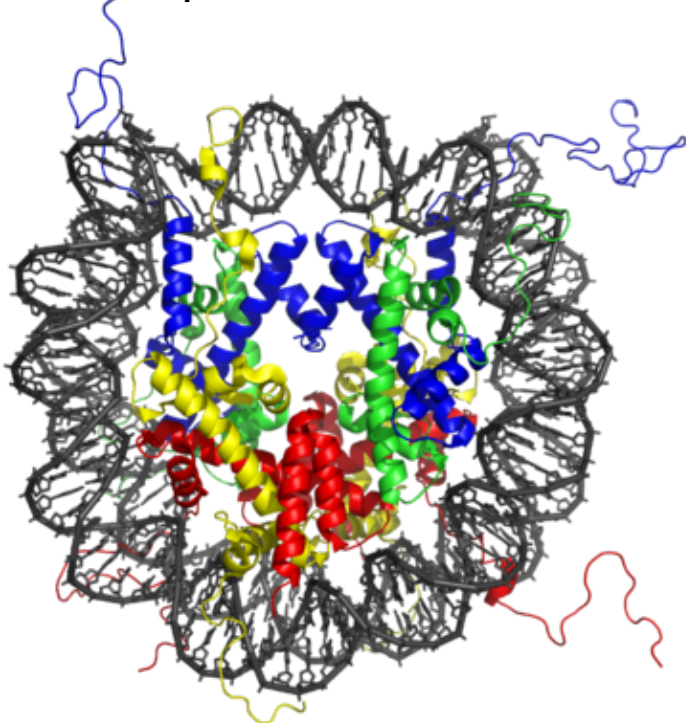


V23: the histone code

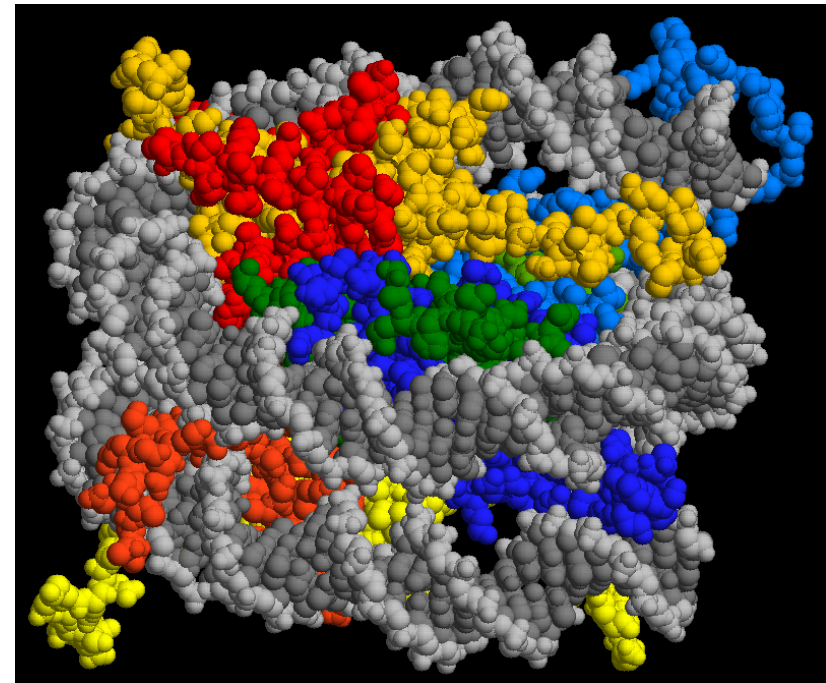
The DNA of eukaryotic organisms is packaged into chromatin, whose basic repeating unit is the **nucleosome**.

A nucleosome is formed by wrapping 147 base pairs of DNA twice around an octamer of four core histones, **H2A** , **H2B** , **H3** and **H4** (2 copies of each one).

X-ray structure of the nucleosome core particle consisting of core histones, and DNA. Top view.



Side view shows two windings of DNA and two histone layers



www.wikipedia.org

Post-translational modifications of histone tails

The disordered histone tails comprise 25-30% of the histone mass.

They extend from the compact histone multimer to provide a platform for various **post-translational modifications (PTMs)**.

These modifications affect the histones' ability to bind DNA and to other histones.

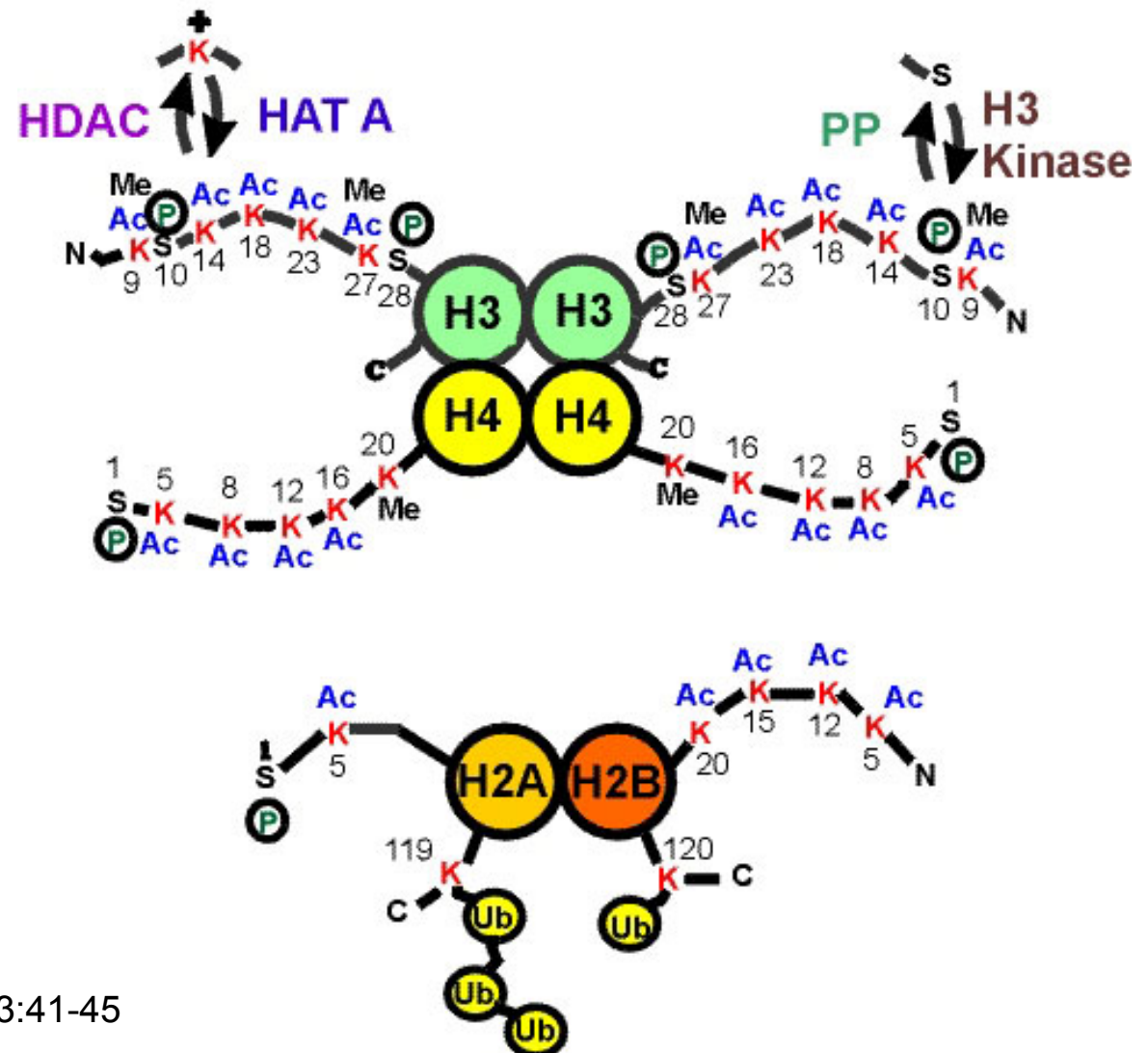
This, in turn affects **gene expression**.

*ACETYLATION AND METHYLATION OF HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS**

By V. G. ALLFREY, R. FAULKNER, AND A. E. MIRSKY

THE ROCKEFELLER INSTITUTE

PNAS 1964;51:786
First report on PTMs of histones



Strahl BD and Allis CD, 2000. Nature 403:41-45

Mode of action of histone PTMs

Histone PTMs exert their effects via two main mechanisms.

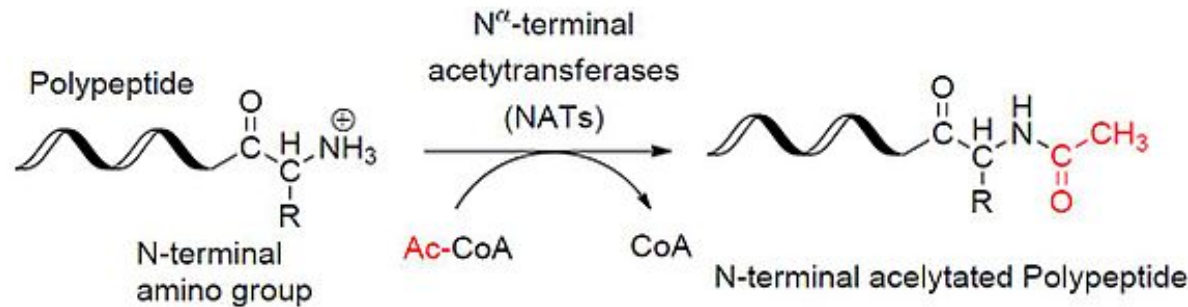
(1) PTMs directly influence the overall structure of chromatin, either over short or long distances.

(2) PTMs regulate (either positively or negatively) the binding of effector molecules.

Bannister, Kouzarides, Cell Res. (2011) 21: 381–395.

PTMs of histone tails

Histone **acetylation** and **phosphorylation** effectively reduce the positive charge of histones.



This potentially disrupts electrostatic interactions between histones and DNA.

This presumably leads to a less compact chromatin structure, thereby facilitating DNA access by protein machineries such as those involved in transcription.

Histone **methylation** mainly occurs on the side chains of lysines and arginines.

Unlike acetylation and phosphorylation, however, histone methylation does not alter the charge of the histone protein.

Bannister, Kouzarides, Cell Res. (2011) 21: 381–395.

By Ybs.Umich - Own work, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=31240656>

H4 tail : conformational dynamics

All histone tails can influence chromatin compaction and accessibility, depending on

- salt concentration,
- construction of the nucleosome arrays, and
- the type of assembly process.

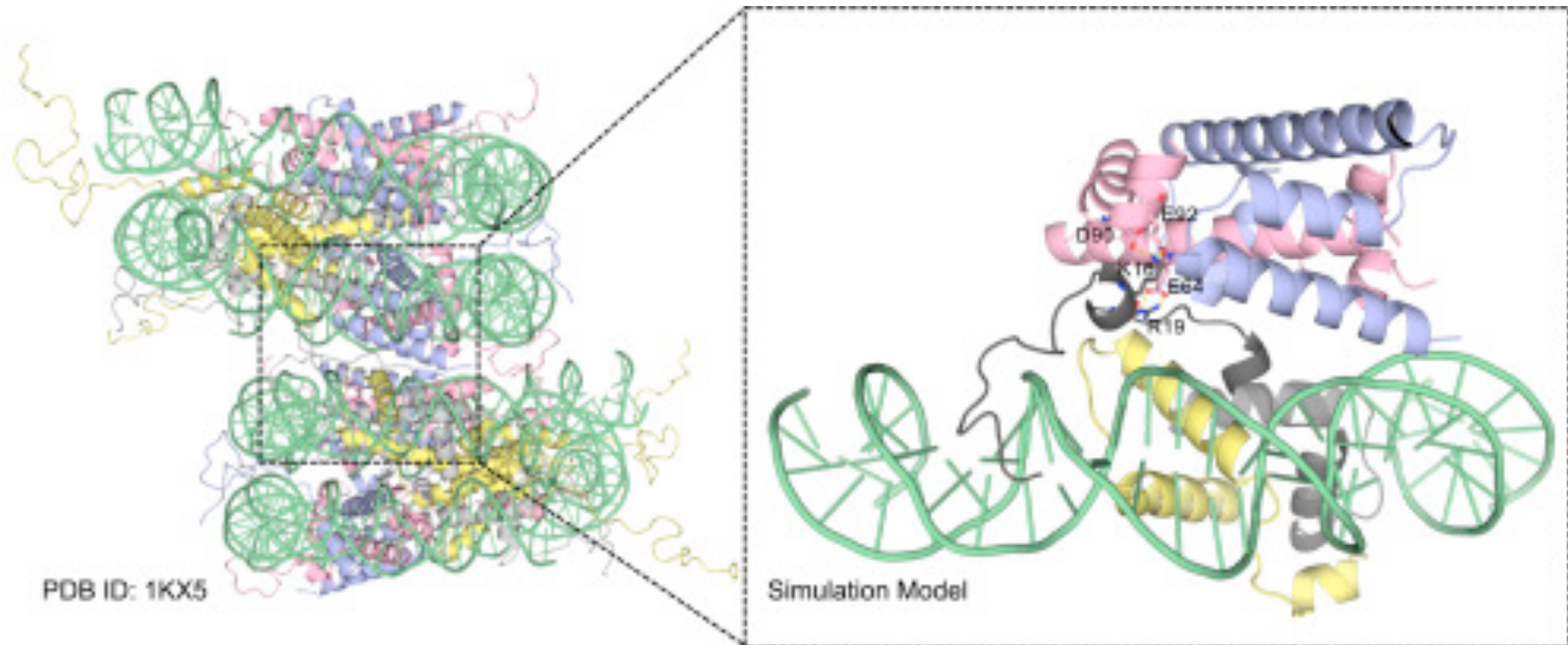
The **H4 tail** probably plays the most important role in inter-nucleosome interaction. Its middle part, the K¹⁶RHRK²⁰ segment forms a positively charged “**basic patch**”.

