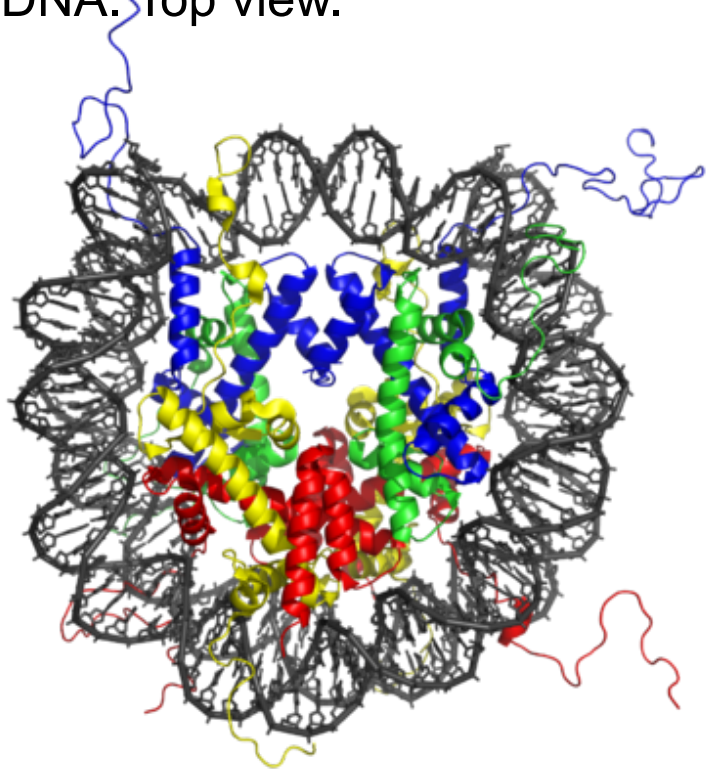
V25: the histone code

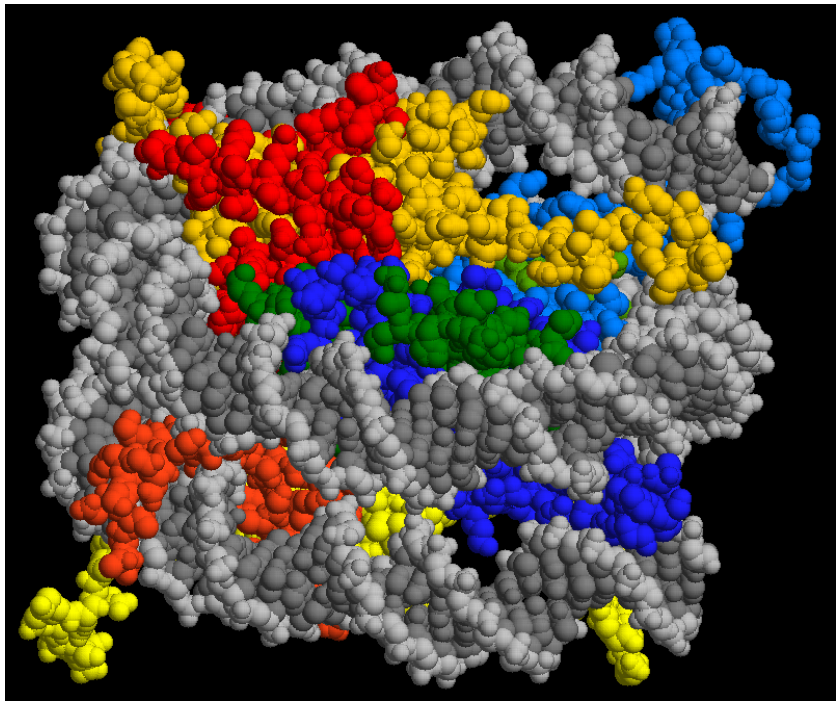
The DNA of eukaryotic organisms is packaged into chromatin, whose basic repeating unit is the **nucleosome**.

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

Basic principles of epigenetics: DNA methylation and histone modfications

The human genome contains ~20 000 genes that must be expressed in specific cells at precise times.

In cells, DNA is wrapped around clusters (octamers) of globular **histone** proteins to form **nucleosomes**.

These nucleosomes of DNA and histones are organized into **chromatin**, the building block of a chromosome.

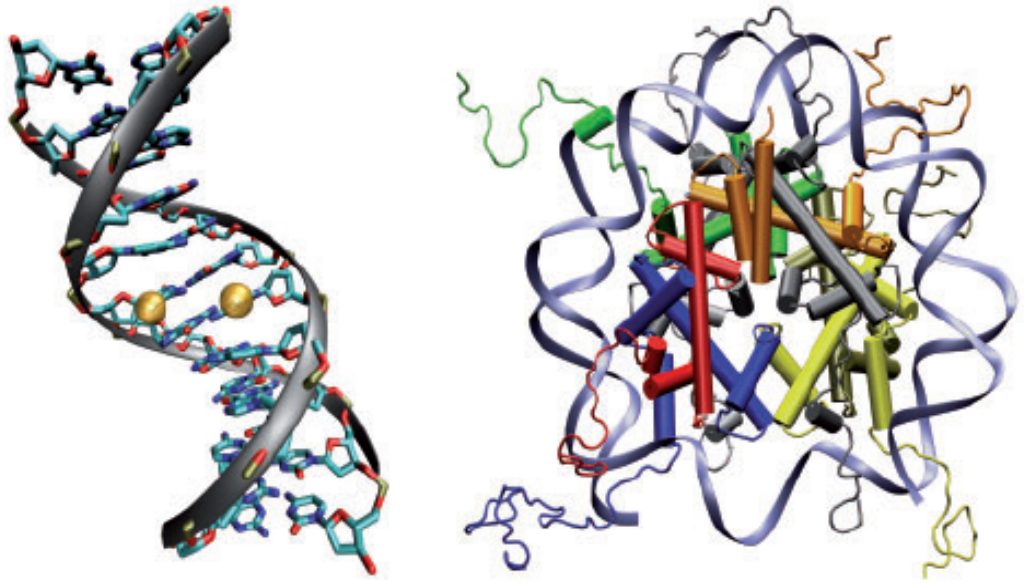


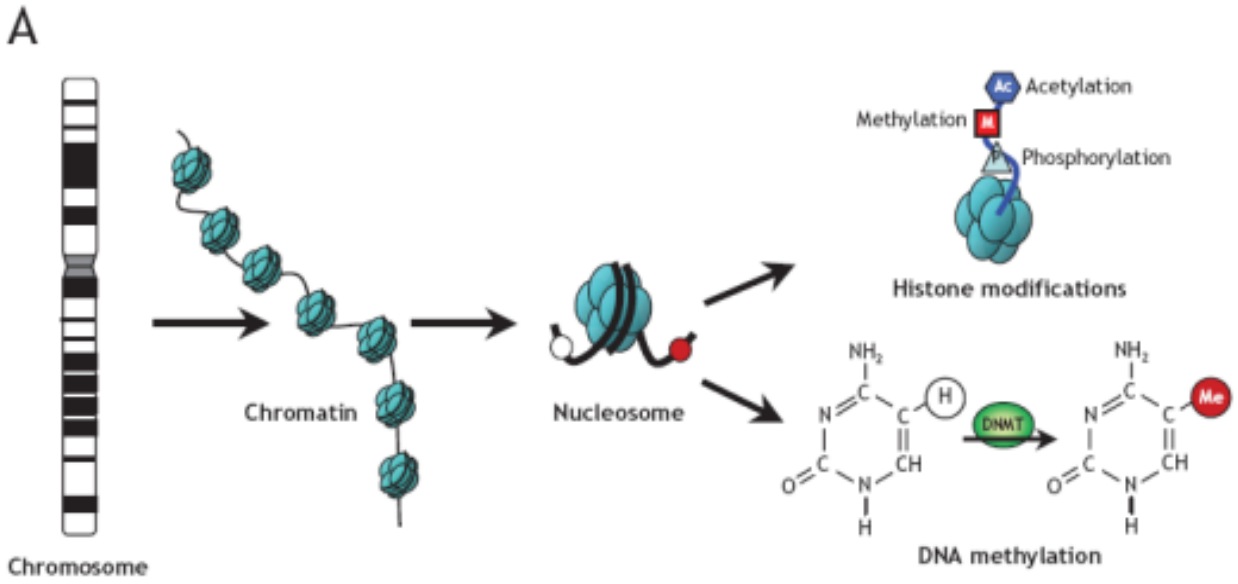
Fig. 1. Carriers of epigenetic information: DNA and nucleosome. The left panel shows a DNA double helix that is methylated symmetrically on both strands (orange spheres) at its center CpG (PDB structure: 329d). DNA methylation is the only epigenetic mechanism that directly targets the DNA. The right panel shows a nucleosome spindle consisting of eight histone proteins (center), around which two loops of DNA are wound (PDB structure: 1KX5). The nucleosome is subject to covalent modifications of its histones and to the binding of non-histone proteins.

Rodenhiser, Mann, CMAJ 174, 341 (2006)

Bock, Lengauer, Bioinformatics 24, 1 (2008)

Epigenetic modifications

Rodenhiser, Mann,
CMAJ 174, 341 (2006)



Reversible and site-specific **histone modifications** occur at multiple sites at the unstructured histone tails through **acetylation**, **methylation** and **phosphorylation**.

DNA methylation occurs at 5-position of cytosine residues within CpG pairs in a reaction catalyzed by DNA methyltransferases (DNMTs).

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.

