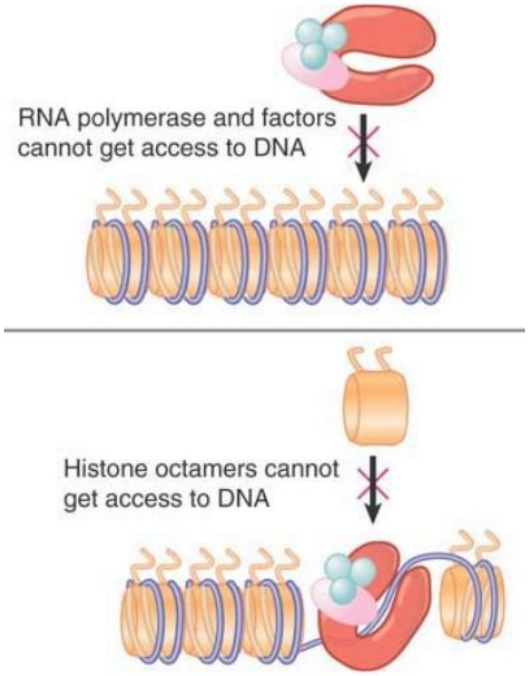
H3K4 methylation

H3K27 acetylation

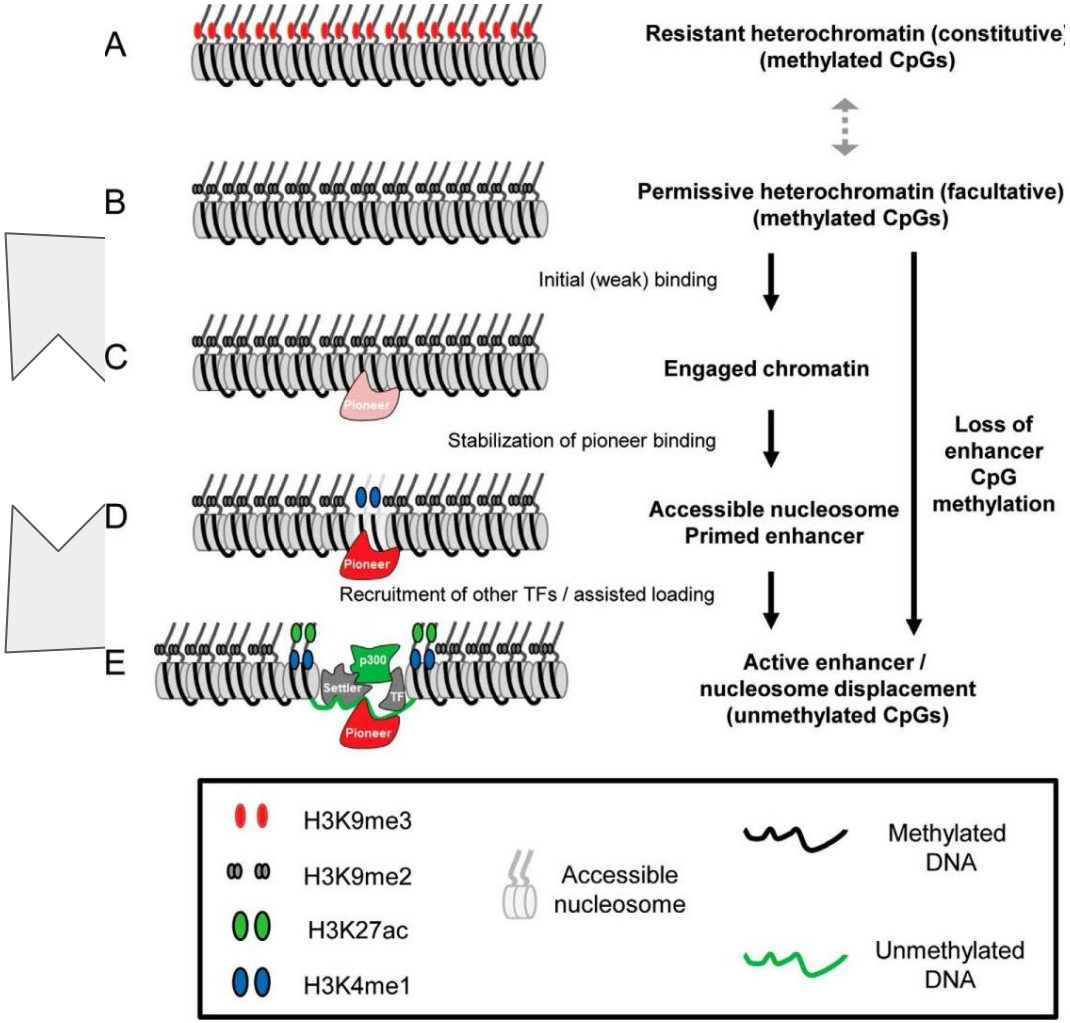
Fate transitions (e.g. differentiation)