On the H2A-H2B dimer, the glutamic acid and aspartic acid residues H2A E56, E61, E64, D90, E91, E92, and H2B E102 and E110 build up a negatively charged area, called the “**acidic patch**”.

Due to the spatial proximity and the electrostatic attraction, stable salt bridges can be formed between these two parts from neighboring nucleosomes

[http://www.cell.com/biophysj/abstract/S0006-3495\(16\)31043-8](http://www.cell.com/biophysj/abstract/S0006-3495(16)31043-8)

Molecular dynamics simulations of H4-H2A/H2B-DNA system



Left: Structure of two nucleosomes from crystal packing

Right: the model structure used in atomistic MD simulations. Water not shown.

(*Green*) DNA; (*yellow*) H3; (*gray*) H4; (*pink*) H2A; and (*blue*) H2B.

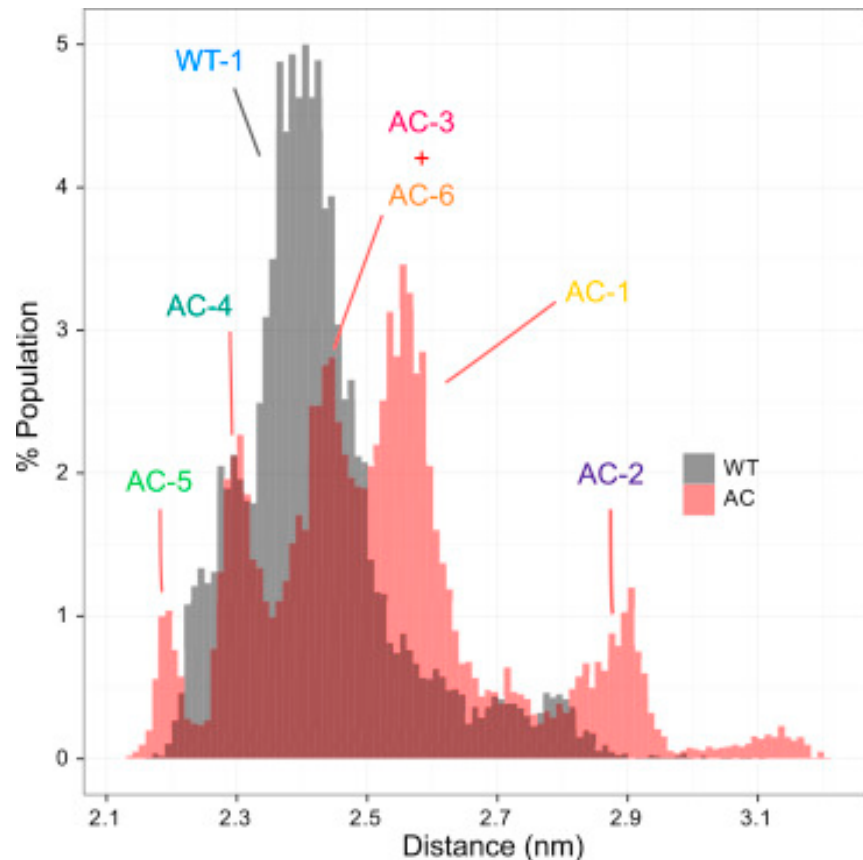
[http://www.cell.com/biophysj/abstract/S0006-3495\(16\)31043-8](http://www.cell.com/biophysj/abstract/S0006-3495(16)31043-8)

Acetylation effects

Distribution of the distance between the H4 tail and the neighboring H2A-H2B dimer in the MD simulations.

The center of mass of the backbone atoms of H4 tail residues 7–17 and H2A-H2B dimer are used for distance measurement.

The middle part of the **AC H4 tail** is generally **further away** from the adjacent H2A-H2B dimer.



The major population of WT is located at ~2.3–2.5 nm, indicating close contact between H4 tail middle part and the neighboring H2A-H2B dimer.

The distribution of AC is broader, ranging from 2.2 to 3.1 nm, and the multiple peaks refer to diverse conformation clusters. The center of the major peak of the AC population (AC-3 and AC-6) is shifted 0.2 nm to the right of the WT center.

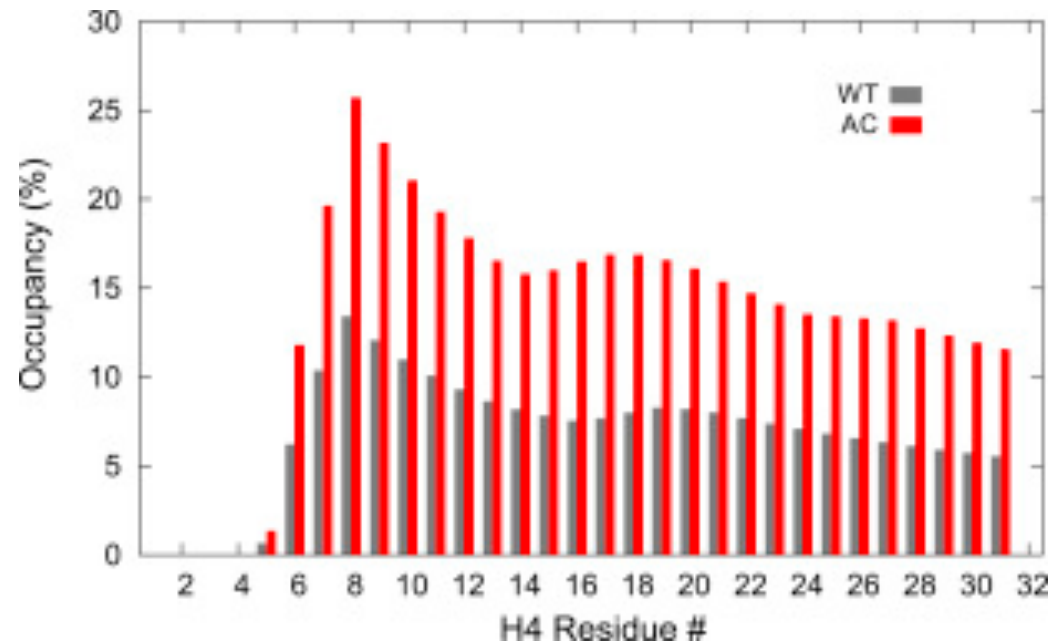
[http://www.cell.com/biophysj/abstract/S0006-3495\(16\)31043-8](http://www.cell.com/biophysj/abstract/S0006-3495(16)31043-8)

Acetylation effects

The H4 tail is basically **disordered** due to active electrostatic interaction with outside partners.

Only some low-frequency **3_{10} -helix** structures (formed by $i+3 \rightarrow i$ hydrogen bonds) were found in the WT system.

In the AC system, the occupancy of 3_{10} -helix structures is twice as high.

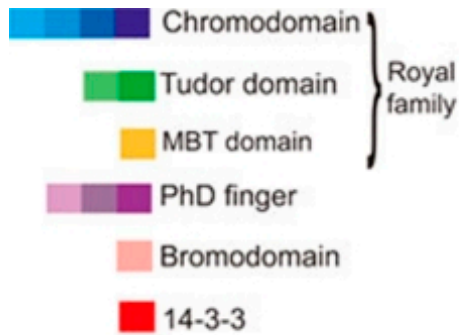


Acetylation disrupts the interaction between the H4 tail and the acidic patch.

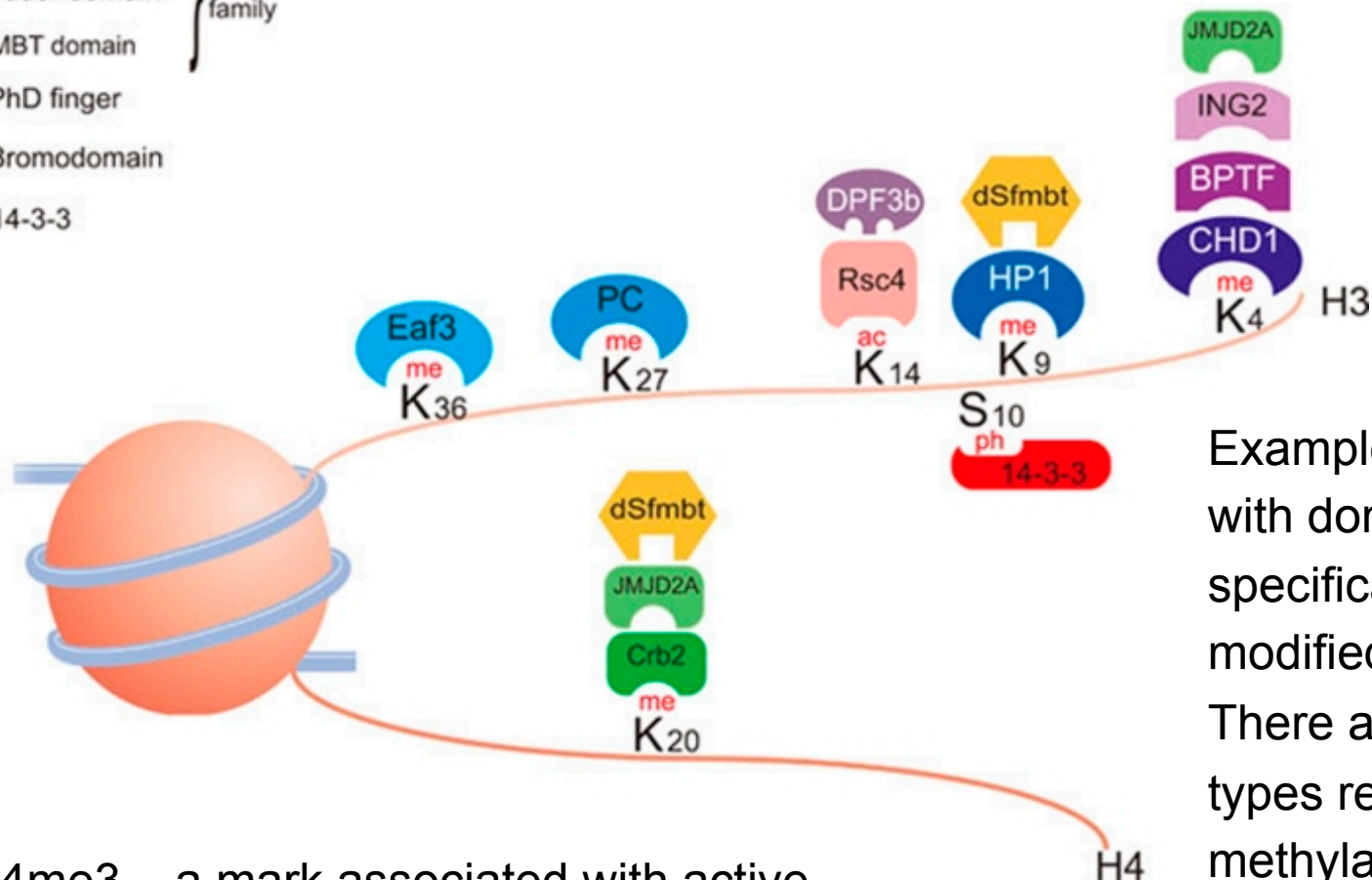
This gives the H4 tail the flexibility to form intratail hydrogen bonds.

The increasing intratail interaction helps to stabilize these structures.