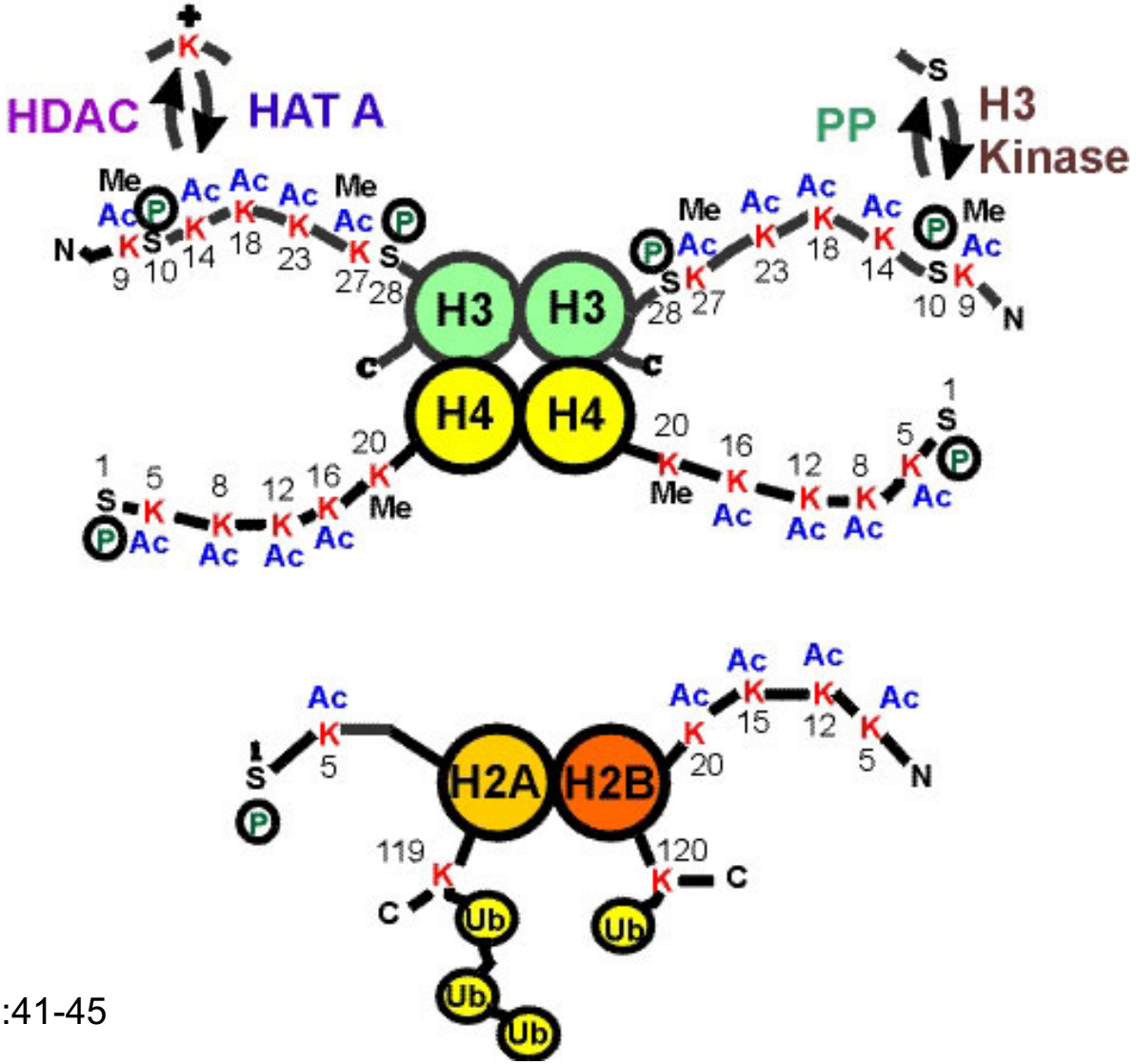
This, in turn affects **gene expression.**

*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.

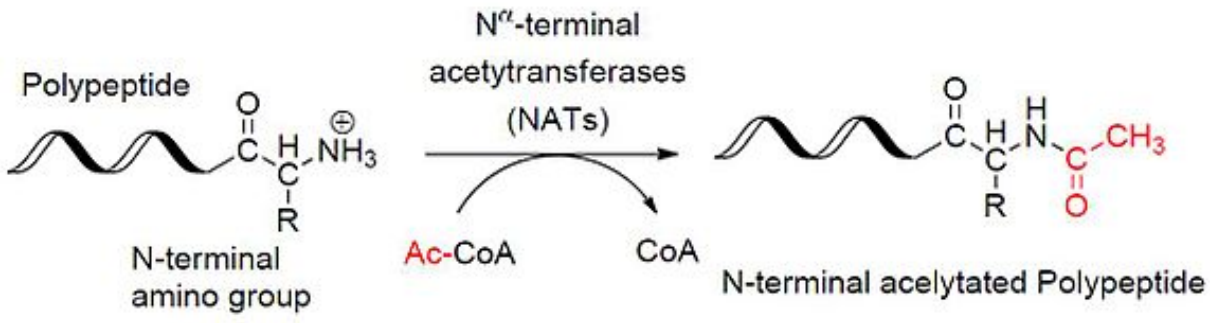
(1) PTMs directly influence the overall structure of chromatin, either over short or long distances.

(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.

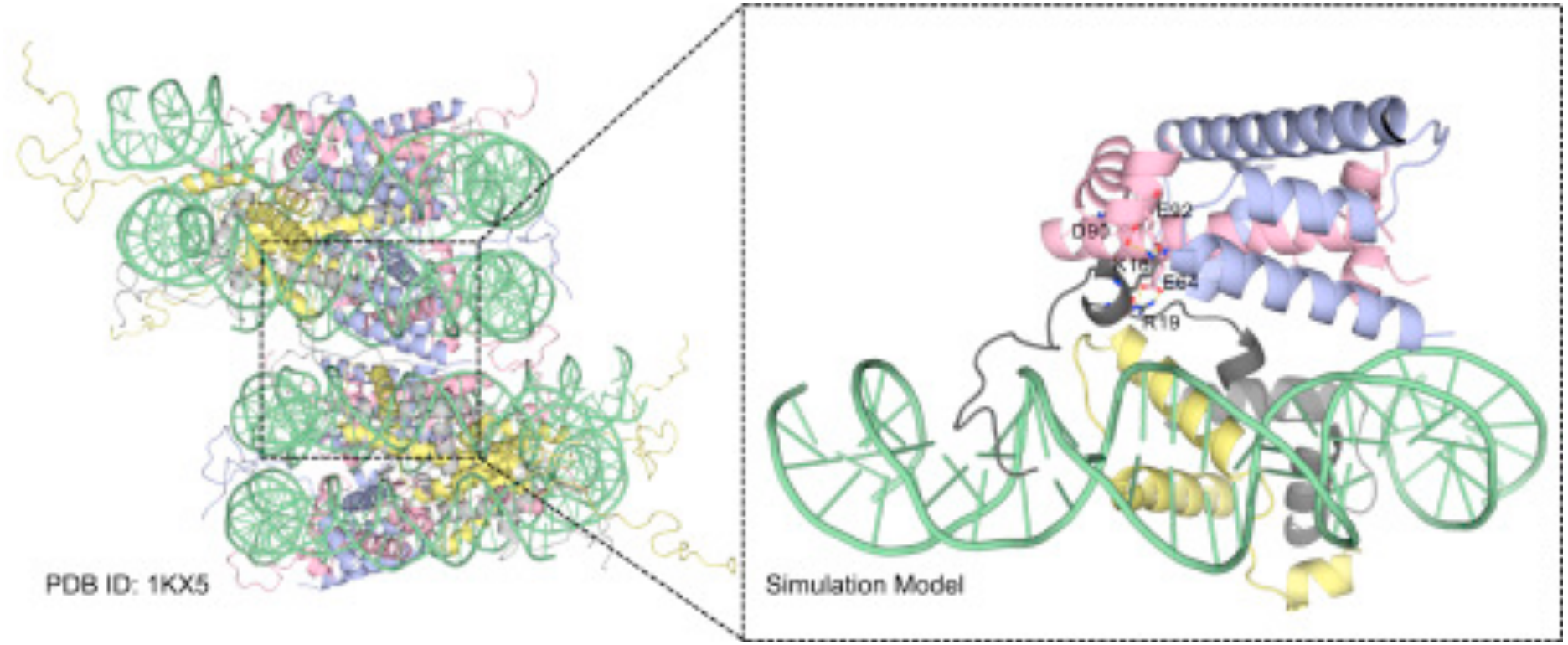
The **H4 tail** probably plays the most important role in inter-nucleosome interaction. Its middle part, the $K^{16}RHRK^{20}$ segment forms a positively charged "**basic patch**".

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

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



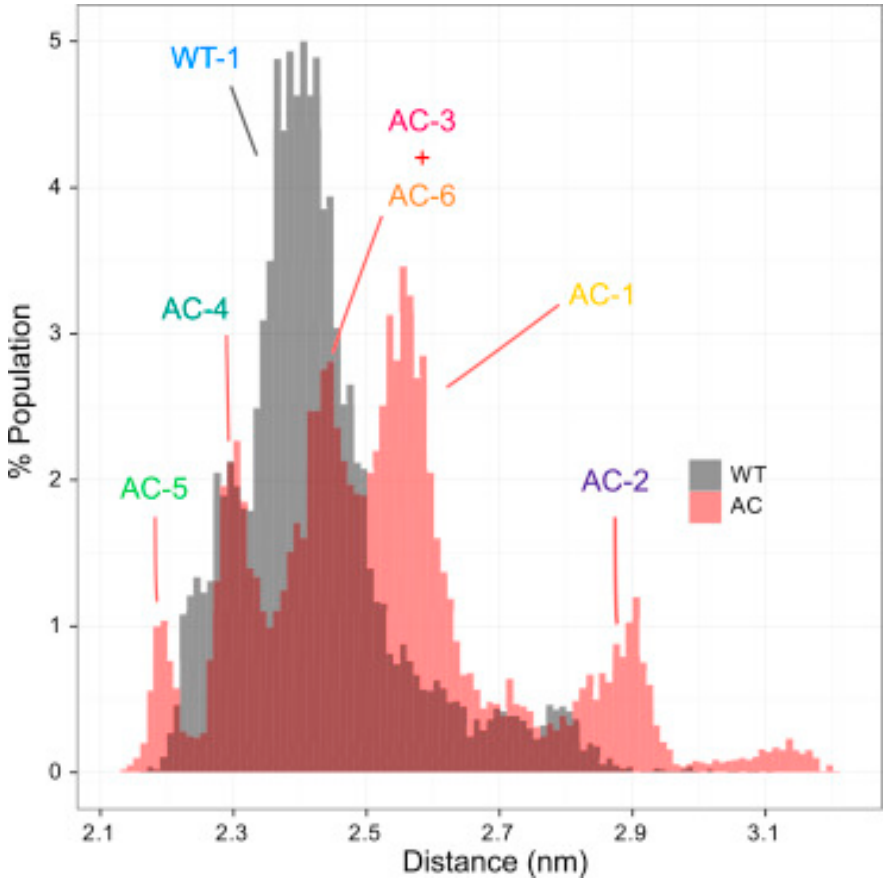
Left: Structure of two nucleosomes from crystal packing

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

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.