Transient responses

Opening chromatin



(Adapted from Krebs, Goldstein and Kilpatrick, Genes XII, 2018)



(Mayran and Drouin, J Biol Chem 2018)

Whereas most TFs cannot bind their target DNA when it's wrapped around nucleosomes, **pioneer factors** can, and can even bind in heterochromatin

Their binding is typically (but not always) independent of (and prior to) other factors

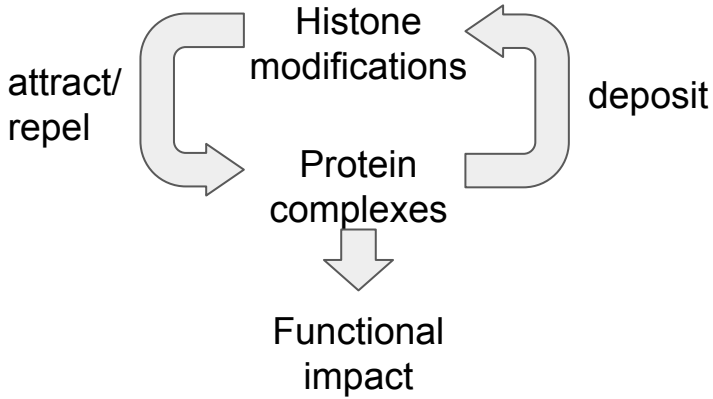
Factor	Binding to heterochromatin	Chromatin activation	Epigenetic memory: DNA demethylation	Cell fate reprogramming	Nucleosome binding
Ascl1/Mash1	102	102		102, 103	
C/EBPα		43		104	
Ebf1	47, 48	47, 48	48		
Esrrb					
Foxa	3	3, 4, 28, 31, 32	4, 69, 85	38, 39	28
Gata	59	59		38, 39	29
GR/AR	18	18			
Klf4	21, 22	21, 22		106, 107	53
Neurod1				50, 70	
Nrf1	70	70	Inhibitory (70)		
Oct4	21, 22	21, 22		106, 107	53
p53	100, 101	100, 101			
Pax7	44, 45	44, 45, 61	45	44	
PU.I	41, 42	41, 42		104	
Sox2	21, 22	21, 22		106, 107	53

(Adapted from Mayran and Drouin, J Biol Chem 2018)

Opening chromatin: pioneering factors or epigenetic mark?

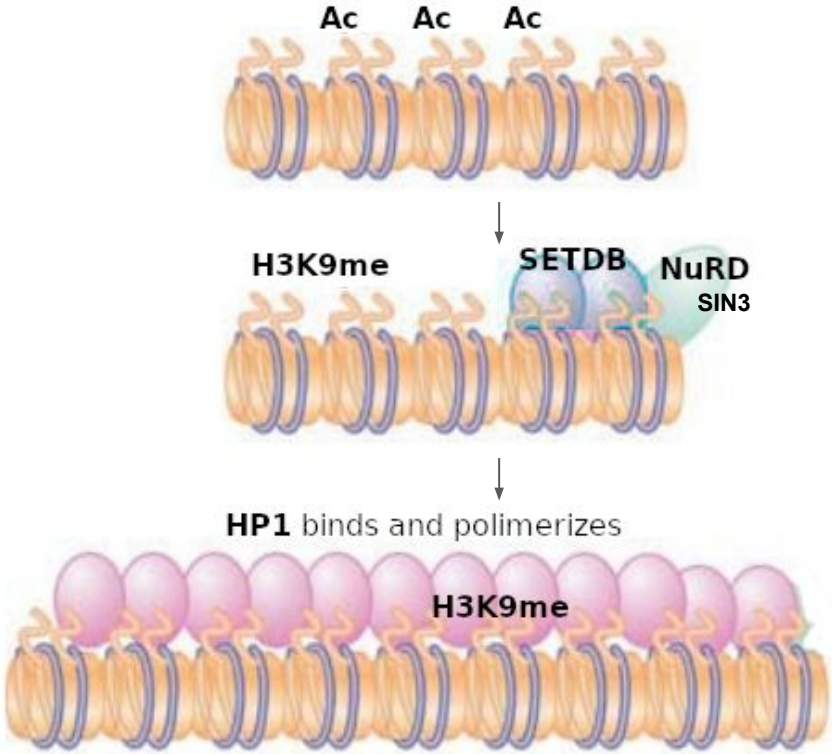
- blocking H3K27ac does not prevent pioneering factors (PF) from opening chromatin
- blocking the PF typically prevents H3K27ac to most sites
- PF binding doesn't always lead to opening, and sometimes require other factors