[http://www.cell.com/biophysj/abstract/S0006-3495\(16\)31043-8](http://www.cell.com/biophysj/abstract/S0006-3495(16)31043-8)



Protein domains bind to modified histones

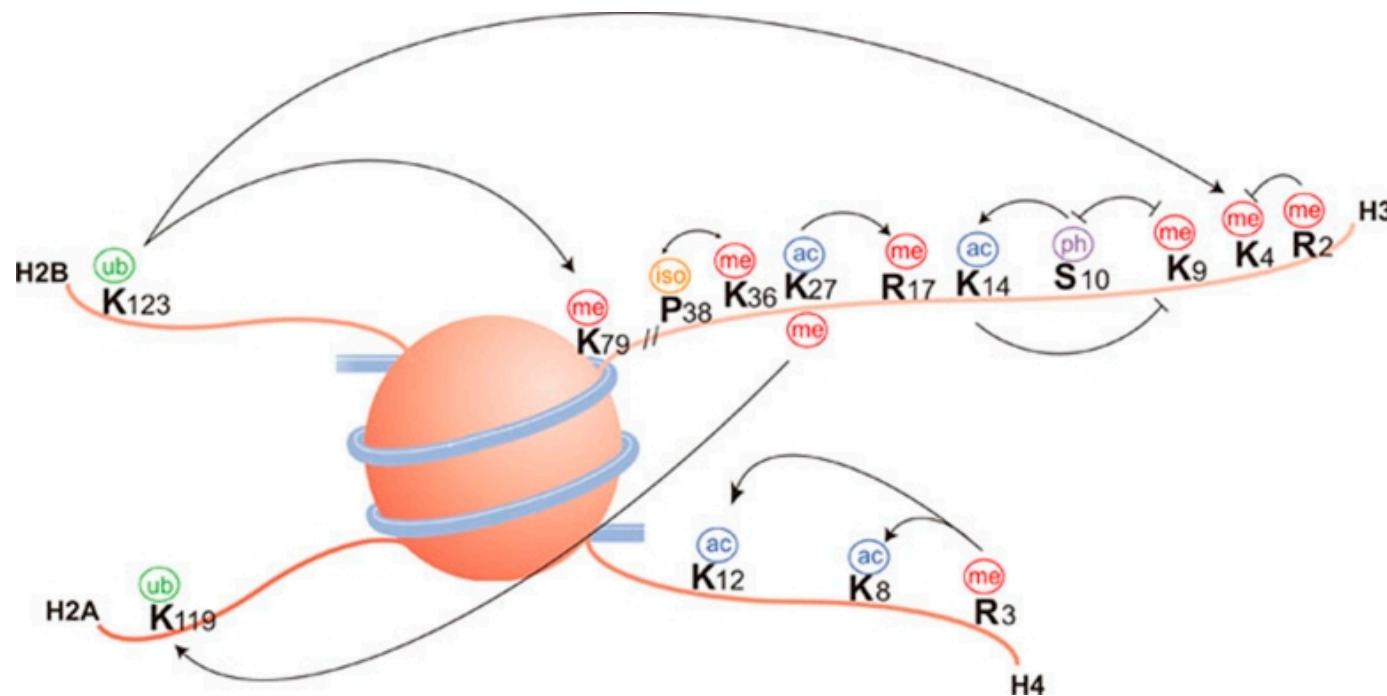


Examples of proteins with domains that specifically bind to modified histones. There are more domain types recognizing lysine methylation than any other PTM.

H3K4me3 – a mark associated with active transcription – is recognized by a PHD finger within the ING family of proteins (ING1-5). The ING proteins in turn recruit additional chromatin modifiers such as HATs and HDACs.

Bannister, Kouzarides
Cell Res. (2011) 21: 381–395.

Histone modification crosstalk



Histone PTMs can positively or negatively affect other PTMs.

A positive effect is indicated by an arrowhead and a negative effect is indicated by a flat head

The large number of histone PTMs enables tight control of chromatin structure. An extra level of complexity exists due to cross-talk between different modifications, which presumably helps to fine-tune the overall control.

Bannister, Kouzarides
Cell Res. (2011) 21: 381–395.

Euchromatin vs. Heterochromatin structure

Eukaryotic genomes can be divided into two geographically distinct environments.

(1) a relatively relaxed environment, containing most of the active genes and undergoing cyclical changes during the cell cycle. These '**open**' regions are referred to as **euchromatin**.

(2) other genomic regions, such as centromeres and telomeres, are relatively compact structures containing mostly inactive genes. These more '**compact**' regions are referred to as **heterochromatin**.

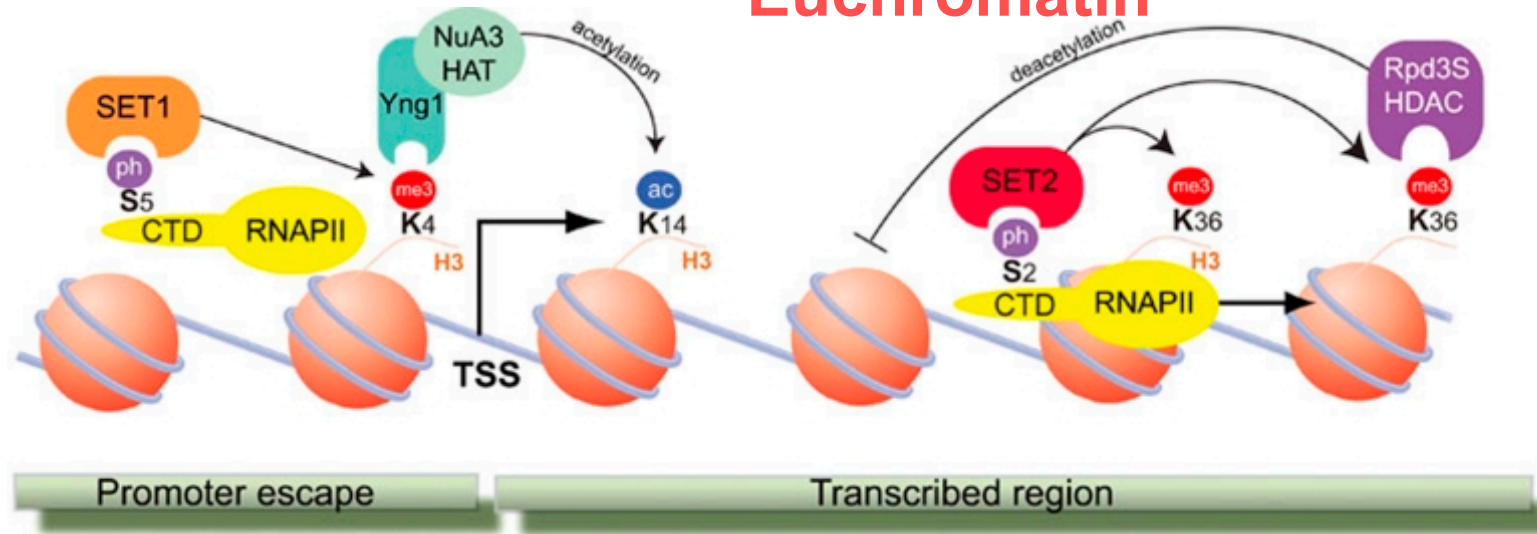
Both heterochromatin and euchromatin are enriched, and indeed also depleted, of certain **characteristic histone PTMs**.

However, there appears to be no simple rules governing the localization of PTMs. There is a high degree of overlap between different chromatin regions.

Nevertheless, there are regions of demarcation between heterochromatin and euchromatin. These '**boundary elements**' are bound by specific factors such as the “insulator” CTCF.

Euchromatin

Interplay of factors at an active gene in yeast.



(Left) The **scSet1** H3K4 methyltransferase binds to serine5 phosphorylated C-terminal domain (CTD) of RNAPII, the **initiating form** of polymerase situated at the TSS.

(Right) In contrast, the **scSet2** H3K36 methyltransferase binds to serine 2 phosphorylated CTD of RNAPII, the transcriptional **elongating form** of polymerase.

Thus, the two enzymes are recruited to genes via interactions with distinct forms of RNAPII

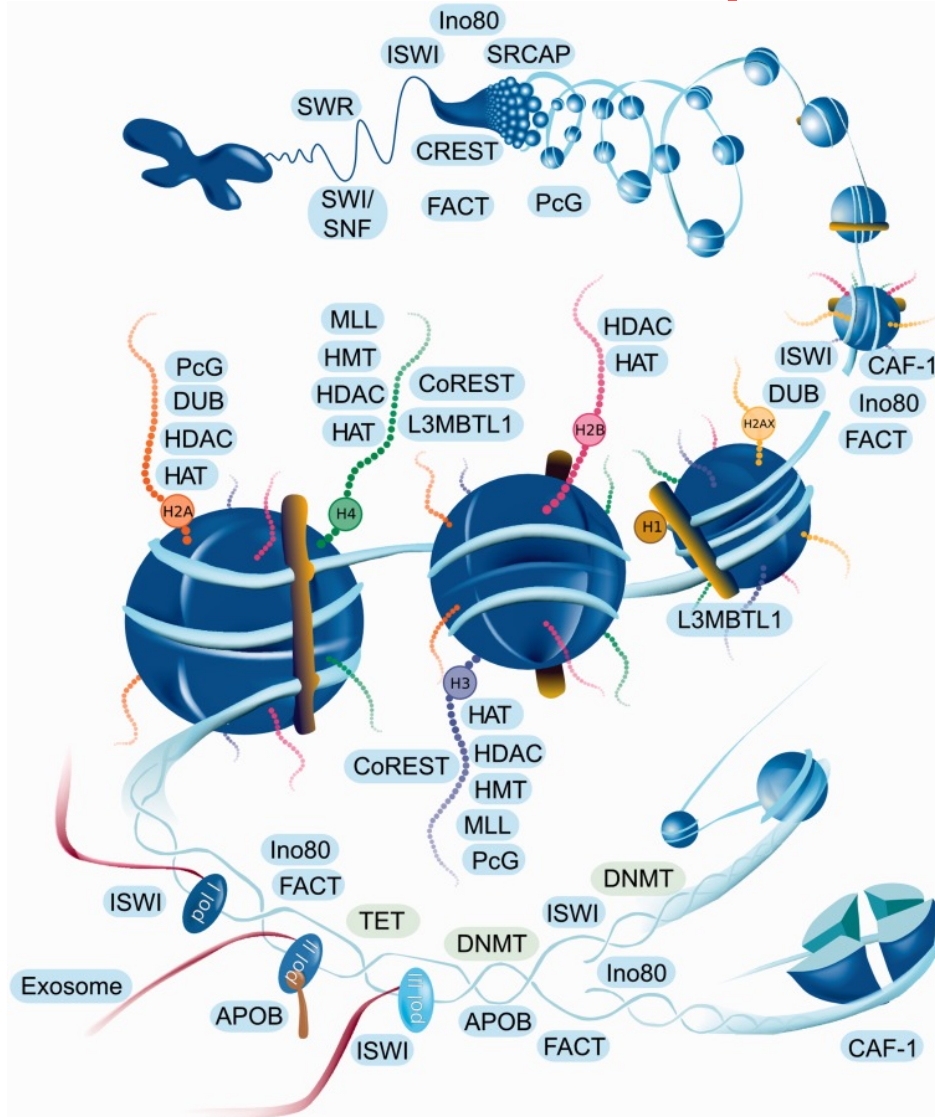
→ the location of the different forms of RNAPII define where the PTMs are placed

Epifactors database

The database EpiFactors stores detailed and curated information about 815 proteins and 69 complexes involved in epigenetic regulation.

http://epifactors.autosome.ru/protein_complexes

MSc thesis topic!



Side view shows two windings of DNA and two histone layers

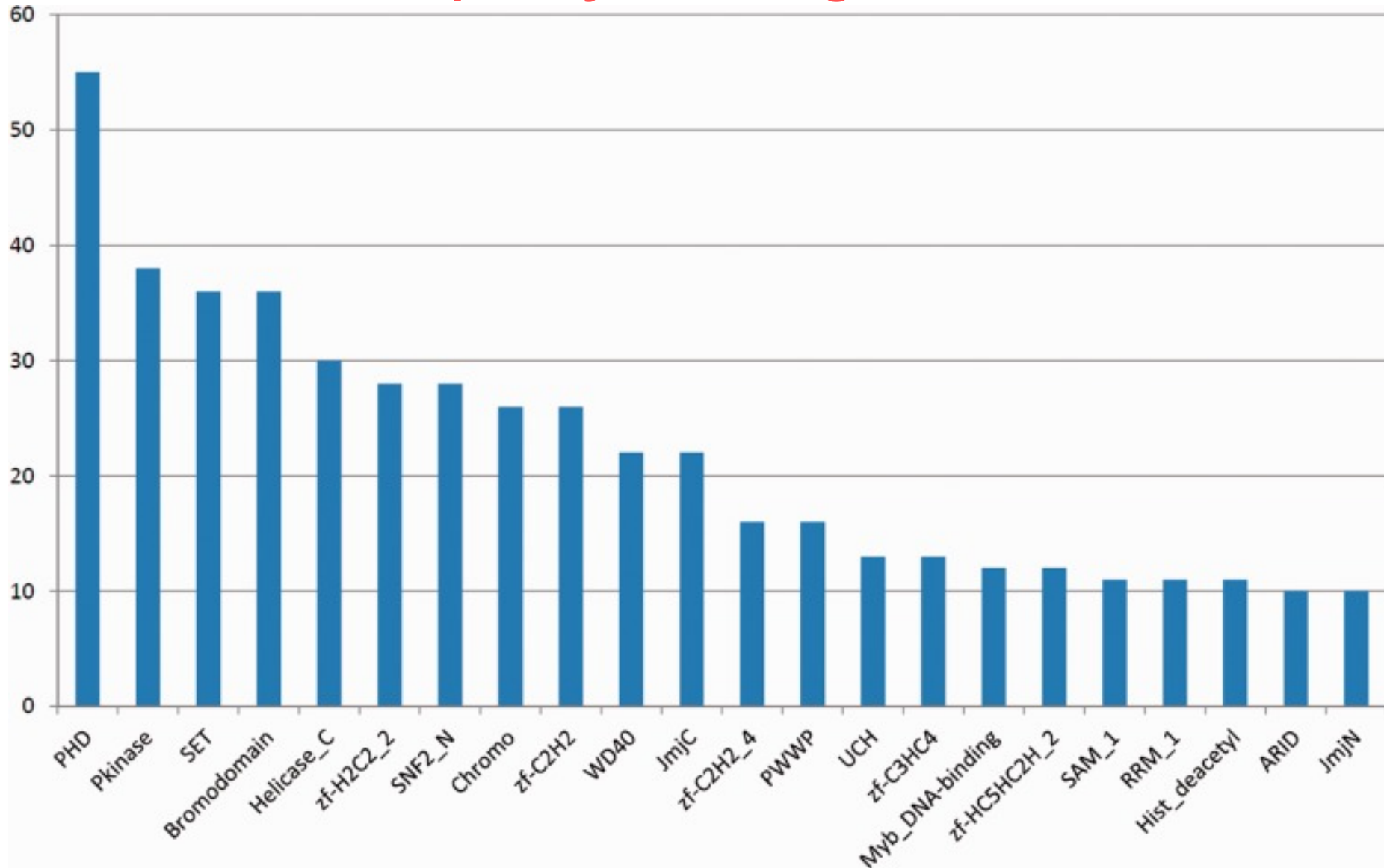
Database (Oxford). 2015; 2015: bav067.