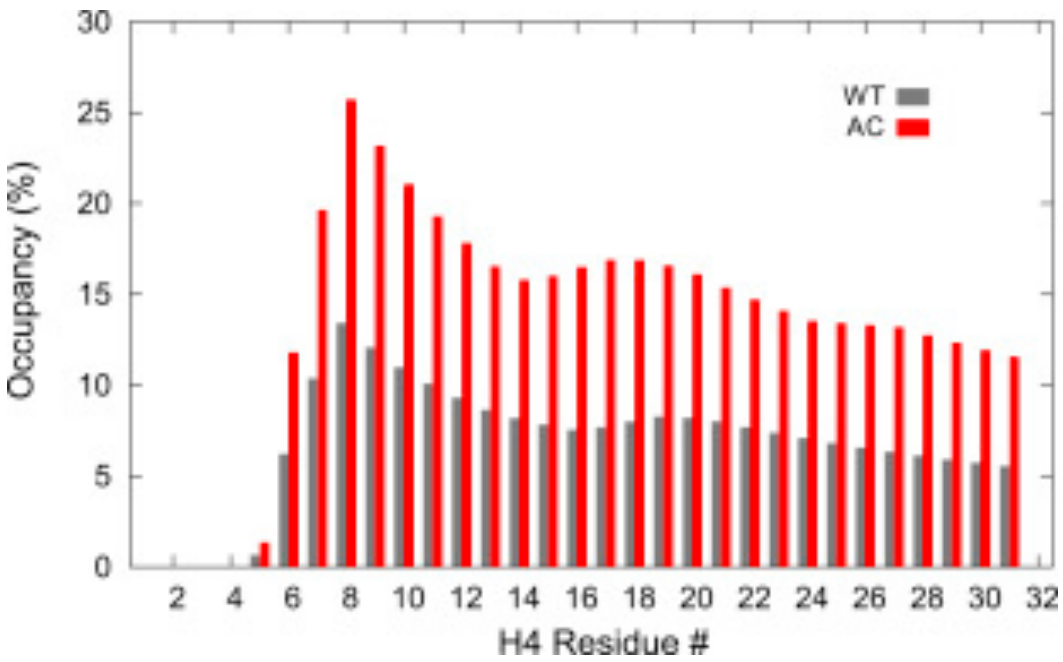
http://www.cell.com/biophysj/abstract/S0006-3495(16)31043-8

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→i hydrogen bonds) were found in the WT system.

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



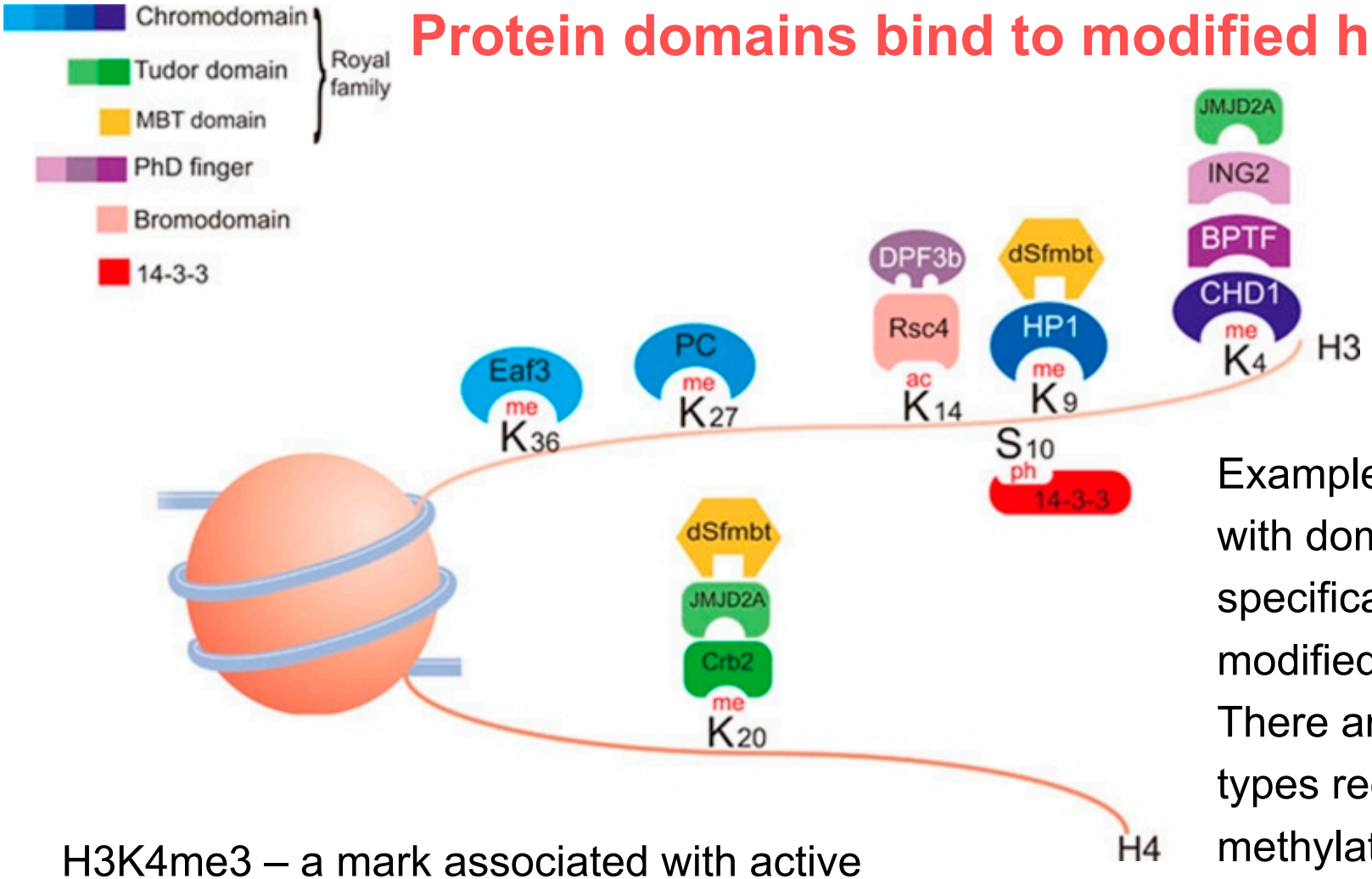
Acetylation disrupts the interaction between the H4 tail and the acidic patch.

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

Protein domains bind to modified histones

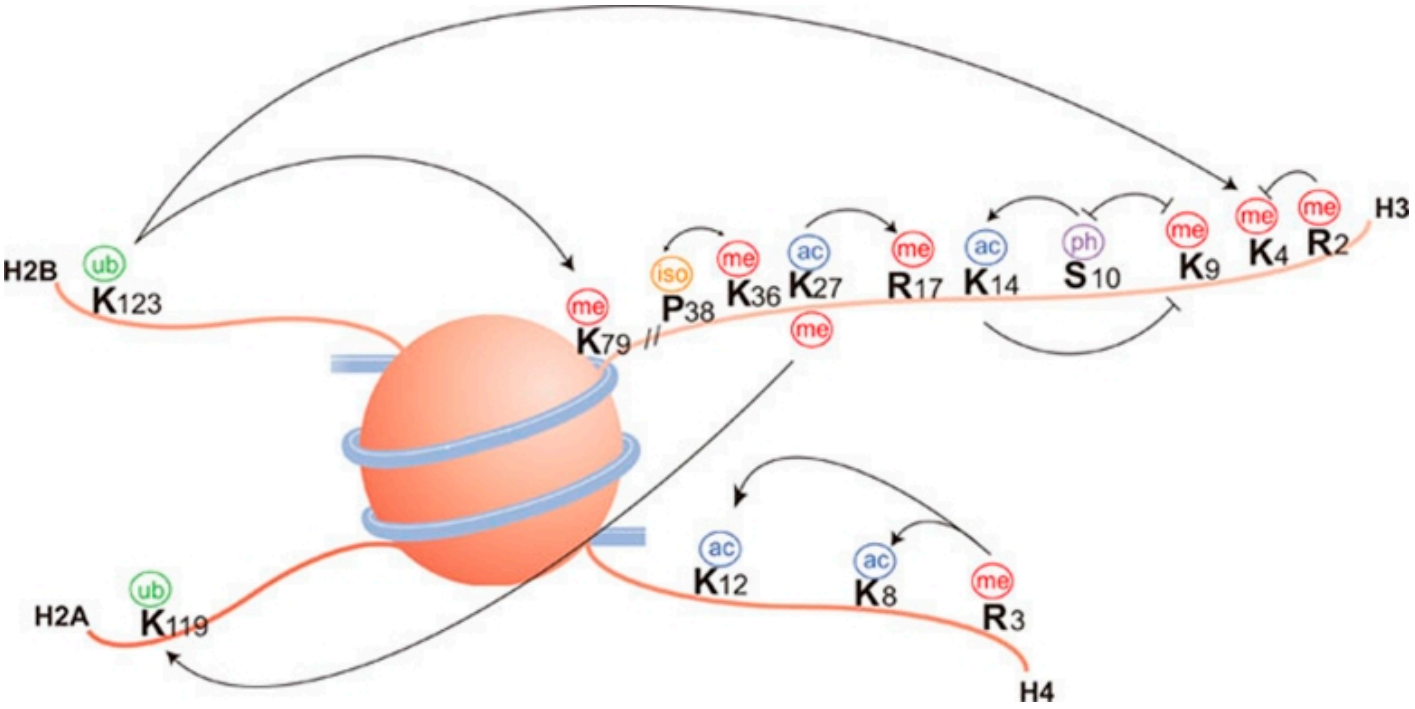


H3K4me3 – a mark associated with active transcription – is recognized by a PHD finger within the ING family of proteins (ING1-5). The ING proteins in turn recruit additional chromatin modifiers such as HATs and HDACs.