(Miao et al., Molecular Cell 2022)



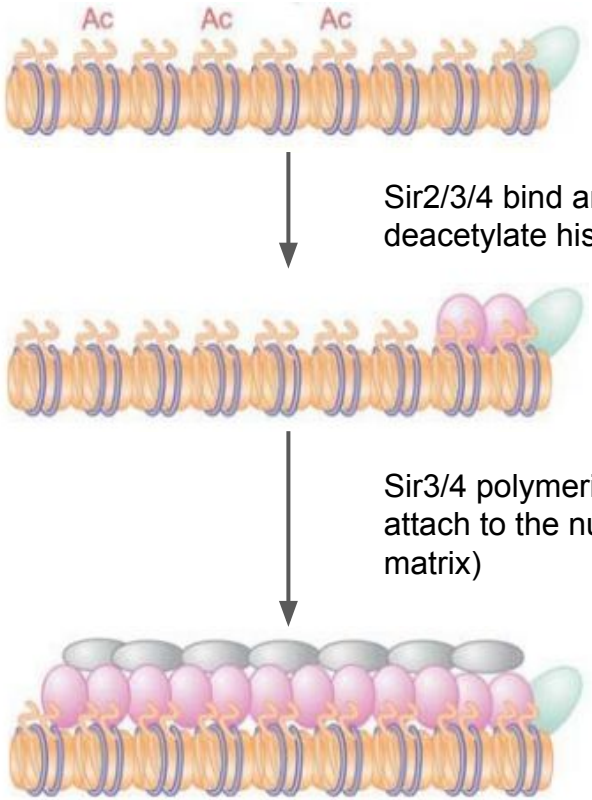
Heterochromatin formation

In mammals:



In yeast:

Rap1 binds to DNA

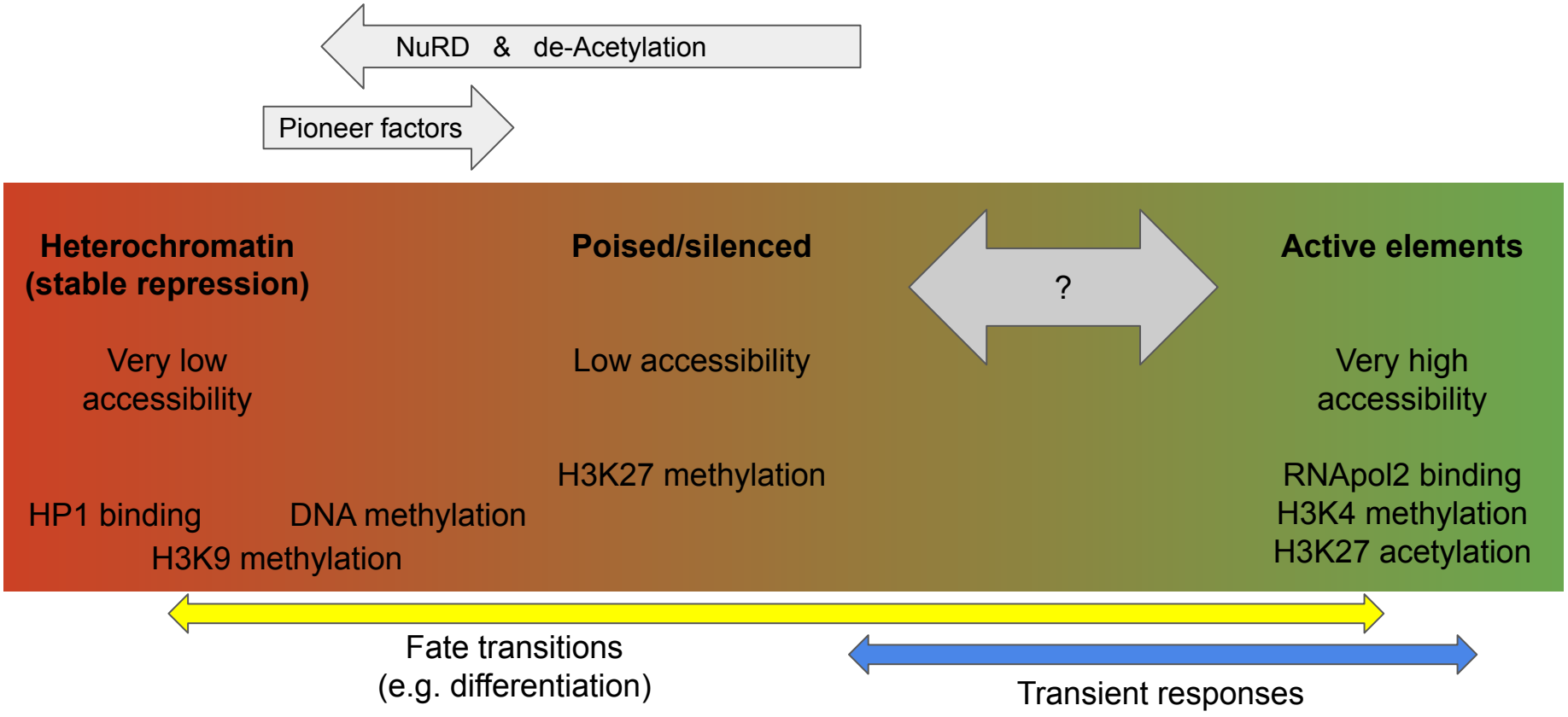


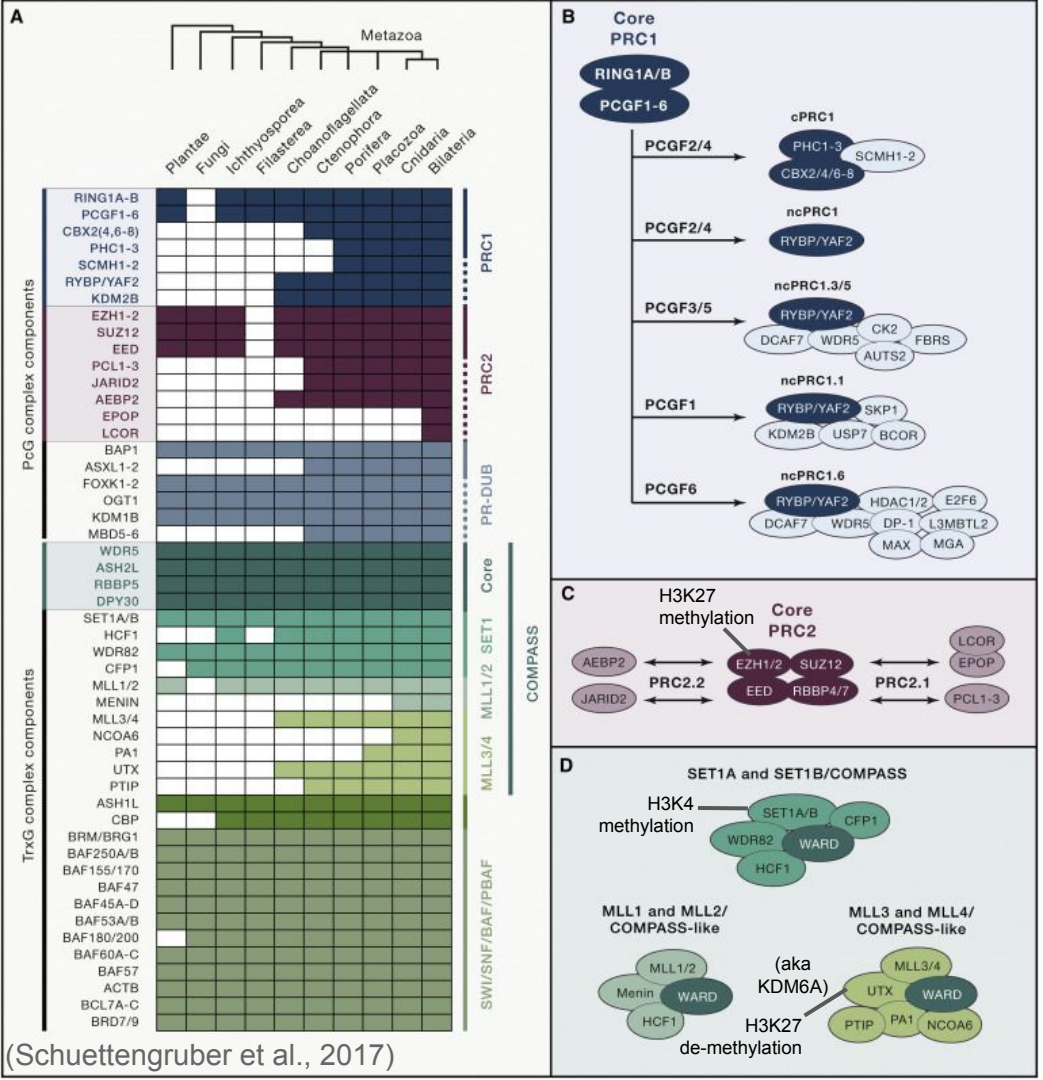
Sir2/3/4 bind and deacetylate histones

Sir3/4 polymerize (and attach to the nuclear matrix)

(Adapted from Krebs, Goldstein and Kilpatrick, Genes XII, 2018)