Frequency of main annotation terms of epifactor proteins

Function	Count	Modification	Count
DNA modification	22	DNA methylation	7
RNA modification	30	DNA demethylation	12
Chromatin remodeling	101	DNA hydroxymethylation	5
Chromatin remodeling cofactor	41	RNA degradation	9
Histone chaperone	26	mRNA editing	10
Histone modification	15	Histone methylation	127
Histone modification cofactor	12	Histone acetylation	139
Histone modification read	90	Histone phosphorylation	55
Histone modification write	158	Histone ubiquitination	61
Histone modification write cofactor	95	Histone sumoylation	2
Histone modification erase	66	Histone citrullination	4
Histone modification erase cofactor	58	TF activator	18
Polycomb group (PcG) protein	29	TF repressor	27
Scaffold protein	12		
TF	53		

Database (Oxford). 2015; 2015: bav067.

Most frequently occurring Pfam domains



Database (Oxford). 2015; 2015: bav067.

ENCODE

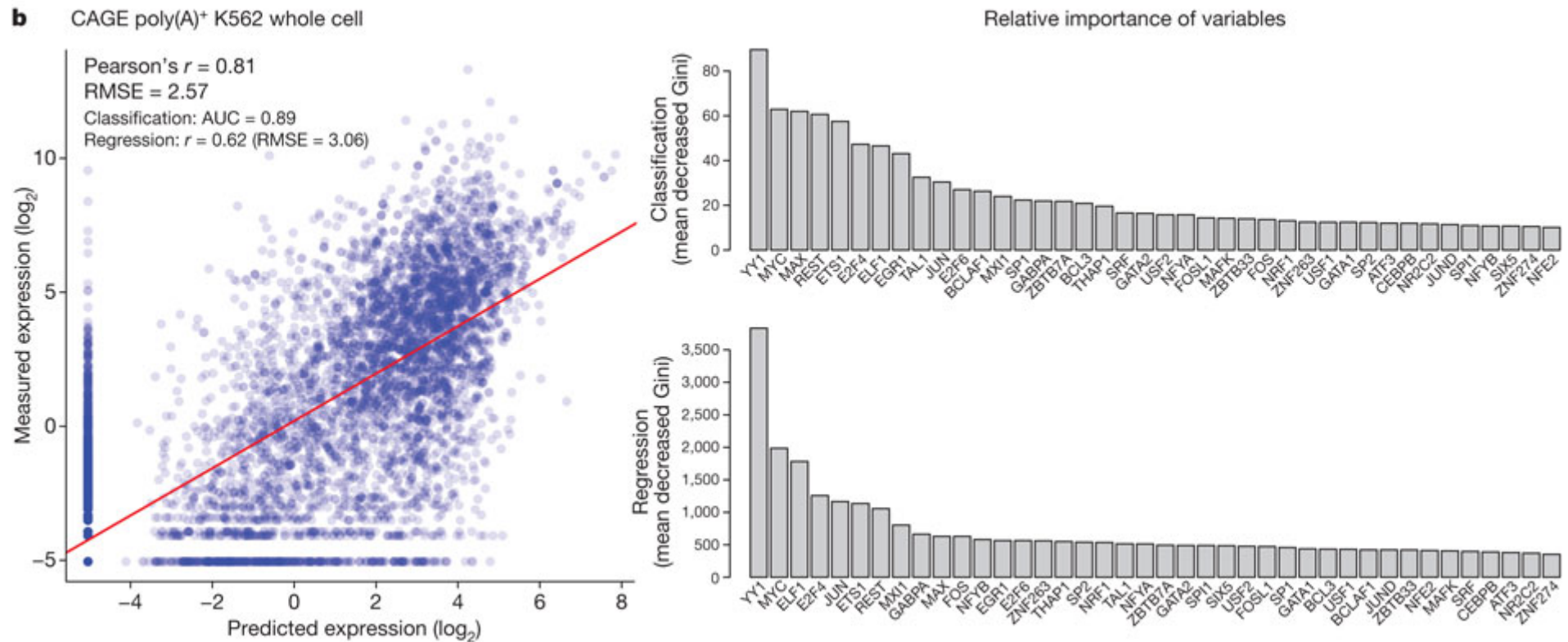


The ENCODE (Encyclopedia of DNA Elements) Consortium is an international collaboration of research groups funded by the National Human Genome Research Institute (NHGRI).

The goal of ENCODE is to build a comprehensive parts list of **functional elements** in the human genome, including elements that act at the protein and RNA levels, and **regulatory elements** that control cells and circumstances in which a gene is active.

ENCODE consortium
Nature 489, 57 (2012)

ENCODE: gene expression – TF binding sites



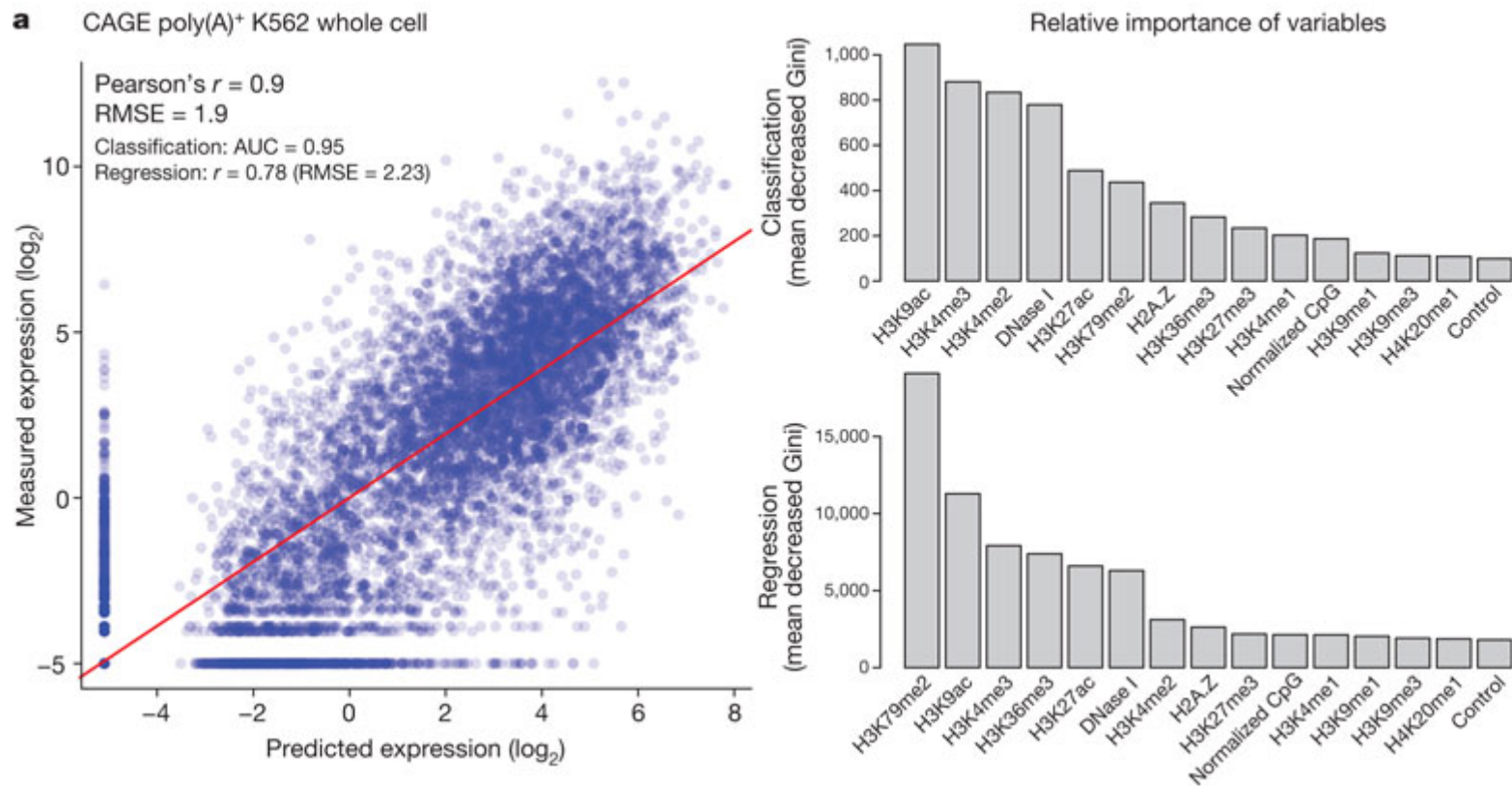
Correlative models between TF binding and RNA production in K562 cells.

(Left) output of the correlation models (x axis) compared to observed values (y axis).

(Right) The bar graphs show the most important TFs.

ENCODE consortium
Nature 489, 57 (2012)

ENCODE: gene expression – histone marks



Correlative models between histone marks and RNA production in K562 cells.

ENCODE consortium
Nature 489, 57 (2012)

ChromHMM

- ChromHMM is a software based on a multivariate **Hidden Markov Model** for learning and characterizing **chromatin states**.
- Input data can be multiple chromatin datasets such as ChIP-seq data of various histone modifications.
- The trained ChromHMM model can be used to systematically annotate a genome in one or more cell types.

Inputs to the initialization procedure:

Let M be the number of marks in the model

Let K be the number of states in the model

Let C denote the set of chromosomes

Let T_c denote the number of bins in chromosome c in C

Let c_t denote the bin t on chromosome c

Let v_{c_t} denote the observation vector at position t on the chromosome c

Let $v_{c_t,m}$ denote the binary 0/1 observation for the m^{th} mark

Let α be a smoothing constant with a default value of 0.02

Outputs of the parameter initialization procedure:

Let $p_{k,m}$ denote the emission probability in state k for mark m .

Let $b_{i,j}$ denote the transition probability from state i to state j .

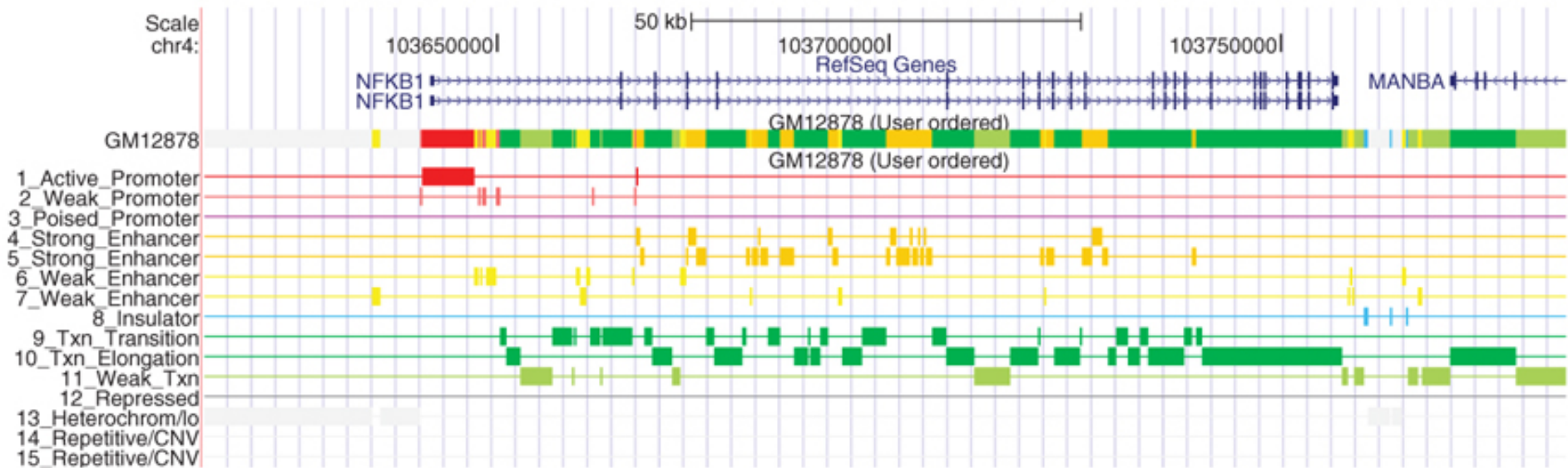
Let a_i denote the probability of starting in state i .