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

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

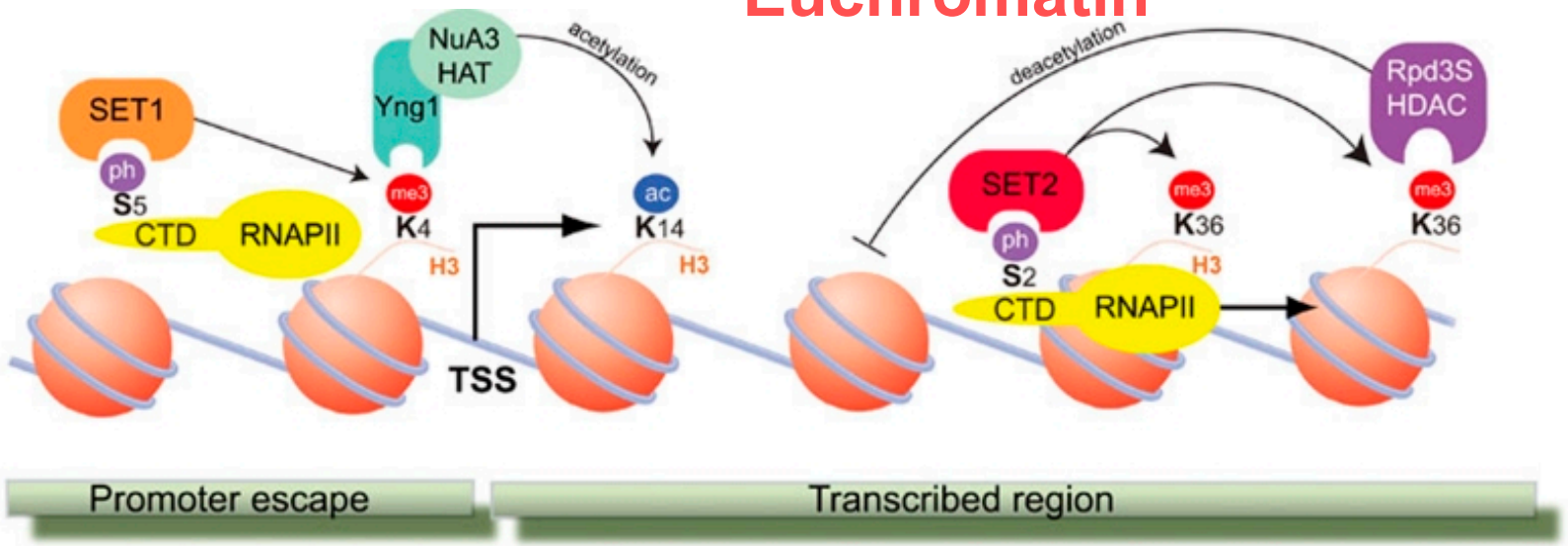
Eukaryotic genomes can be divided into two geographically distinct environments.
(1) a relatively relaxed environment, containing most of the active genes and undergoing cyclical changes during the cell cycle. These **'open'** regions are referred to as **euchromatin**.
(2) other genomic regions, such 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.

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

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.

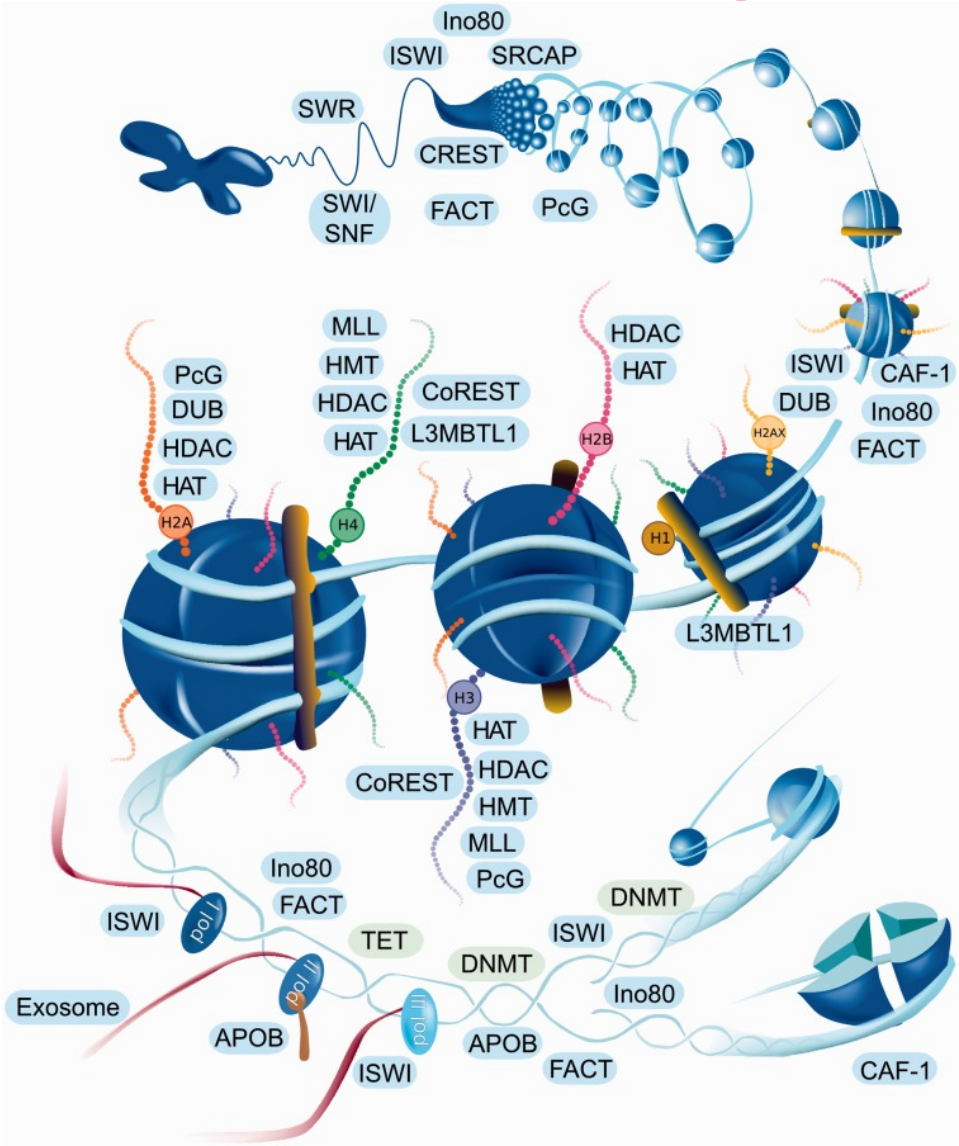
(Right) In contrast, the **scSet2** H3K36 methyltransferase binds to serine 2 phosphorylated CTD of RNAPII, the transcriptional **elongating form** of polymerase.

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

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

Epifactors database



Side view shows two windings of DNA and two histone layers

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!

Database (Oxford). 2015; 2015: bav067.

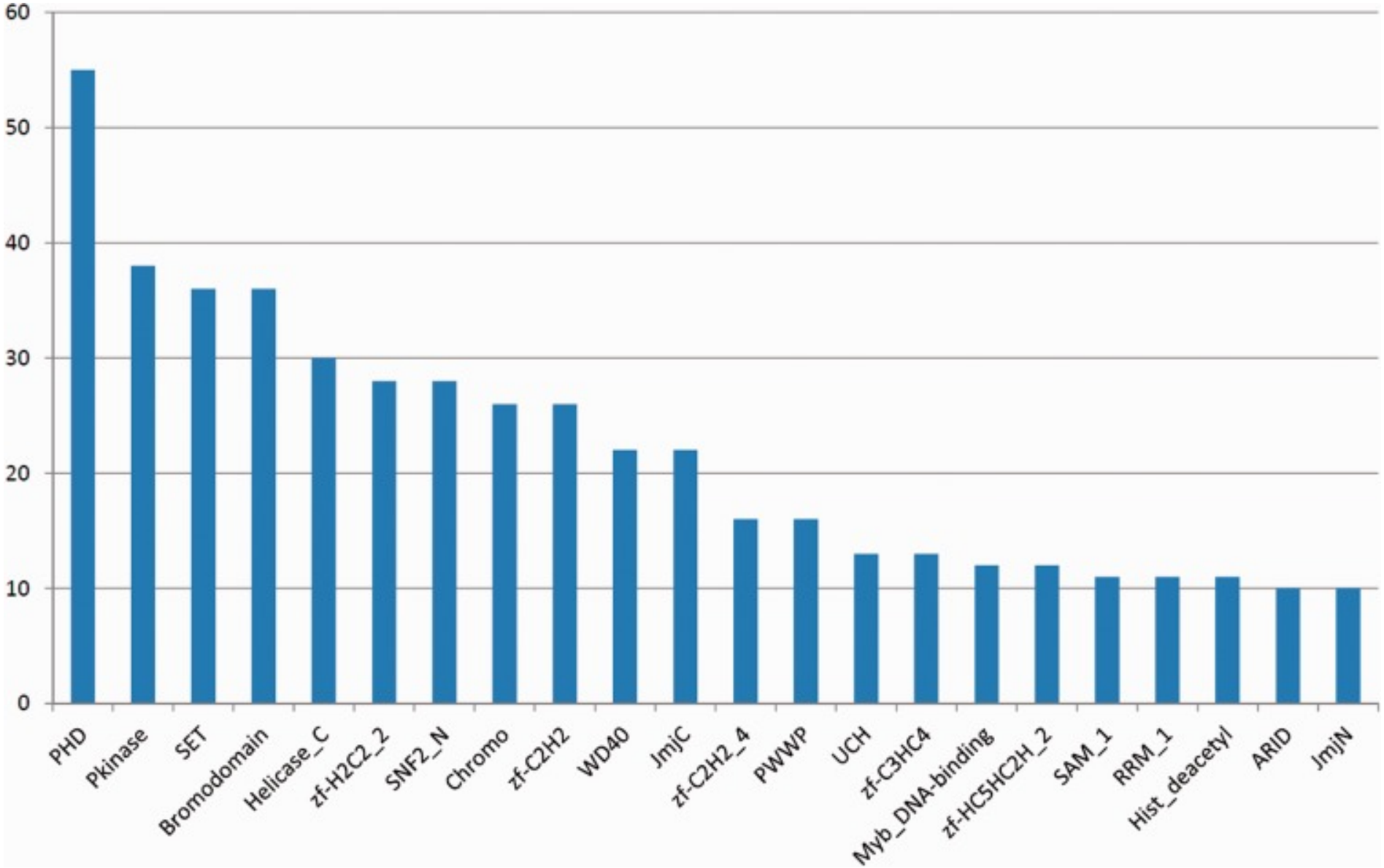
Frequency of main annotation terms of epifactor proteins

Function	Count
DNA modification	22
RNA modification	30
Chromatin remodeling	101
Chromatin remodeling cofactor	41
Histone chaperone	26
Histone modification	15
Histone modification cofactor	12
Histone modification read	90
Histone modification write	158
Histone modification write cofactor	95
Histone modification erase	66
Histone modification erase cofactor	58
Polycomb group (PcG) protein	29
Scaffold protein	12
TF	53

Modification	Count
DNA methylation	7
DNA demethylation	12
DNA hydroxymethylation	5
RNA degradation	9
mRNA editing	10
Histone methylation	127
Histone acetylation	139
Histone phosphorylation	55
Histone ubiquitination	61
Histone sumoylation	2
Histone citrullination	4
TF activator	18
TF repressor	27

Database (Oxford). 2015; 2015: bav067.