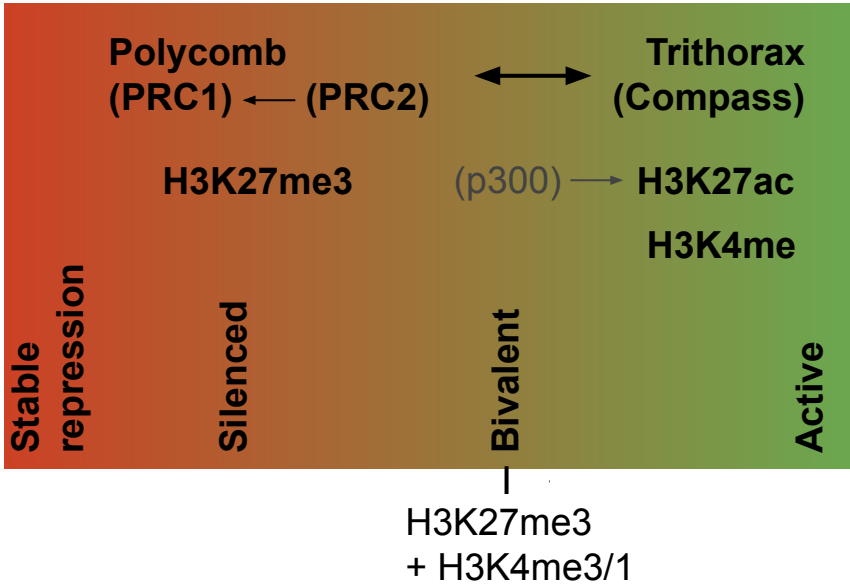
Opening and closing chromatin



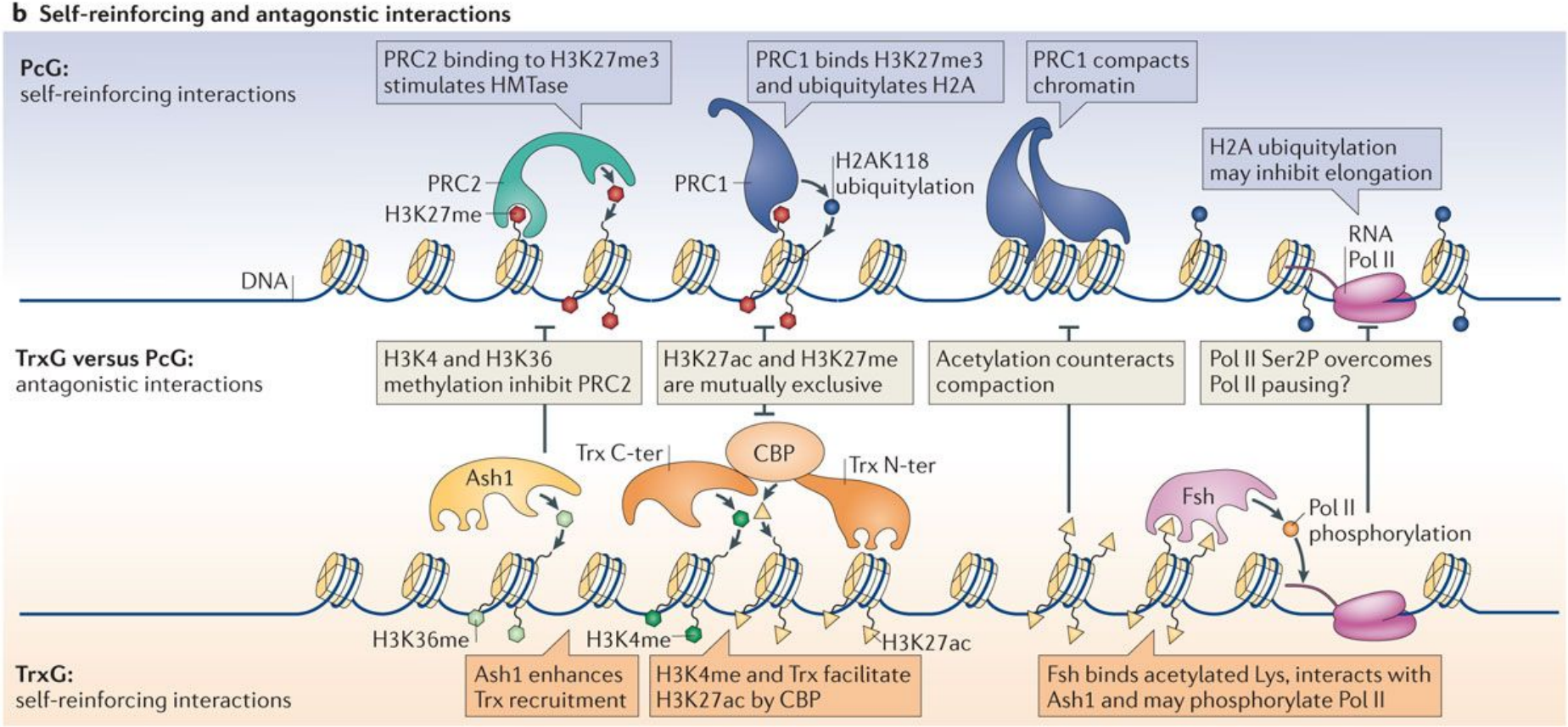


(Schuettengruber et al., 2017)