Manolis Kellis
MIT

Ernst, Kellis,
Nature Methods 9, 215 (2012)

ChromHMM



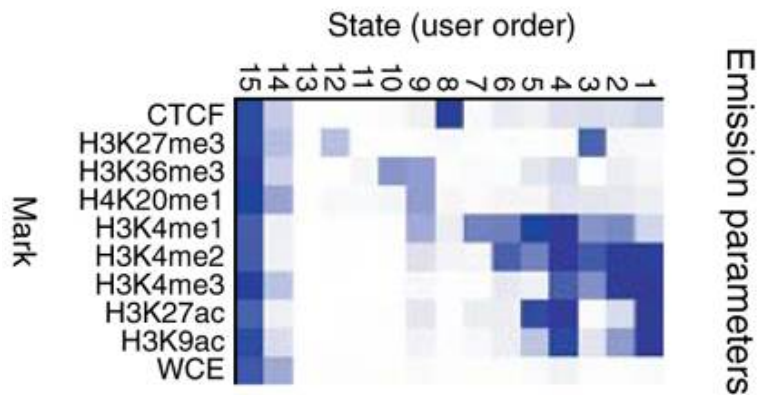
Example of chromatin-state annotation tracks produced from ChromHMM and visualized in the UCSC genome browser.

Shown as example is the NFKB1 (subunit of nuclear factor kappa B, this TF controls more than 200 genes).

Active promoter, **transcription + elongation**, **insulator** before next gene MANBA

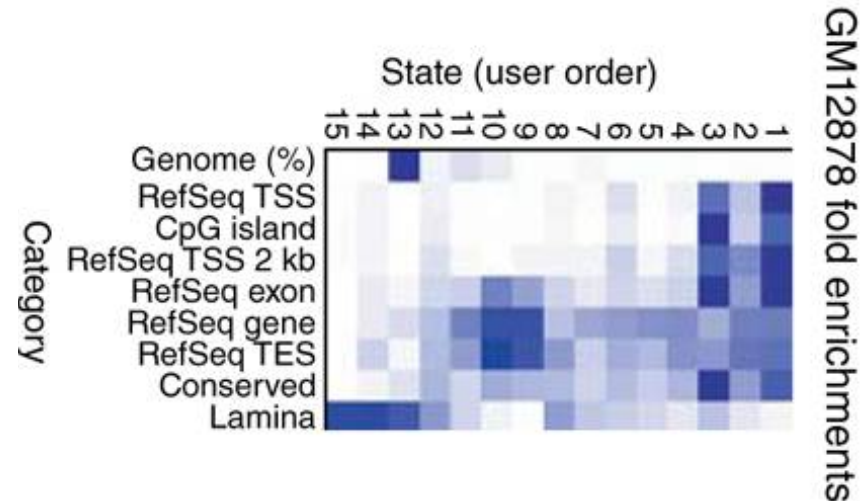
Ernst, Kellis,
Nature Methods 9, 215 (2012)

ChromHMM



(left) which PTMs contribute to which states.

1_Active_Promoter
2_Weak_Promoter
3_Poised_Promoter
4_Strong_Enhancer
5_Strong_Enhancer
6_Weak_Enhancer
7_Weak_Enhancer
8_Insulator
9_Txn_Transition
10_Txn_Elongation
11_Weak_Txn
12_Repressed
13_Heterochrom/lo
14_Repetitive/CNV
15_Repetitive/CNV



(right) Relative percentage of the genome represented by each chromatin state.

TSS, transcription start site;

TES, transcript end site;

GM12878 is a lymphoblastoid cell line.

Ernst, Kellis,
Nature Methods 9, 215 (2012)

Relate histone modifications to expression

- (i) Is there a quantitative relationship between histone modifications levels and transcription?
- (ii) Are there histone modifications that are more important than others to predict transcript levels?
- (iii) Are there different requirements for different promoter types?
- (iv) Are the relationships general?



Martin Vingron
MPI Berlin

The numbers of tags for each histone modification or variant, found in a region of 4,001 base pairs surrounding the transcription start sites of 14,802 RefSeq genes, was used as an estimation of the level of histone modifications.

Karlic et al.,
PNAS 107, 2926 (2010)

Relate histone modifications to expression

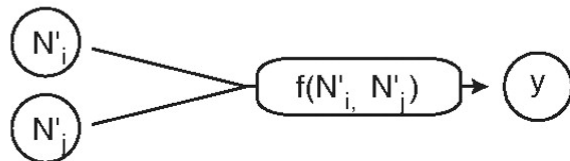
One-modification model (41 models)

$$f(N'_i) = a + b_i * N'_i$$



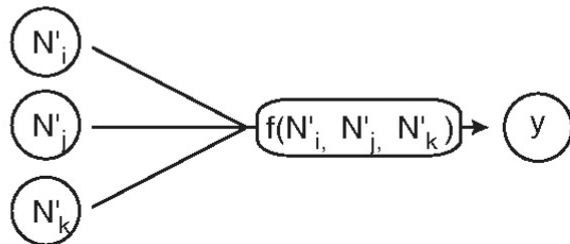
Two-modifications model (820 models)

$$f(N'_i, N'_j) = a + b_i N'_i + b_j N'_j$$



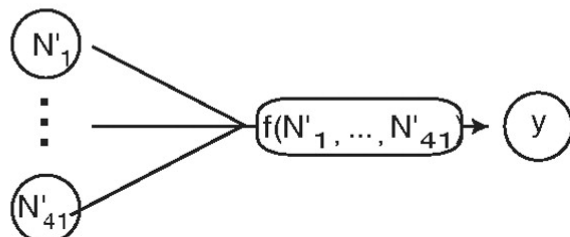
Three-modifications model (10,660 models)

$$f(N'_i, N'_j, N'_k) = a + b_i N'_i + b_j N'_j + b_k N'_k$$



Full model (1 model)

$$f(N'_1, \dots, N'_{41}) = a + b_1 N'_1 + \dots + b_{41} N'_{41}$$



Models are formulated as equations that **linearly** relate the levels of histone modifications to the measured expression value.

N'_i : transformed levels of histone modification i

$$N'_i = \log(N_i + \alpha_i) \text{ (vector of length } L\text{)}$$

N_i : number of tags in each promoter

y : expression values (vector of length L).

In the **one-modification models**, i can be any of the 39 modifications or two control IgG antibodies.

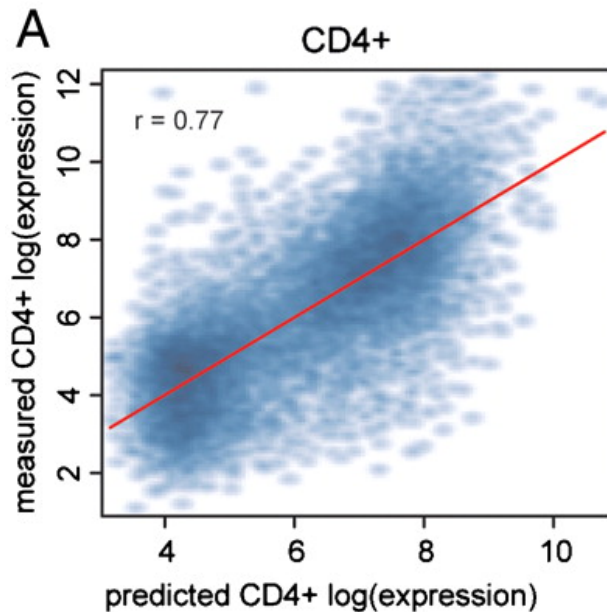
In the **two-modifications models**, i and j cover all combinations of two modifications without repetition.

In the **three-modifications models**, i , j , and k cover all combinations of three modifications without repetition.

The **full model** incorporates all 41 variables.

Karlic et al.,
PNAS 107, 2926 (2010)

Linear model for expression



Predicted expression values in CD4+ T-cells using the **full linear model** on the x axis and the measured expression values in CD4+ T-cells on the y axis.

The shades of blue indicate the density of points; the darker color, the more points.

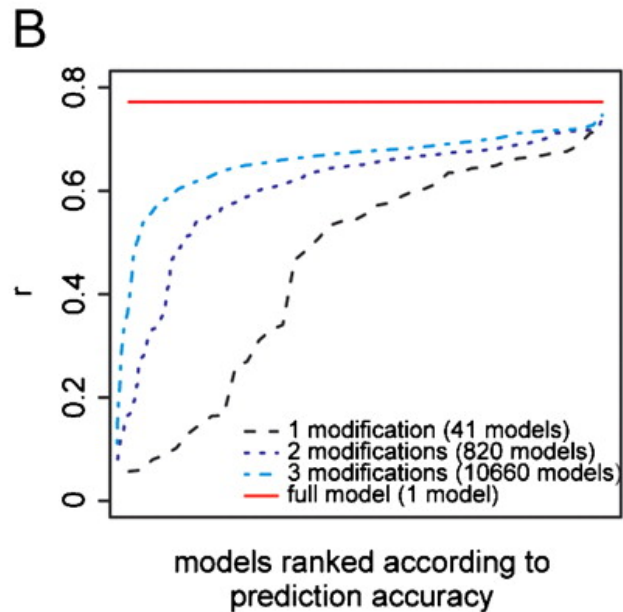
Red line : linear fit between predicted and measured expression ($y = 0.99x + 0.02$), which are highly correlated ($r = 0.77$)

→ a quantitative relationship exists between levels of histone modifications at the promoter and gene expression levels

(see slide 18 from ENCODE project)

Karlic et al.,
PNAS 107, 2926 (2010)

Linear model for expression



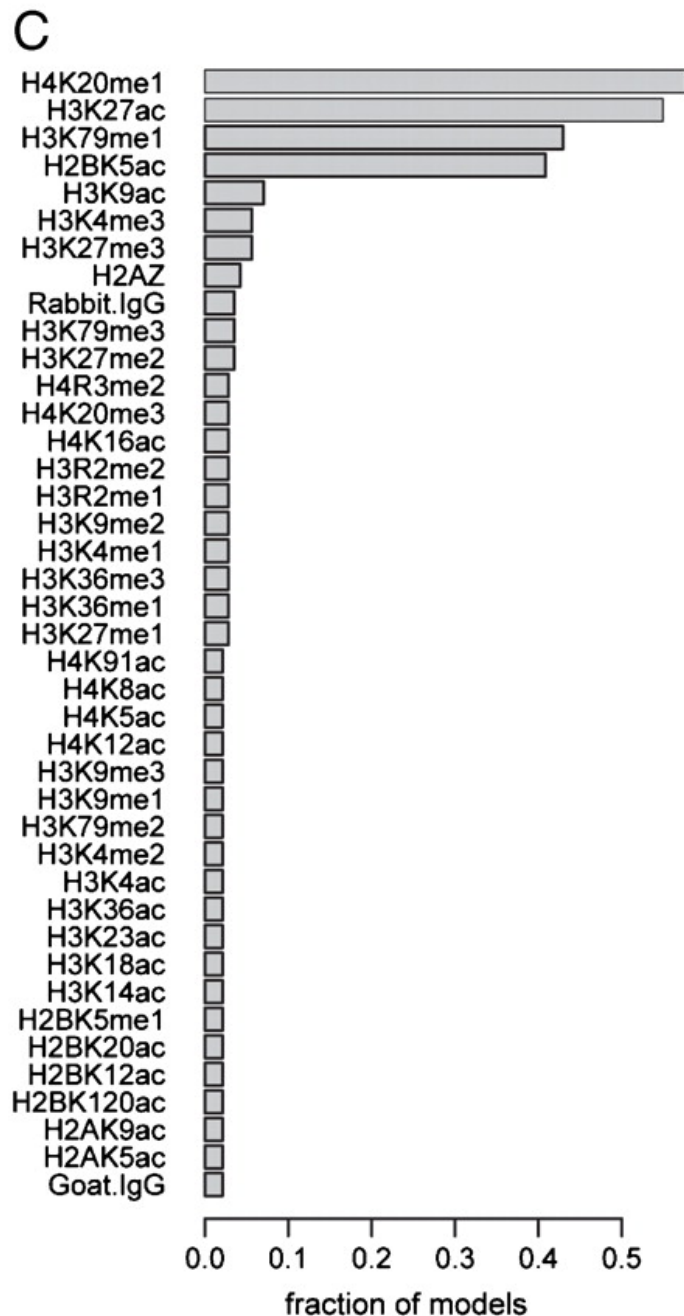
Comparison of prediction accuracy between all possible one-modification, two-modifications, three-modifications models, and the full model for CD4+ T-cells.