Most frequently occurring Pfam domains



Database (Oxford). 2015; 2015: bav067.

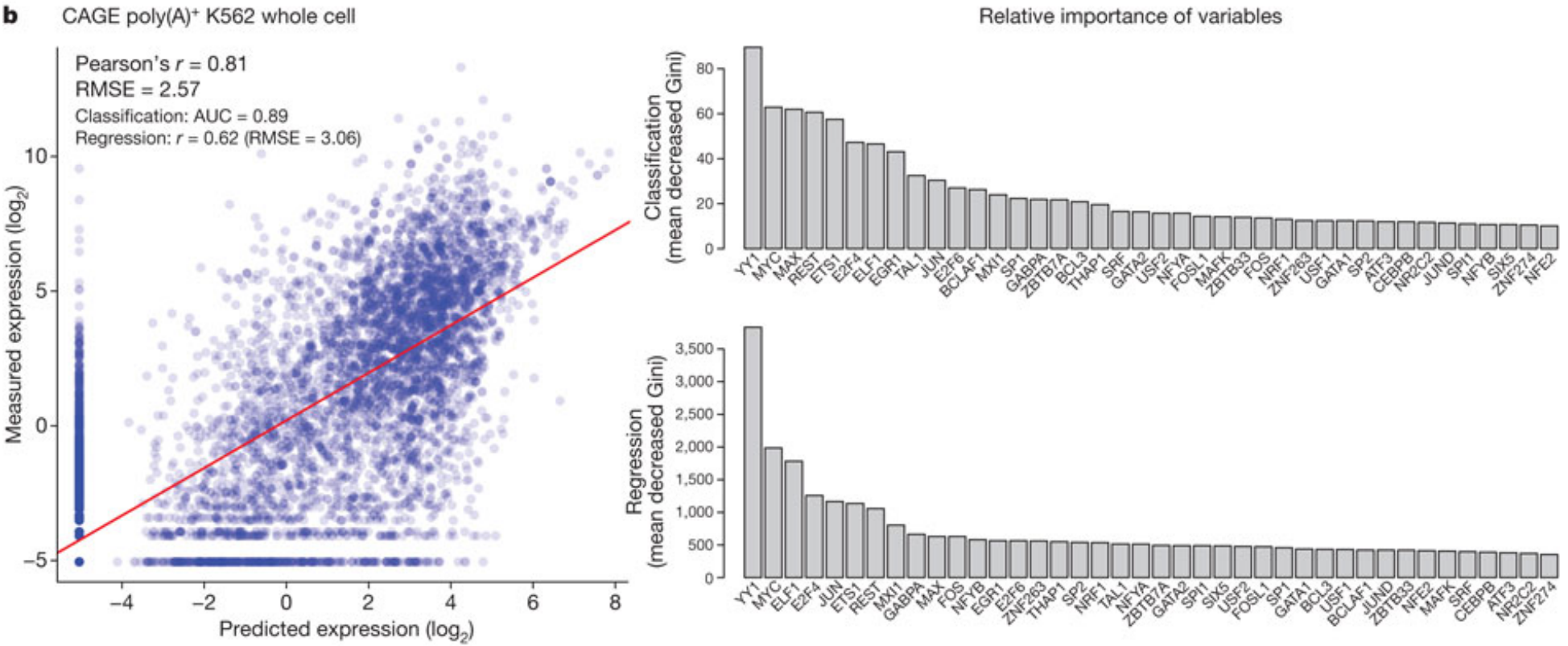
ENCODE

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



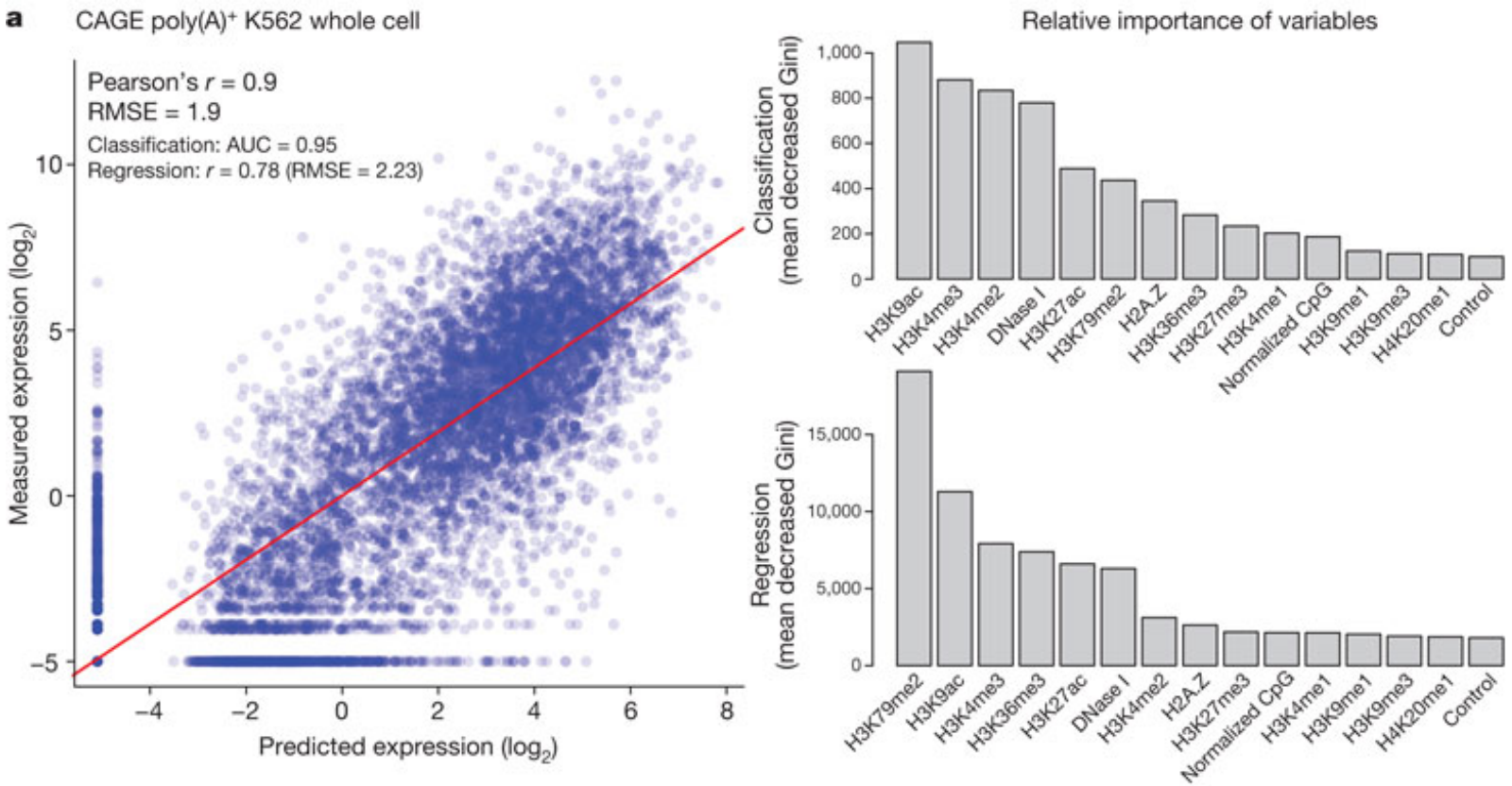
Correlative models between TF binding and RNA production in K562 cells.

(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

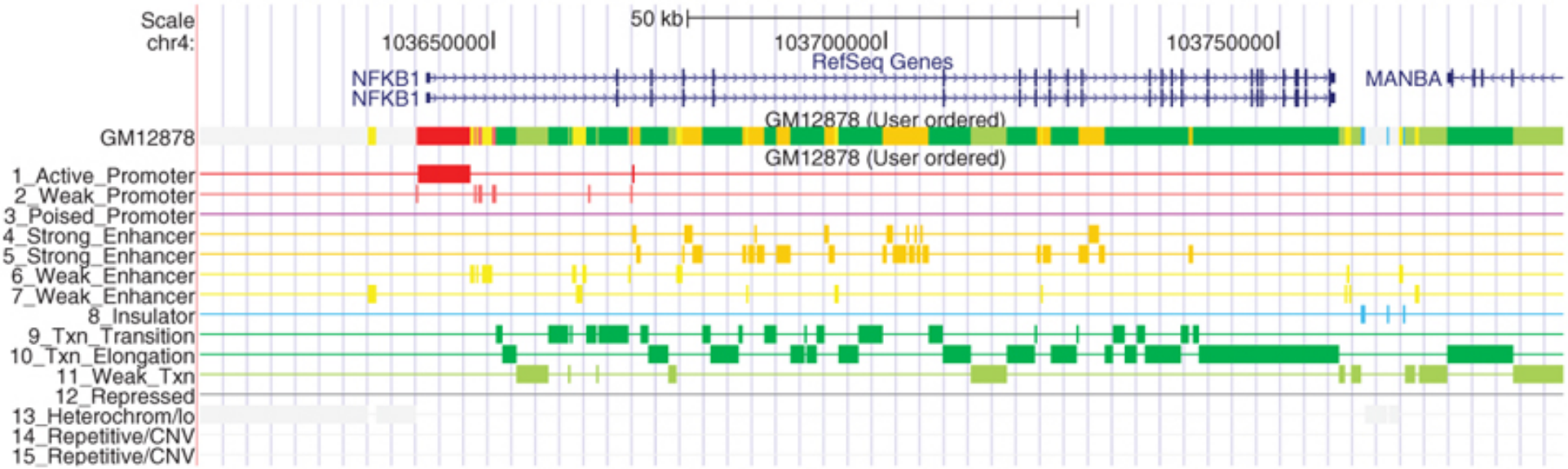
Outputs of the parameter initialization procedure:

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.