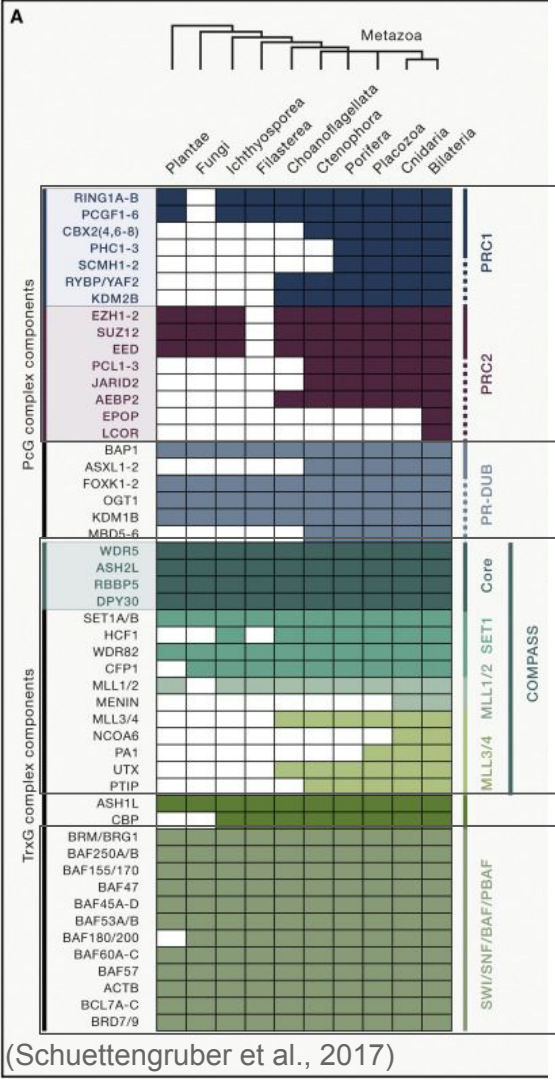
The conserved competition between Polycomb (repressive) and Trithorax (activating) protein groups regulates a very large variety of phenomena



Competition between Polycomb (PcG) and Trithorax (Trx) protein groups



(Steffen and Ringrose 2014)



(Schuettengruber et al., 2017)