Models are sorted by ascending prediction accuracy along the x axis. The best models using only a small subset of modifications almost reach the prediction accuracy of the full linear model.

The top one-modification ($r_{\max} = 0.72$, H3K27ac), two-modifications ($r_{\max} = 0.74$, H3K27ac + H4K20me1) and three-modifications models ($r_{\max} = 0.75$, H3K27ac + H3K4me1 + H4K20me1) are very well correlated to expression.

Karlic et al.,
PNAS 107, 2926 (2010)

Linear model for expression



Bar plot showing the frequency of appearance of different histone PTMs in best scoring three-modifications models (142 models) for CD4+ T-cells.

Best scoring models are defined as reaching at least 95% of prediction accuracy of the full linear model.

Not all modifications are equally important, possibly because of a high degree of redundancy.

Karlic et al.,
PNAS 107, 2926 (2010)

Promoter methylation

Next, the authors separated the promoters into 2779 LCPs (**low CpG-content** promoters) and 7089 HCPs (**high CpG-content** promoters). Promoters with normalized CpG content > 0.4 are classified as HCP and the others as LCP.

This was motivated by the fact that the nucleosomes in HCPs are almost always decorated with **H3K4me3**, whereas nucleosomes in LCPs carry this modification only when they are expressed.

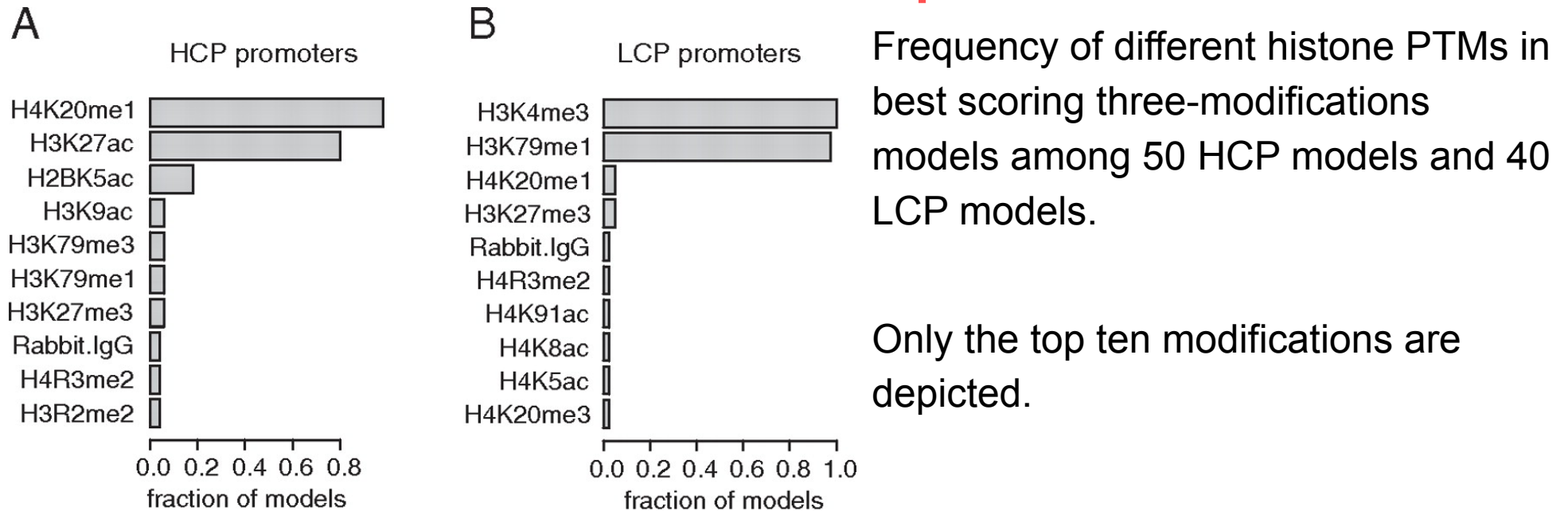
H3K4me3 is thought to be a mark of transcription initiation.

The authors reasoned that if these promoters are differently marked by histone modifications then the predictive power of histone modifications should also differ between these two groups of promoters.

Derive separate linear models for both groups.

Karlic et al.,
PNAS 107, 2926 (2010)

Linear model for expression



(A) H4K20me1 and H3K27ac (and possibly H2BK5ac) are significantly over-represented among the best scoring models for HCPs

(p-values hypergeometric test $9.97\text{e-}43$, $2.58\text{e-}31$, and 0.003)

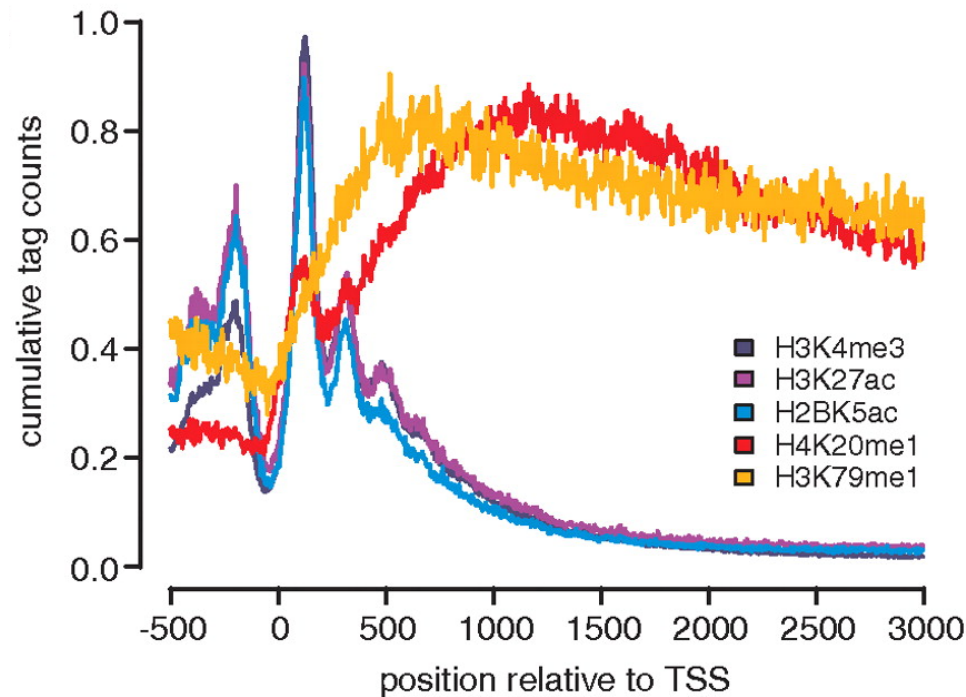
(B) H3K4me3 and H3K79me1 are significantly overrepresented in the LCPs

(p-values of the hypergeometric test $9.71\text{e-}36$ and $2.1\text{e-}34$)

→ different modifications are important for the prediction of expression of genes in these two groups.

Karlic et al.,
PNAS 107, 2926 (2010)

Linear model for expression



H3K4me3, **H3K27ac**, and **H2BK5ac** have the highest levels at the promoter, with the highest peaks around 100 base pairs downstream of the TSS.

H3K79me1 is enriched along the gene body, and **H4K20me1** shows two distinct patterns: a peak close to the promoter at a similar position to H3K4me3 and H3K27ac, and a further enrichment across the gene body region.

Karlic et al.,
PNAS 107, 2926 (2010)

Test whether model is transferable to other cell types

Apply trained CD4+ model to CD36+ and CD133+ cells.

The gene expression profiles of CD36+ and CD133+ cells are highly correlated to CD4+ T-cells ($r = 0.79$ and $r = 0.82$, respectively).

Thus, the prediction was restricted to genes with a fold change higher than five.

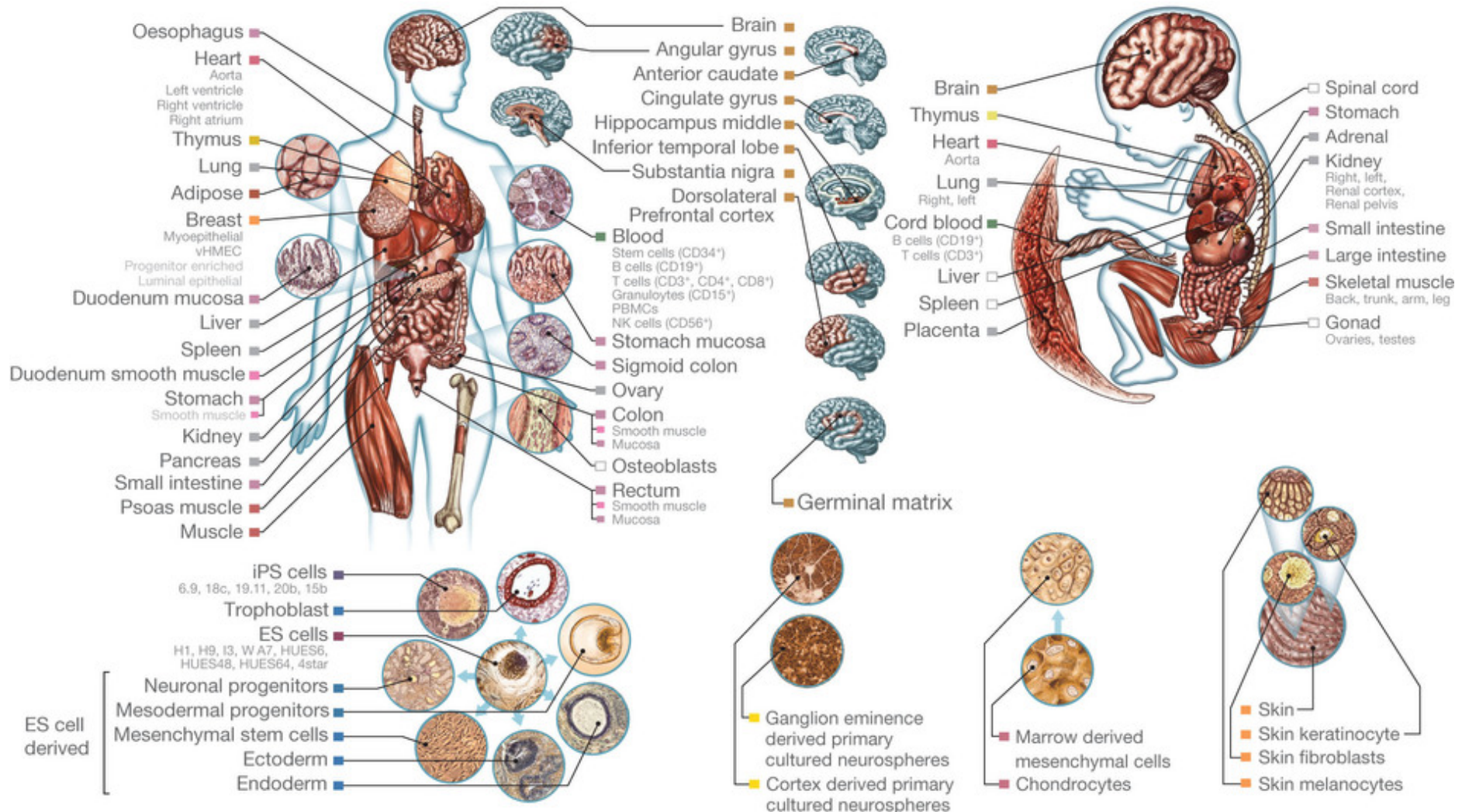
They found high correlation of predicted and measured expression values for both CD36+ ($r = 0.75$) and CD133+ ($r = 0.63$) cells.

This suggests that the relationship between histone modifications and gene expression is general and not dependent on the cellular context.

Karlic et al.,
PNAS 107, 2926 (2010)

Roadmap: Integrative analysis of 111 epigenomes

How does the epigenomic landscape contribute to cellular circuitry, lineage specification, and the onset and progression of human disease?



Roadmap Epigenomics Consortium
Nature 518, 317 (2015).