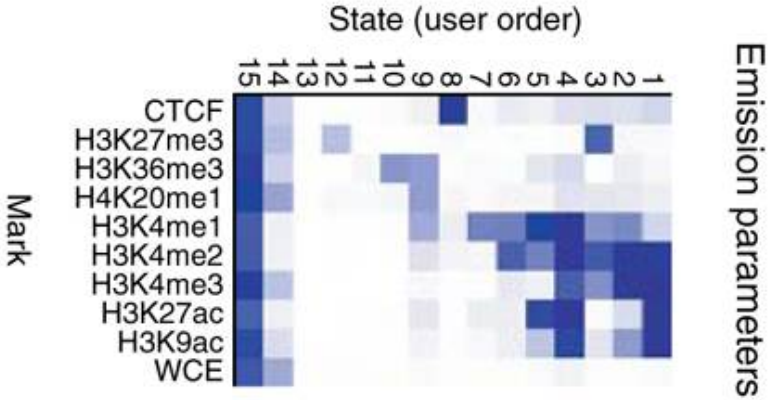
Shown as example is the NFKB1 (subunit of nuclear factor kappa B, this TF controls more than 200 genes).

Active promoter, **transcription transcription + elongation**,

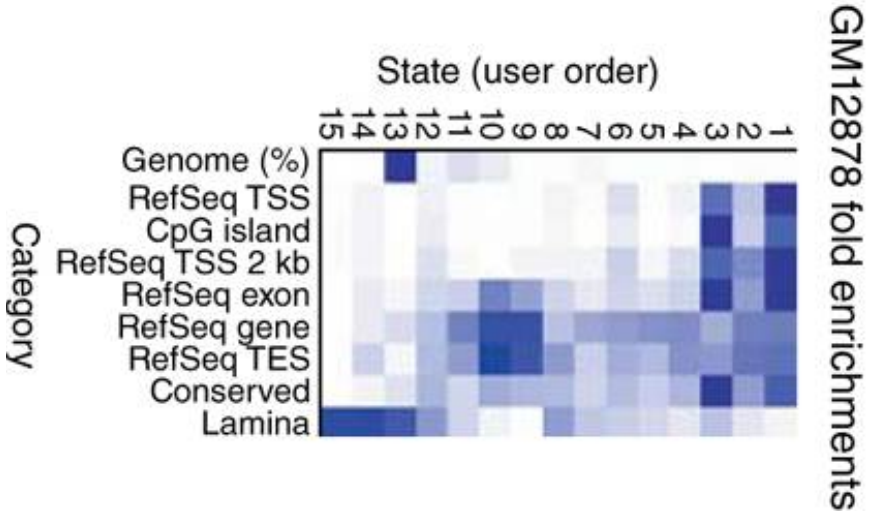
insulator before next gene MANBA

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

ChromHMM



(**left**) which PTMs contribute to which states.



(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?

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.

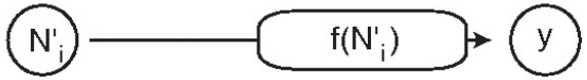
Martin Vingron
MPI Berlin

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

Relate histone modifications to expression

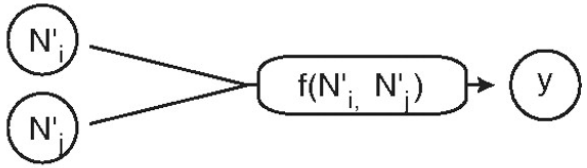
One-modification model (41 models)

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



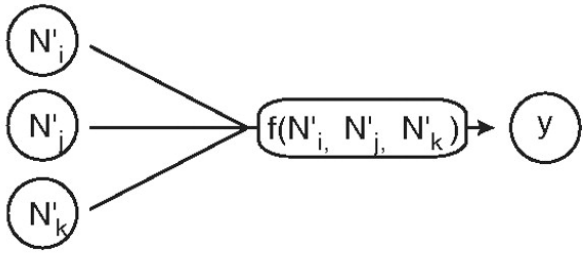
Two-modifications model (820 models)

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



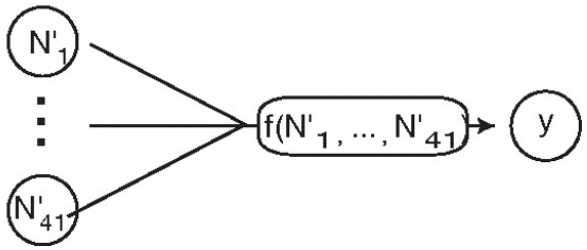
Three-modifications model (10,660 models)

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



Full model (1 model)

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



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

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

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.

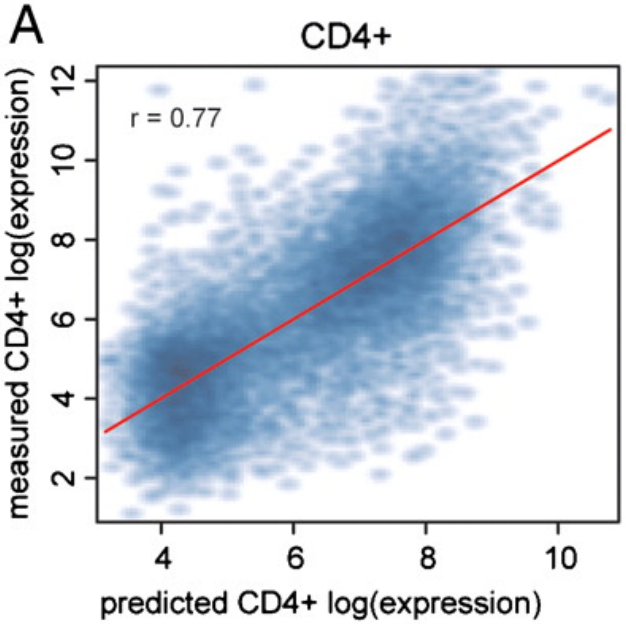
In the **two-modifications models**, i and j cover all combinations of two modifications without repetition.

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.

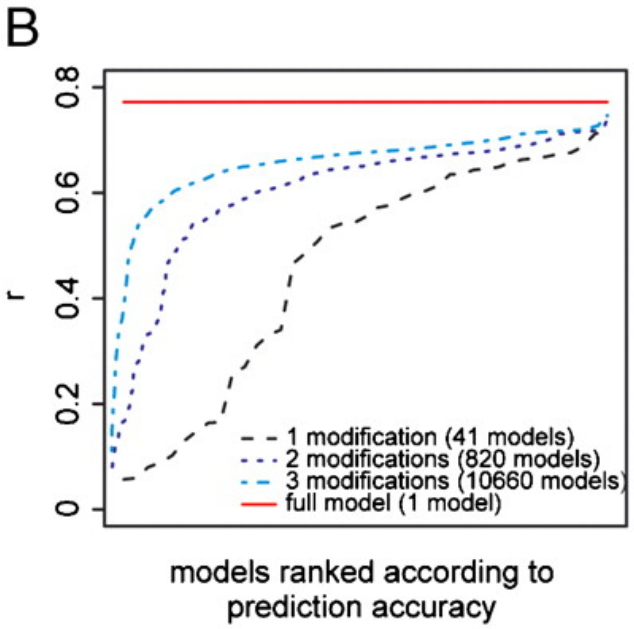
Red line : linear fit between predicted and measured expression ($y = 0.99x + 0.02$), which are highly correlated ($r = 0.77$)

→ 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



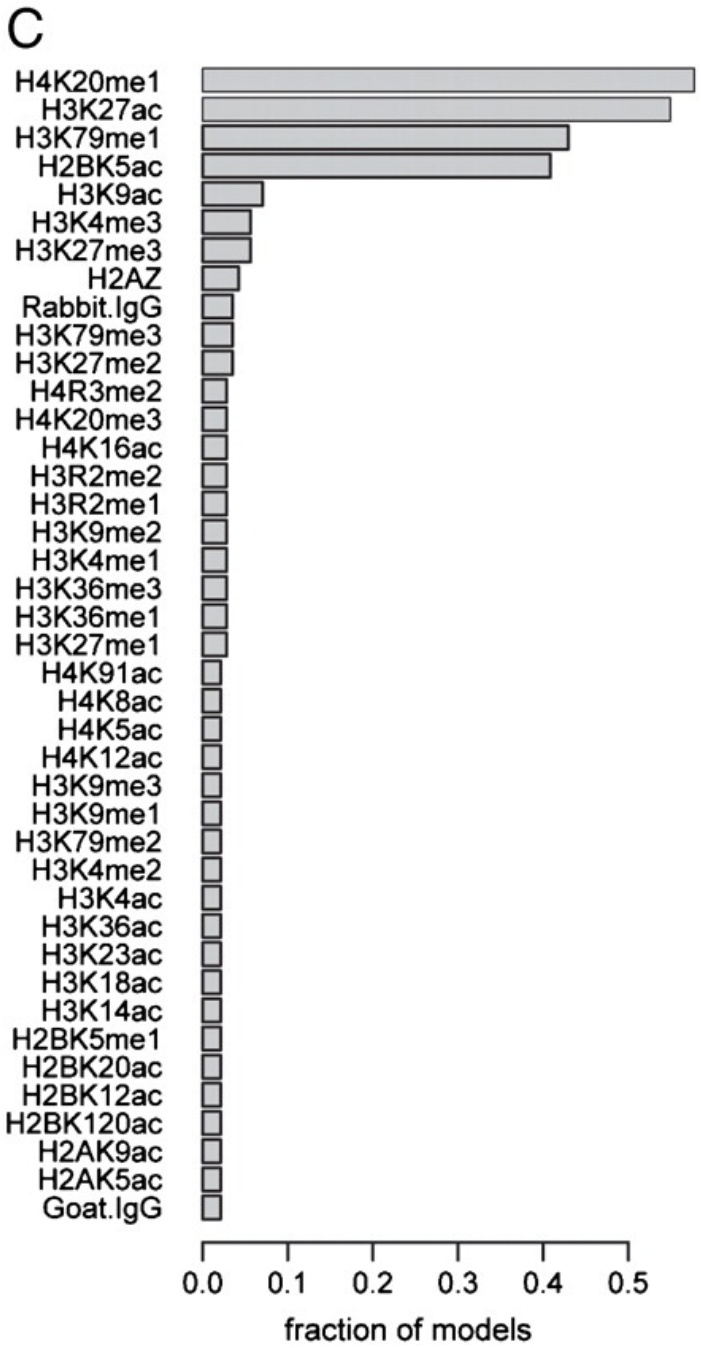
Comparison of prediction accuracy between all possible one-modification, two-modifications, three-modifications models, and the full model for CD4+ T-cells.