Polycomb repressing complexes

COMPASS complexes

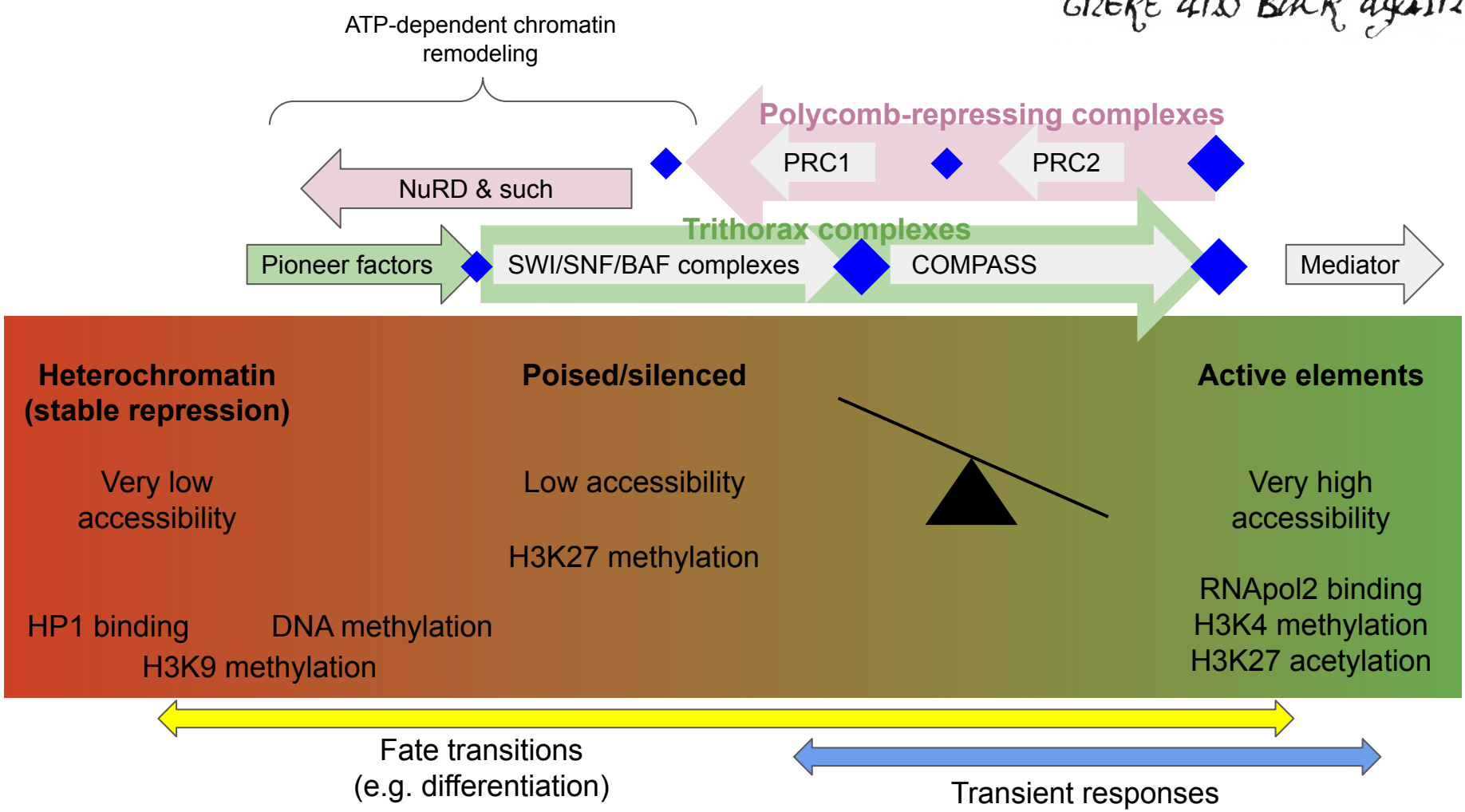
Nucleosome remodeling complexes

Direct and constant competition

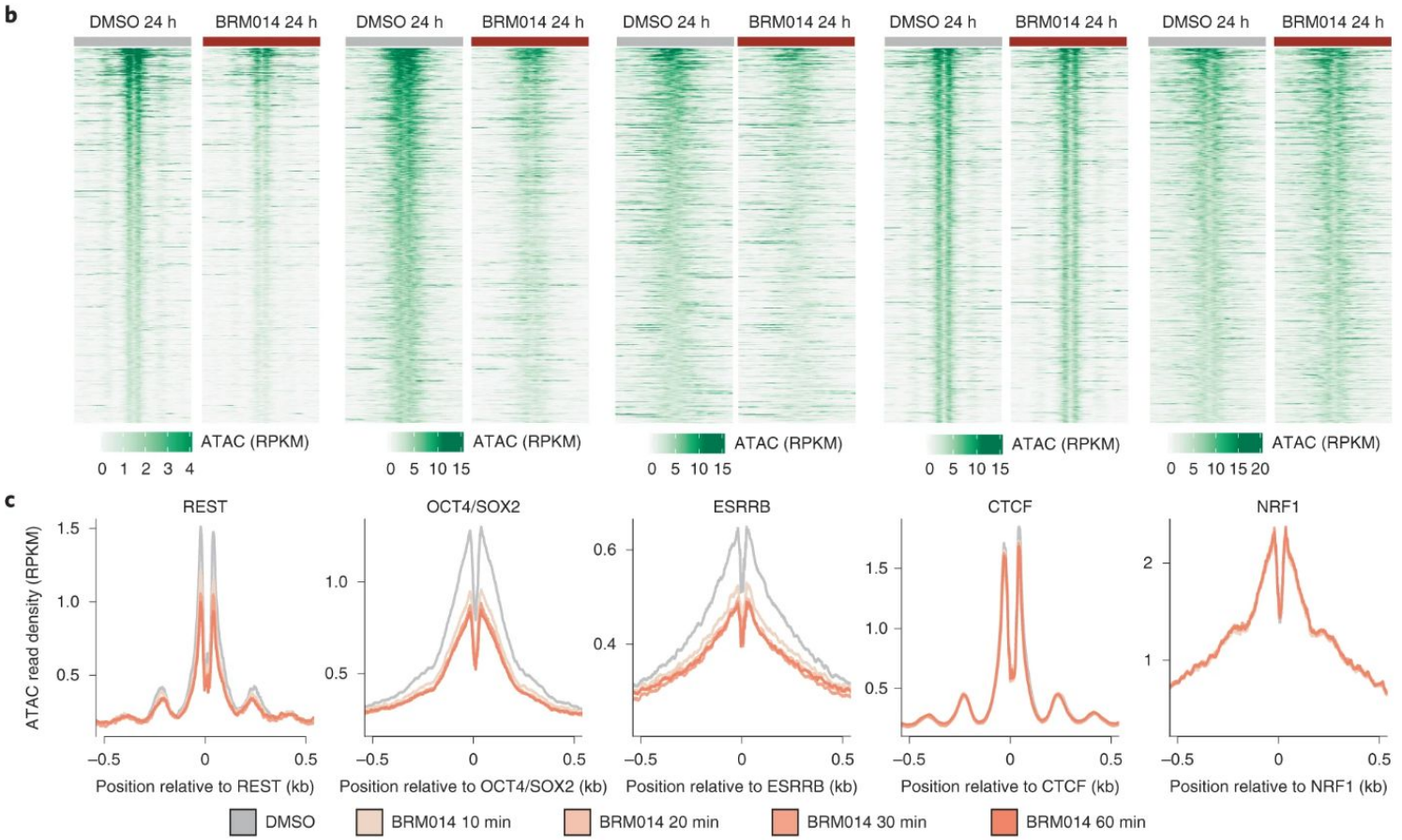
Prior

The conserved competition between Polycomb (repressive) and Trithorax (activating) protein groups regulates a very large variety of phenomena

there and back again...



Inhibition of SWI/SNF activity instantly impairs accessibility at dependent TF-binding sites



(Adapted from Iurlaro et al., Nat Gen 2021)

Practical:

Clustering epigenomic signals & GO enrichments in genomic regions

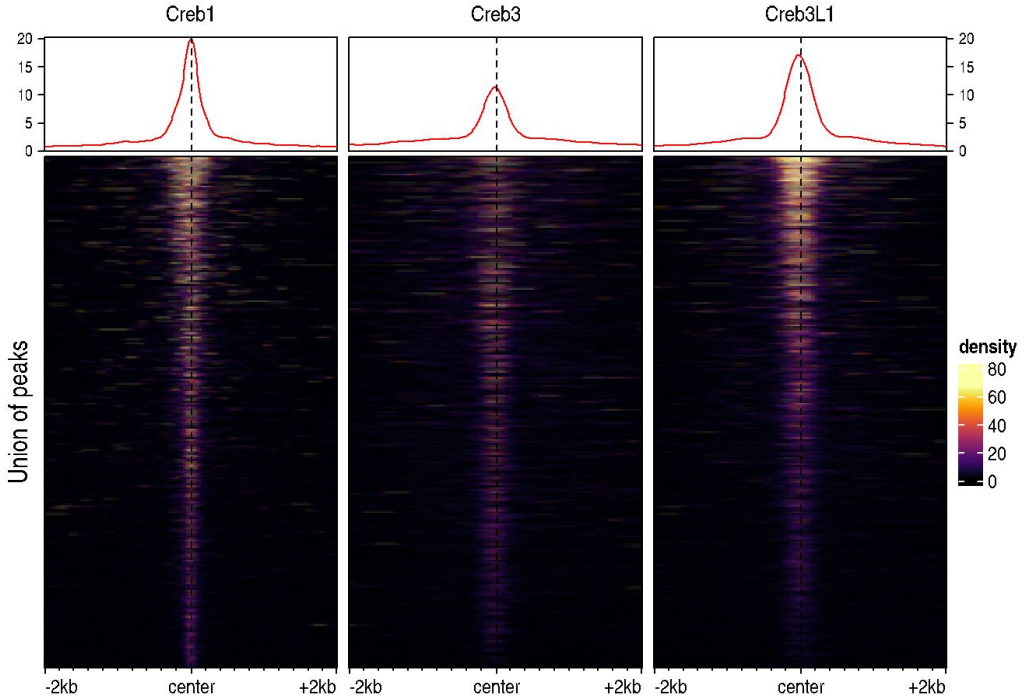
Assignment

- Download and decompress the following archive:
  - https://ethz-ins.org/content/w10.assignment.zip
- This contains the bigwig files and peaks (bed) files for three TFs of the CREB family (all restricted to chr1; aligned against the hg38 genome)
- Use clustering and visualization to illustrate the relationship between the binding of the different proteins
- Use enrichment analysis (either GO or motif) on at least one of the clusters
- Write a paragraph describing your results

- Save your assignment in a R markdown named `assignment.Rmd`, render it, and push the html file to this folder in your github repository

Assignment

Simply plotting the regions, the signals seem very similar… but are they?



Tip: focus on high-confidence peaks to define the universe of regions, e.g.:

```
peaks <- list.files(pattern="bed$")
# we first import the peaks
peaks <- lapply(peaks, rtracklayer::import.bed)
# we'll focus on the high-quality peaks
peaks <- lapply(peaks, FUN=function(x) x[x$score>800])
# we get the union of non-redundant regions
regions <- reduce(unlist(GRangesList(peaks)))
```