Mapped modifications

H3K4me3 - associated with promoter regions

H3K4me1 - associated with enhancer regions

H3K36me3 - associated with transcribed regions

H3K27me3 - associated with Polycomb repression

H3K9me3 - associated with heterochromatin regions

H3K27ac and H3K9ac, associated with increased activation of enhancer and promoter regions

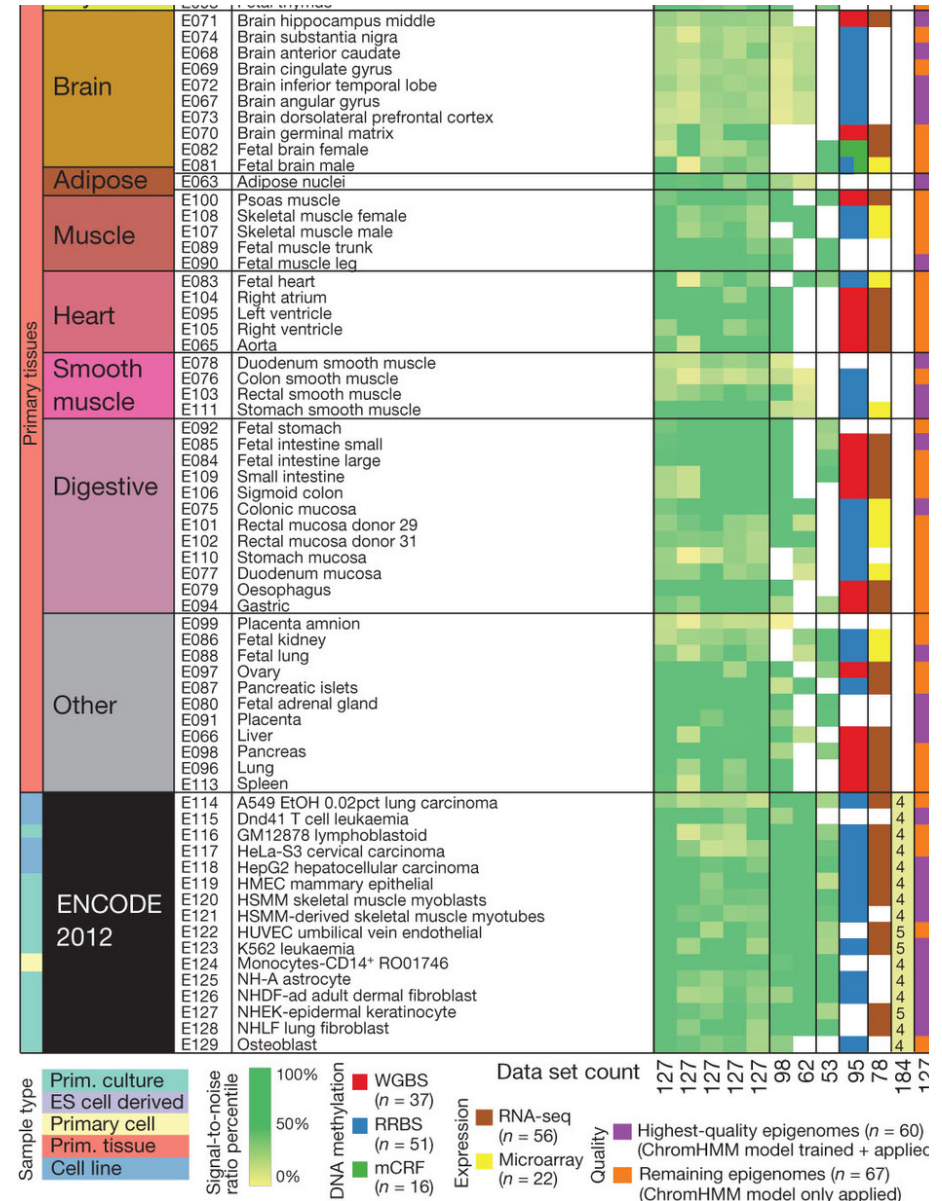
DNase hypersensitivity denoting regions of accessible chromatin commonly associated with regulator binding

DNA methylation, typically associated with repressed regulatory regions or active gene transcripts

Roadmap Epigenomics Consortium
Nature 518, 317 (2015).

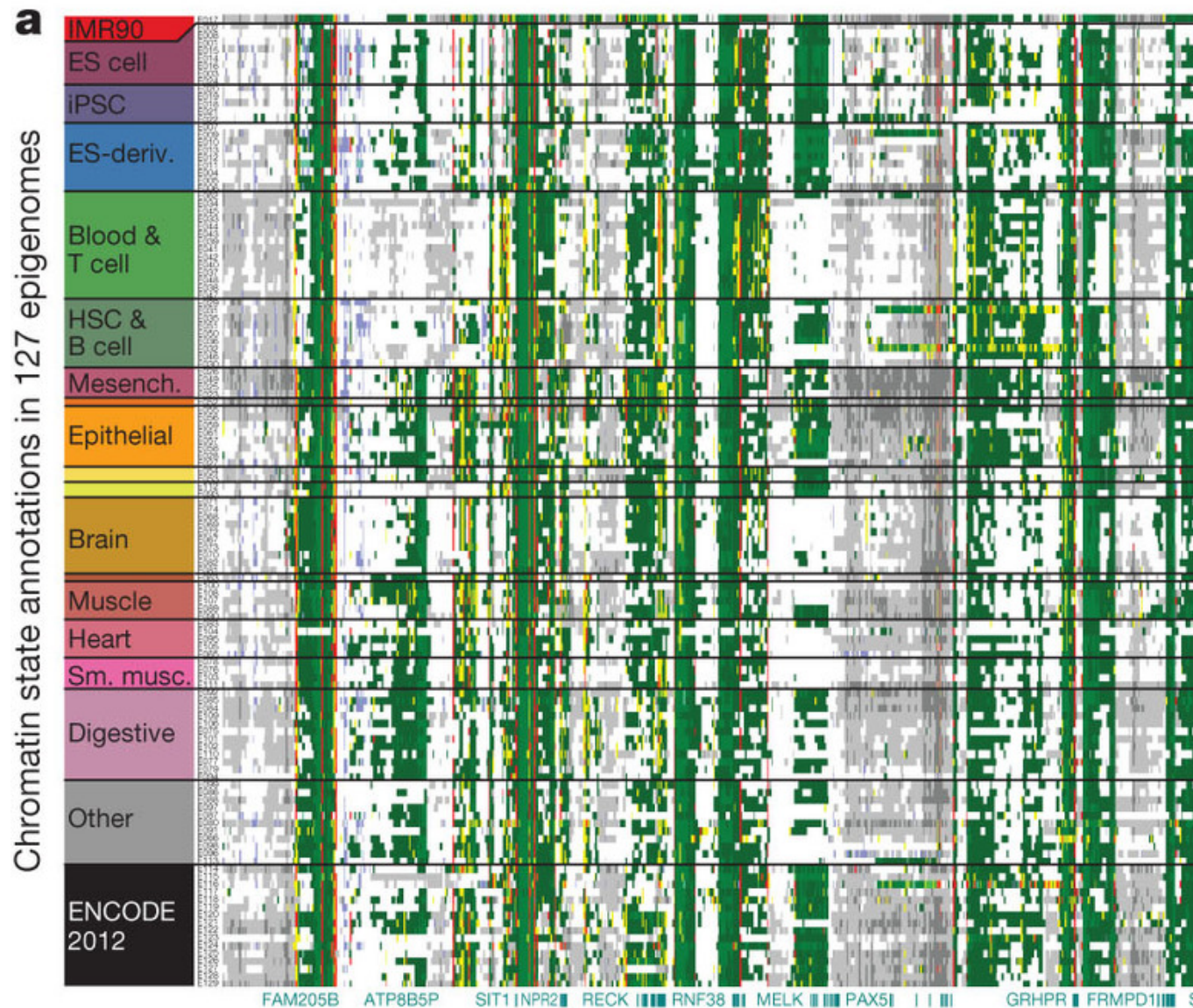
Data sets available for 111 epigenomes

a	b	c	d	e	f	g	h	i	j	k						
Sample type	Cell type/ tissue group	EID	Epigenome name	H3K4me1	H3K4me3	H3K36me3	H3K27me3	H3K27ac	H3K9ac	DNase-Seq	DNA methyl	Gene expr.	Addtl marks	Chrom. states		
Primary cultures	IMR90	E017	IMR90 fetal lung fibroblasts										21			
	ES cell	E002	ES-WA7 cells											21		
		E008	H9 cells											21		
		E001	ES-I3 cells											21		
		E015	HUES6 cells											21		
		E014	HUES48 cells											21		
		E016	HUES64 cells											21		
	iPSC	E003	H1 cells											20		
		E024	ES-UCSF4 cells											20		
		E020	iPS-20b cells											20		
ES cell derived	ES-deriv.	E019	iPS-18 cells											20		
		E018	iPS-15b cells											20		
		E021	iPS DF 6.9 cells											20		
		E022	iPS DF 19.11 cells											20		
		E007	H1 derived neuronal progenitor cultured cells											13		
		E009	H9 derived neuronal progenitor cultured cells											1		
	Blood & T cell	E010	H9 derived neuron cultured cells											1		
		E013	HUES64 derived CD56 ⁺ mesoderm											11		
		E012	HUES64 derived CD56 ⁺ ectoderm											15		
		E011	HUES64 derived CD184 ⁺ endoderm											13		
		E004	H1 BMP4 derived mesendoderm											13		
		E005	H1 BMP4 derived trophoblast											13		
Primary cells	HSC & B cell	E006	H1 derived mesenchymal stem cells											13		
		E062	Primary mononuclear cells (from PB)											13		
		E034	Primary T cells from primary blood (from PB)											13		
		E045	Primary T cells effector/memory enriched (PB)											13		
		E033	Primary T cells from cord blood											13		
		E044	Primary T regulatory cells (from PB)											13		
		E043	Primary T helper cells (from PB)											13		
		E039	Primary T helper naive cells (from PB)											13		
		E041	Primary T helper cells PMA-I stimulated											13		
		E042	Primary T helper 17 cells PMA-I stimulated											13		
Primary cultures	Mesench.	E040	Primary T helper memory cells (from PB)											13		
		E037	Primary T helper memory cells (from PB)											13		
		E048	Primary T CD8 ⁺ memory cells (from PB)											13		
		E038	Primary T helper naive cells (from PB)											13		
		E047	Primary T CD8 ⁺ naive cells (from PB)											13		
		E029	Primary monocytes (from PB)											13		
		E031	Primary B cells from cord blood											13		
		E035	Primary haematopoietic stem cells (HSCs)											13		
		E051	Primary HSCs G-CSF-mobilized male											13		
		E050	Primary HSCs G-CSF-mobilized female											13		
Primary cultures	Myosat.	E036	Primary HSCs short term culture											13		
		E032	Primary B cells (from PB)											13		
		E046	Primary natural killer cells (from PB)											13		
		E030	Primary neutrophils (from PB)											13		
		E026	Bone marrow derived MSCs											13		
		E049	Mesenchymal stem cell deriv. chondrocyte											13		
		E025	Adipose-derived mesenchymal stem cells											13		
		E023	Mesenchymal stem cell derived adipocyte											13		
		E052	Muscle satellite											1		
		Primary cultures	Epithelial	E055	Foreskin fibroblast											1
E056	Foreskin fibroblast													1		
E059	Foreskin melanocyte													1		
E061	Foreskin melanocyte													1		
E057	Foreskin keratinocyte													1		
E058	Foreskin keratinocyte													1		
E028	Breast vHMEC mammary epithelial													1		
E027	Breast myoepithelial													1		
Neurosph.	E054			Ganglion eminence derived neurospheres											1	
	E053			Cortex derived neurospheres											1	
Thymus	E112	Thymus											1			
	E093	Fetal thymus											1			



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Integrative analysis of 111 epigenomes