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.

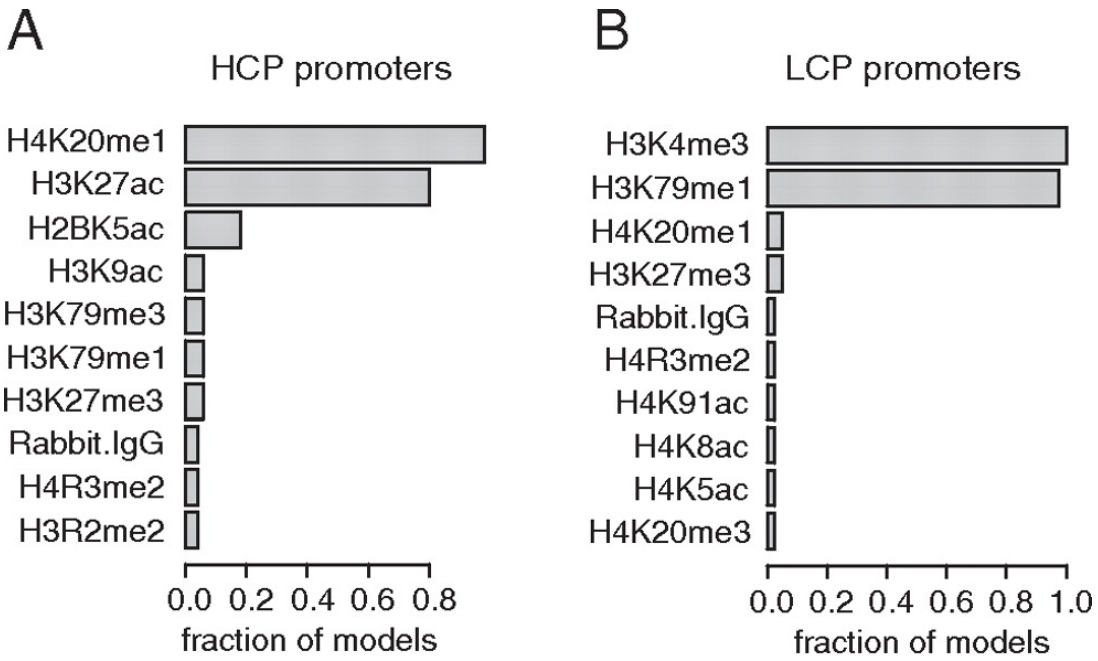
H3K4me3 is thought to be a mark of transcription initiation.

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



Frequency of different histone PTMs in best scoring three-modifications models among 50 HCP models and 40 LCP models.

Only the top ten modifications are depicted.

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

(p-values hypergeometric test 9.97e-43, 2.58e-31, and 0.003)

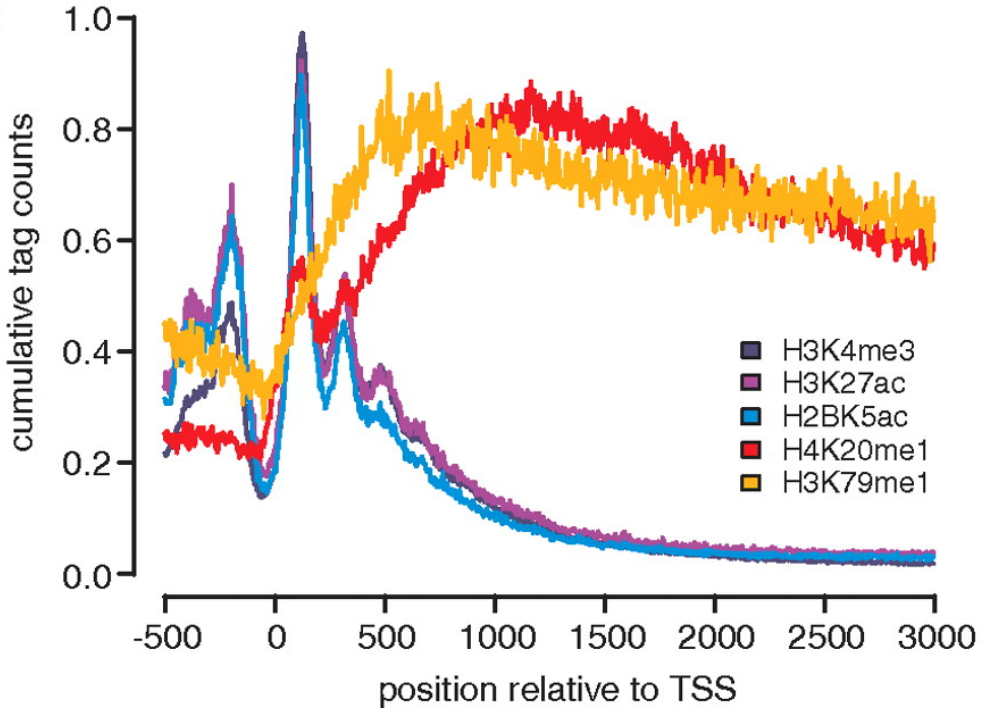
(B) H3K4me3 and H3K79me1 are significantly overrepresented in the LCPs

(p-values of the hypergeometric test 9.71e-36 and 2.1e-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



Normalized cumulative tag counts in the region of -500 base pairs to 3,000 base pairs surrounding the transcription start site of RefSeq genes in CD4+ T-cells for the 5 important modifications identified by our analysis.

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

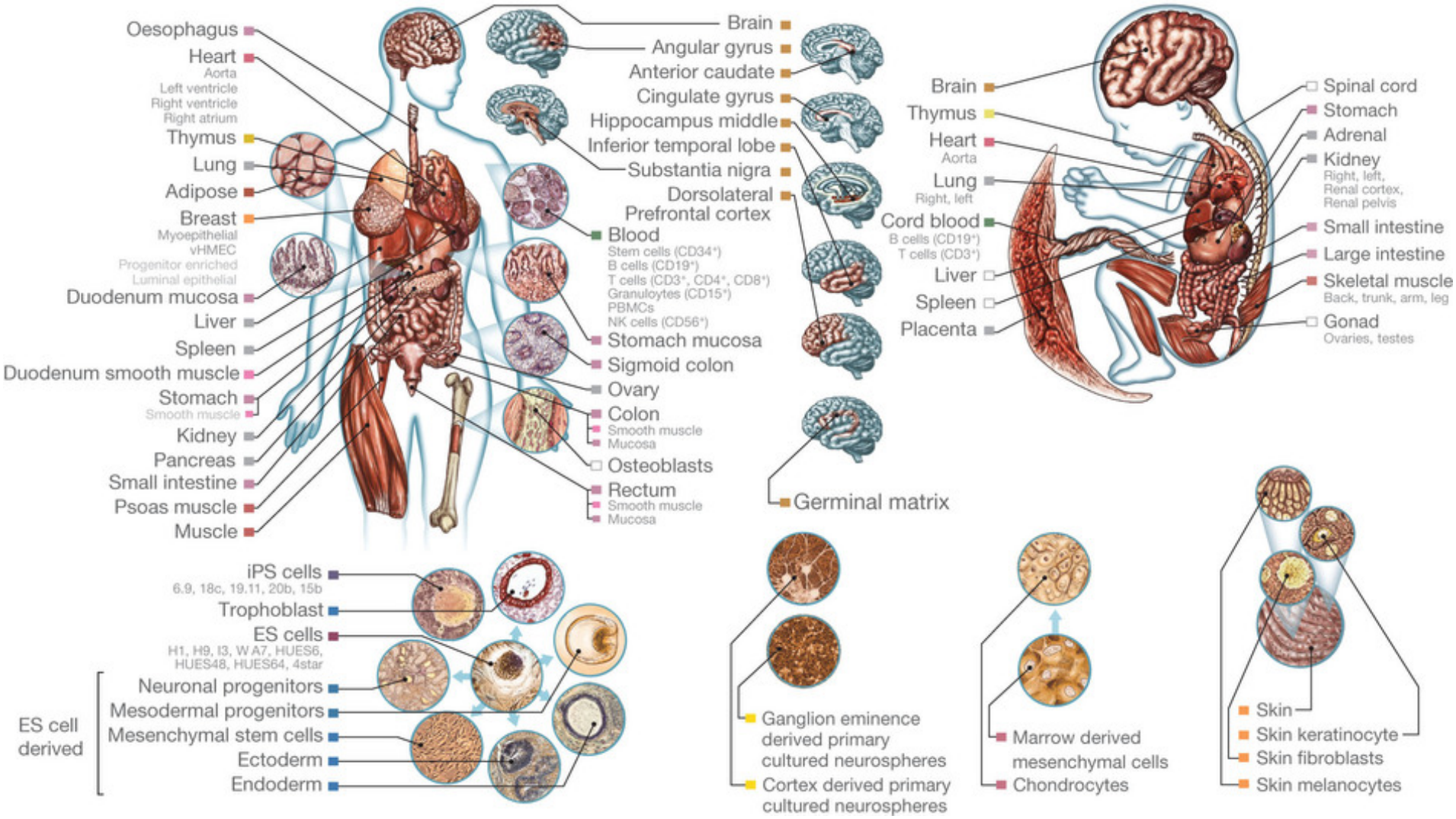
They found high correlation of predicted and measured expression values for both CD36+ (r = 0.75) and CD133+ (r = 0.63) cells.