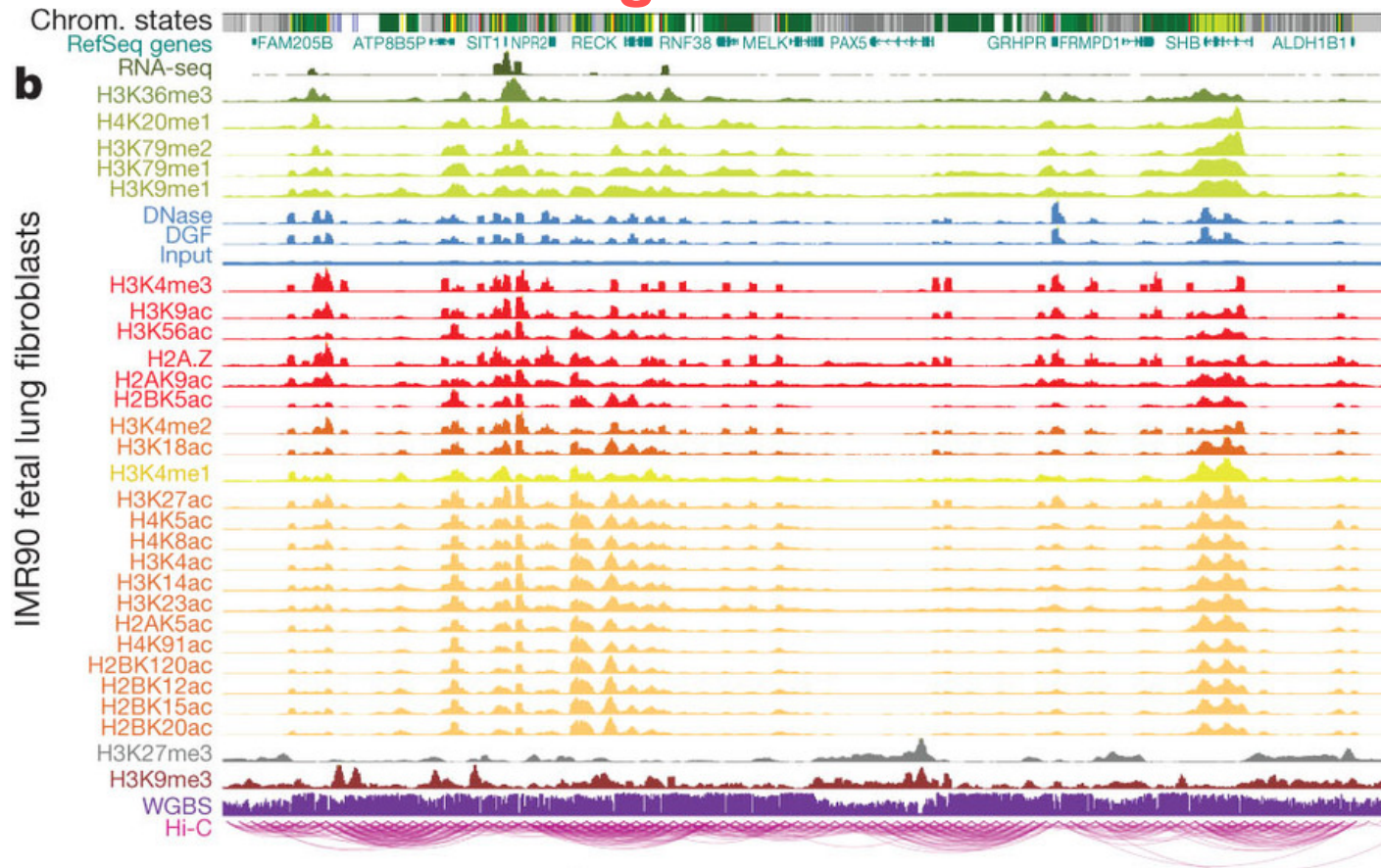


Chromatin state annotations across 127 reference epigenomes (rows) in a ~3.5-Mb region on chromosome 9.

Promoters are primarily constitutive (i.e. unchanged) (red vertical lines), while **enhancers** are highly dynamic (dispersed yellow regions).

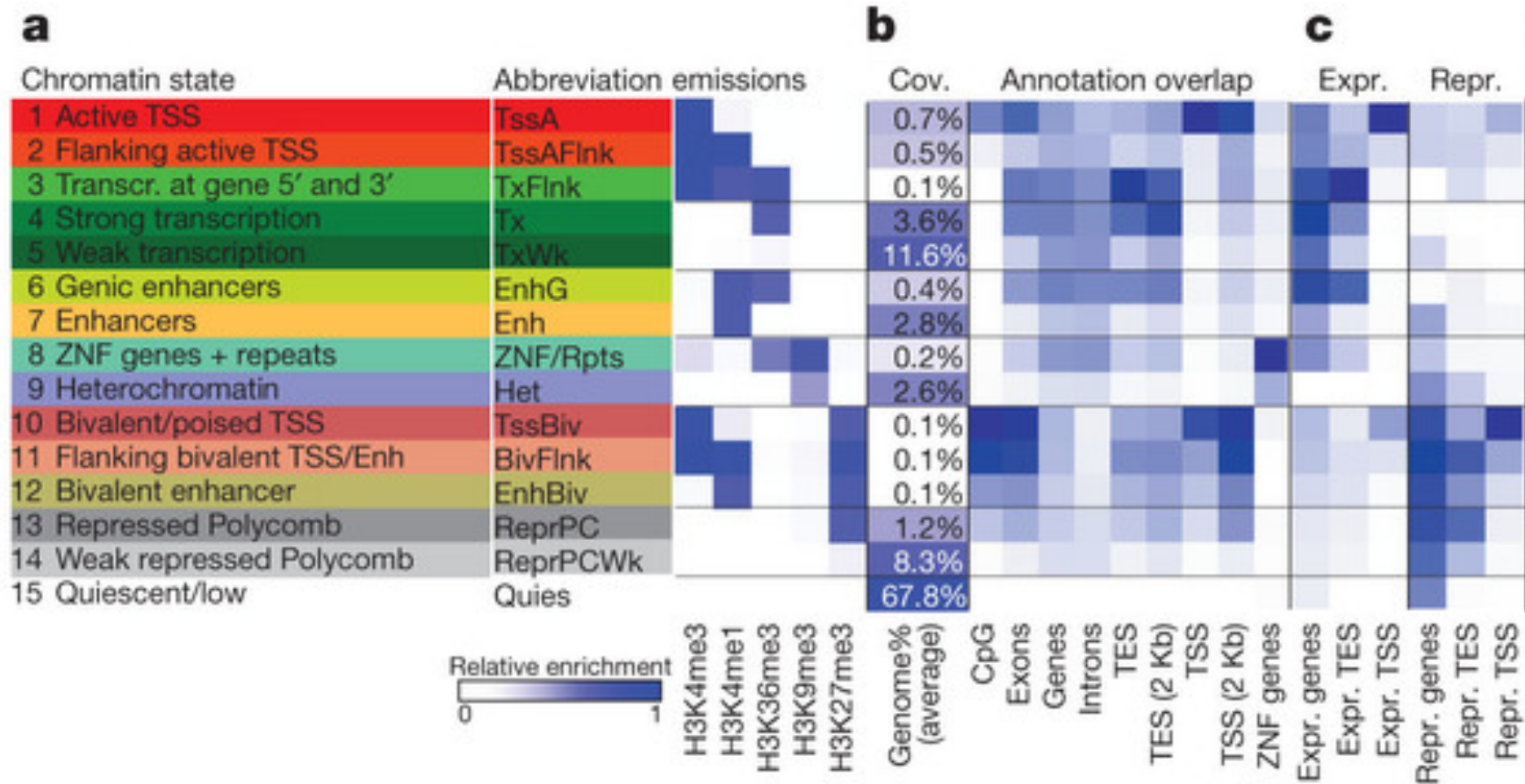
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Signal tracks for IMR90



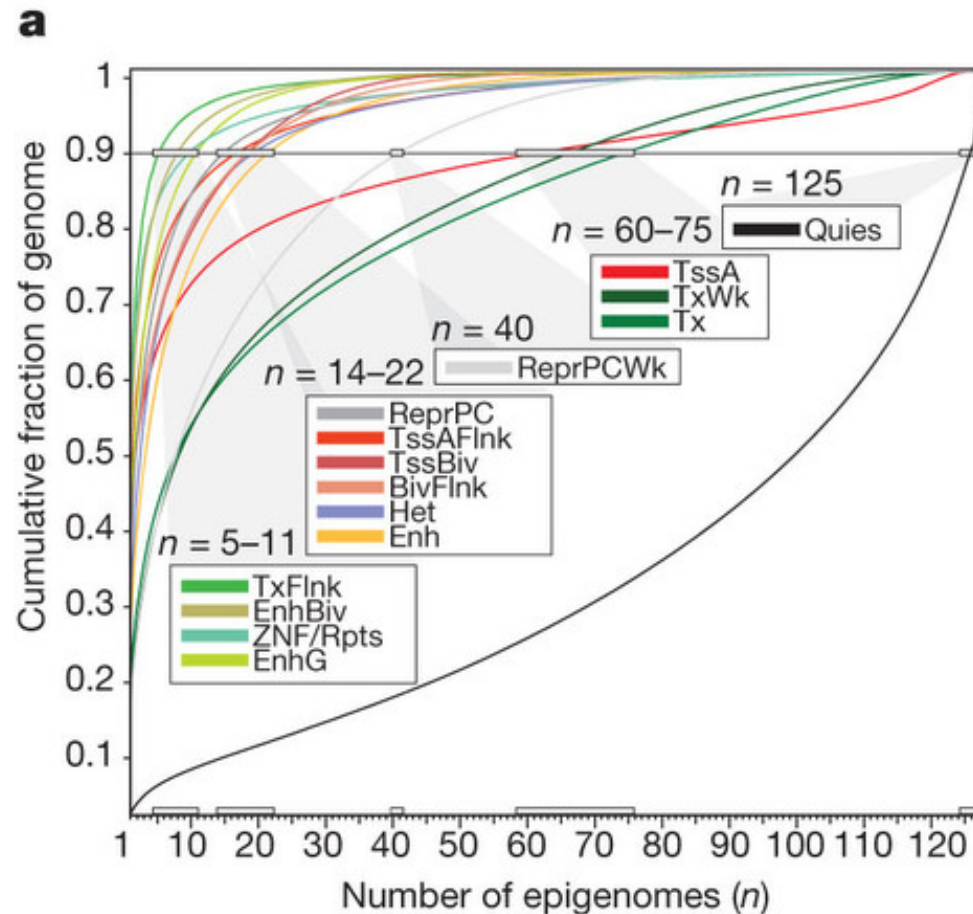
Signal tracks for IMR90 (fetal lung fibroblast) showing RNA-seq, a total of 28 histone modification marks, whole-genome bisulfite DNA methylation, DNA accessibility, digital genomic footprints (DGF), input DNA and chromatin conformation information.

Training of recurring 15-states chromatin model



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Consistency of chromatin states across genomic positions



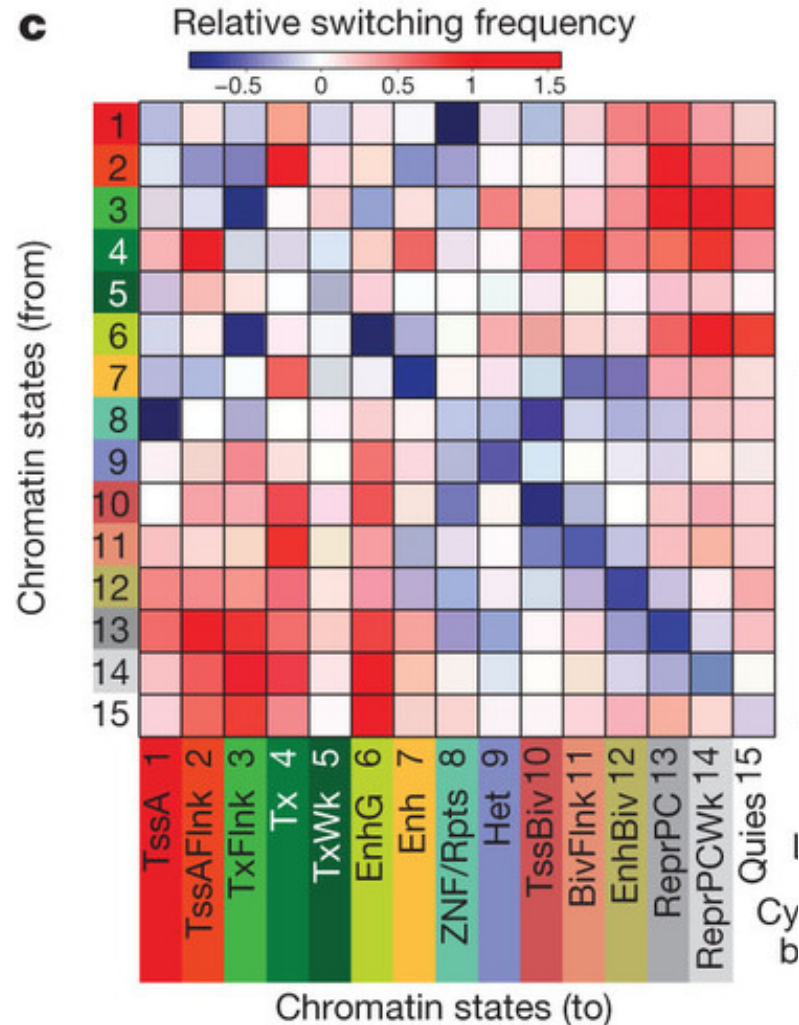
H3K4me1-associated states (including TxFlnk, EnhG, EnhBiv and Enh) are the most **tissue specific**, with 90% of instances present in at most 5–10 epigenomes, followed by bivalent promoters (TssBiv) and repressed states (ReprPC, Het).

In contrast, active promoters (TssA) and transcribed states (Tx, TxWk) were highly **constitutive**, with 90% of regions marked in as many as 60–75 epigenomes.

Quiescent regions were the most constitutive, with 90% consistently marked in most of the 127 epigenomes.

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Relative switching between states



More frequent switching found between active states and repressed states.

This is consistent with activation and repression of regulatory regions.

Summary

Combinations of histone modification marks are highly informative of the methylation and accessibility levels of different genomic regions, while the converse is not always true.

Genomic regions vary greatly in their association with active marks.

Approximately 5% of each epigenome is marked by enhancer or promoter signatures on average, which show increased association with expressed genes, and increased evolutionary conservation.

Two-thirds of each reference epigenome on average are quiescent, and enriched in gene-poor and stably repressed regions.

Even though promoter and transcription associated marks are less dynamic than enhancer marks, each mark recovers biologically meaningful cell-type groupings when evaluated in relevant chromatin states.