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

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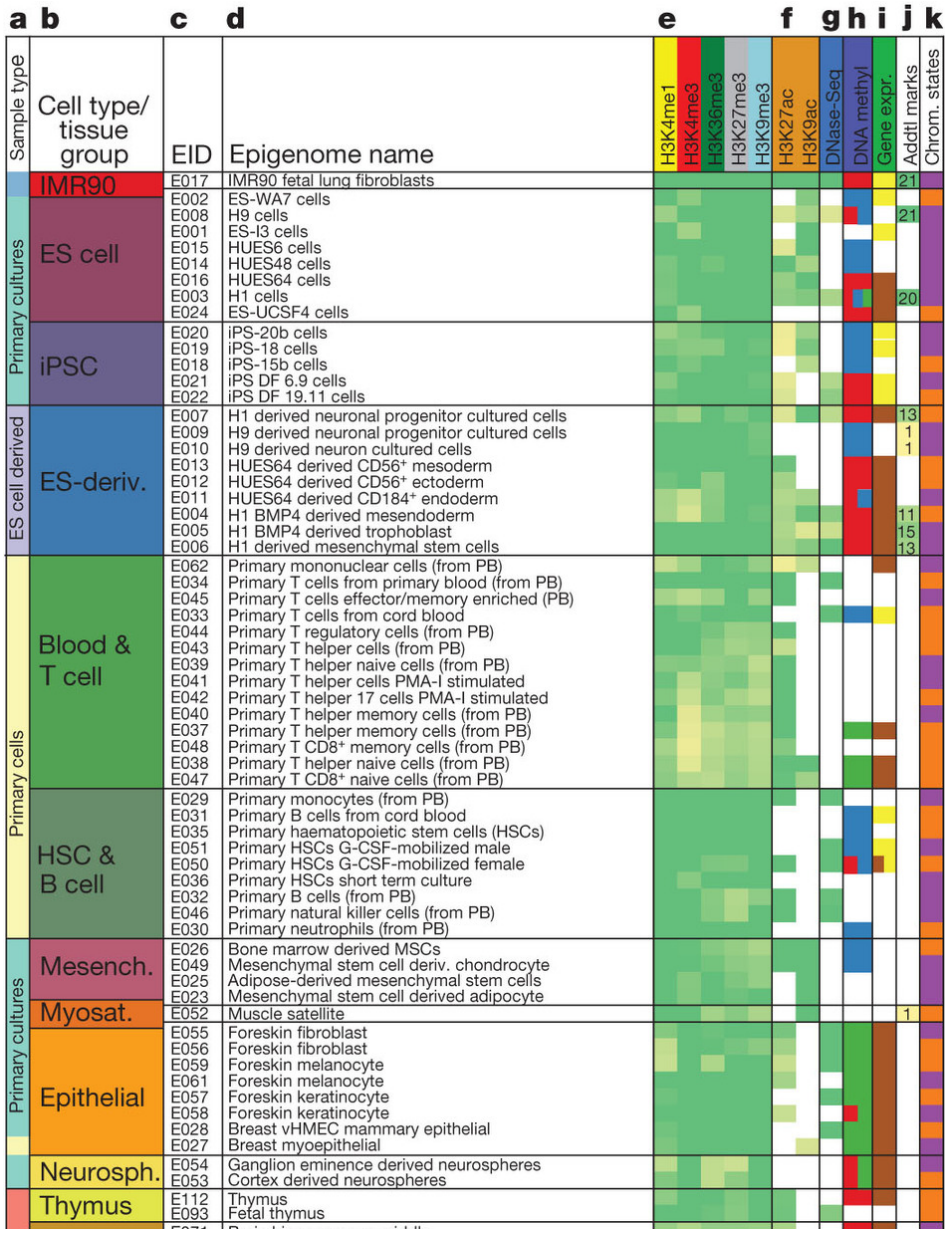
Data sets available for 111 epigenomes



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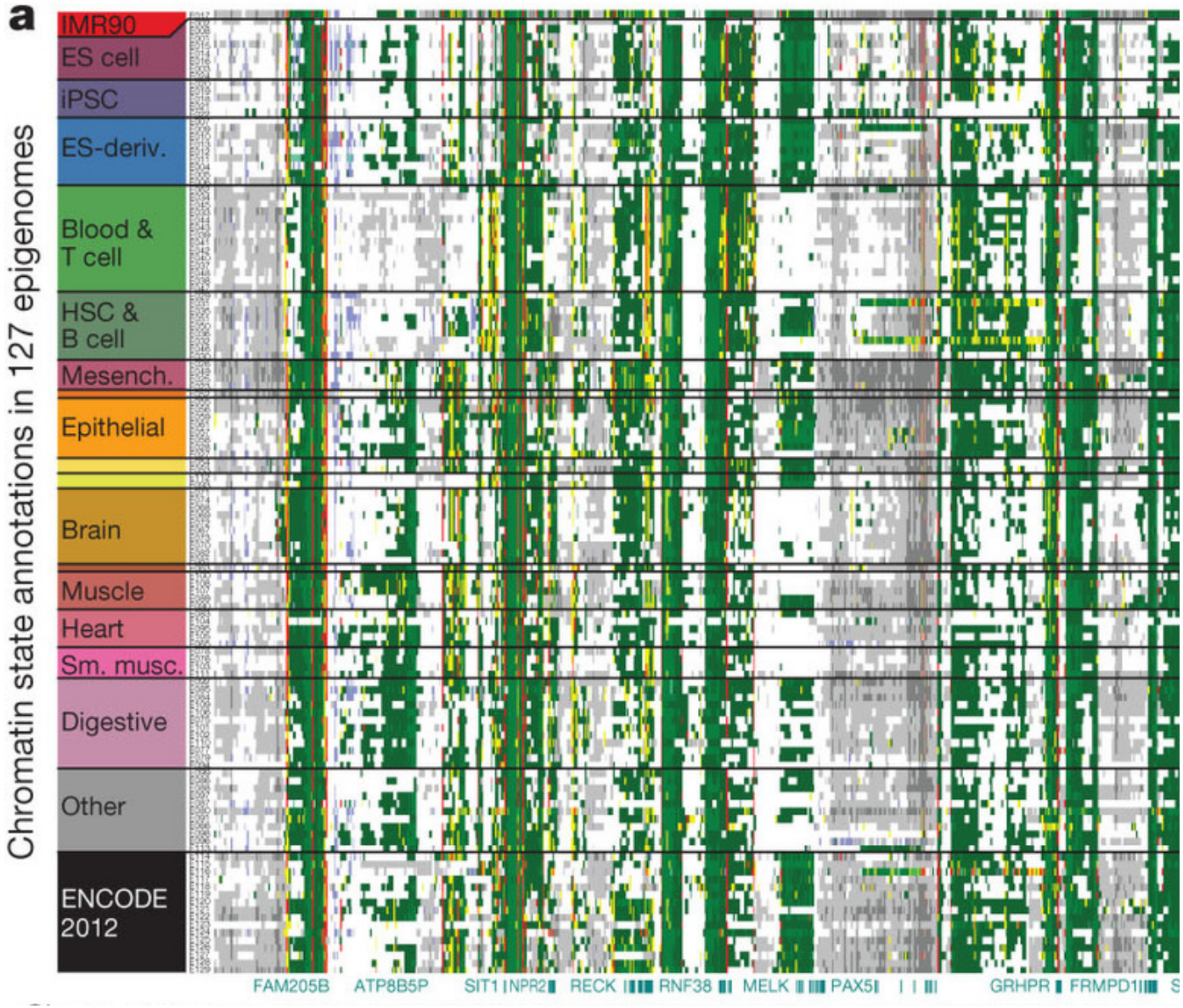
Nature 518, 317 (2015).

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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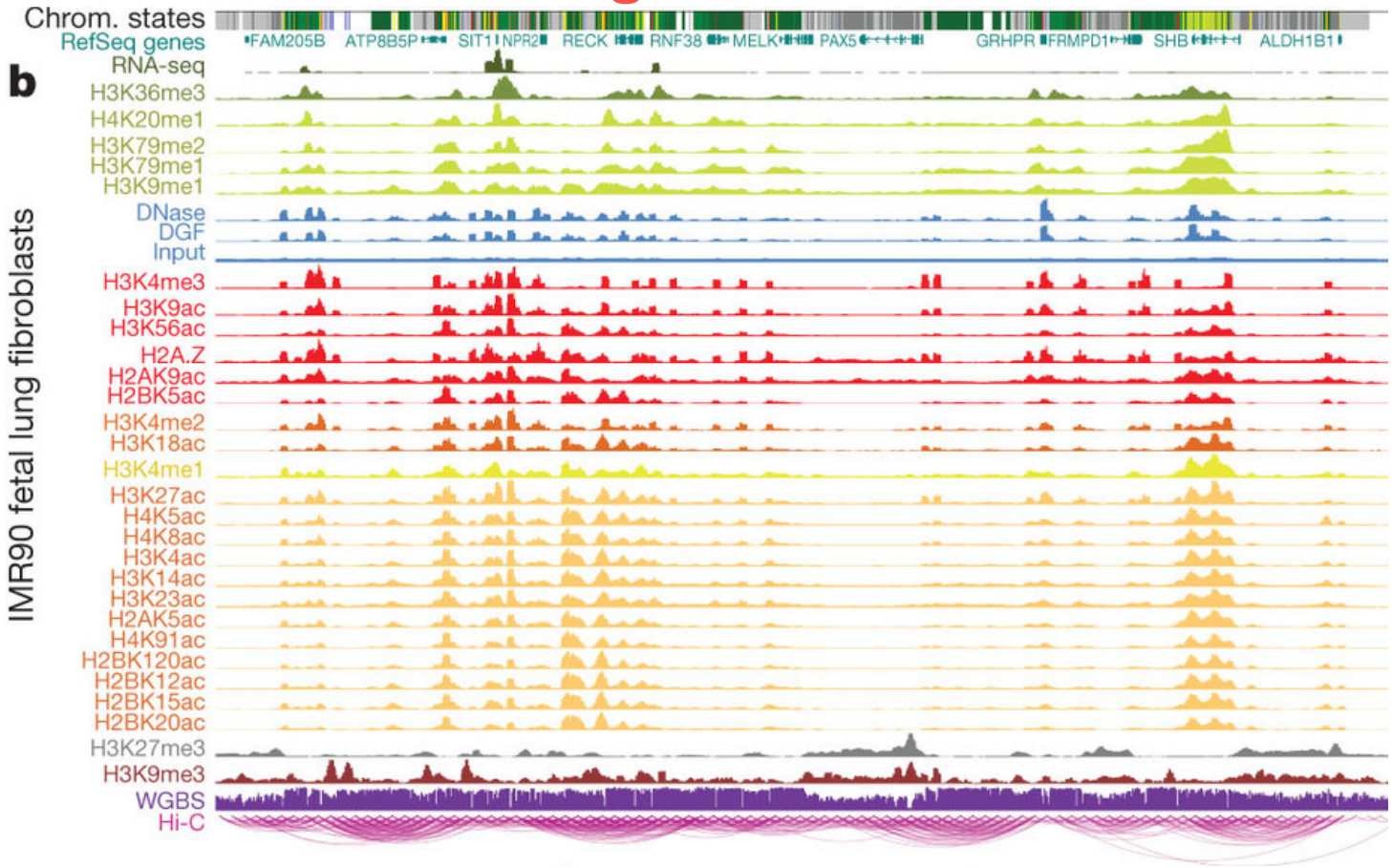
Signal tracks for IMR90



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.

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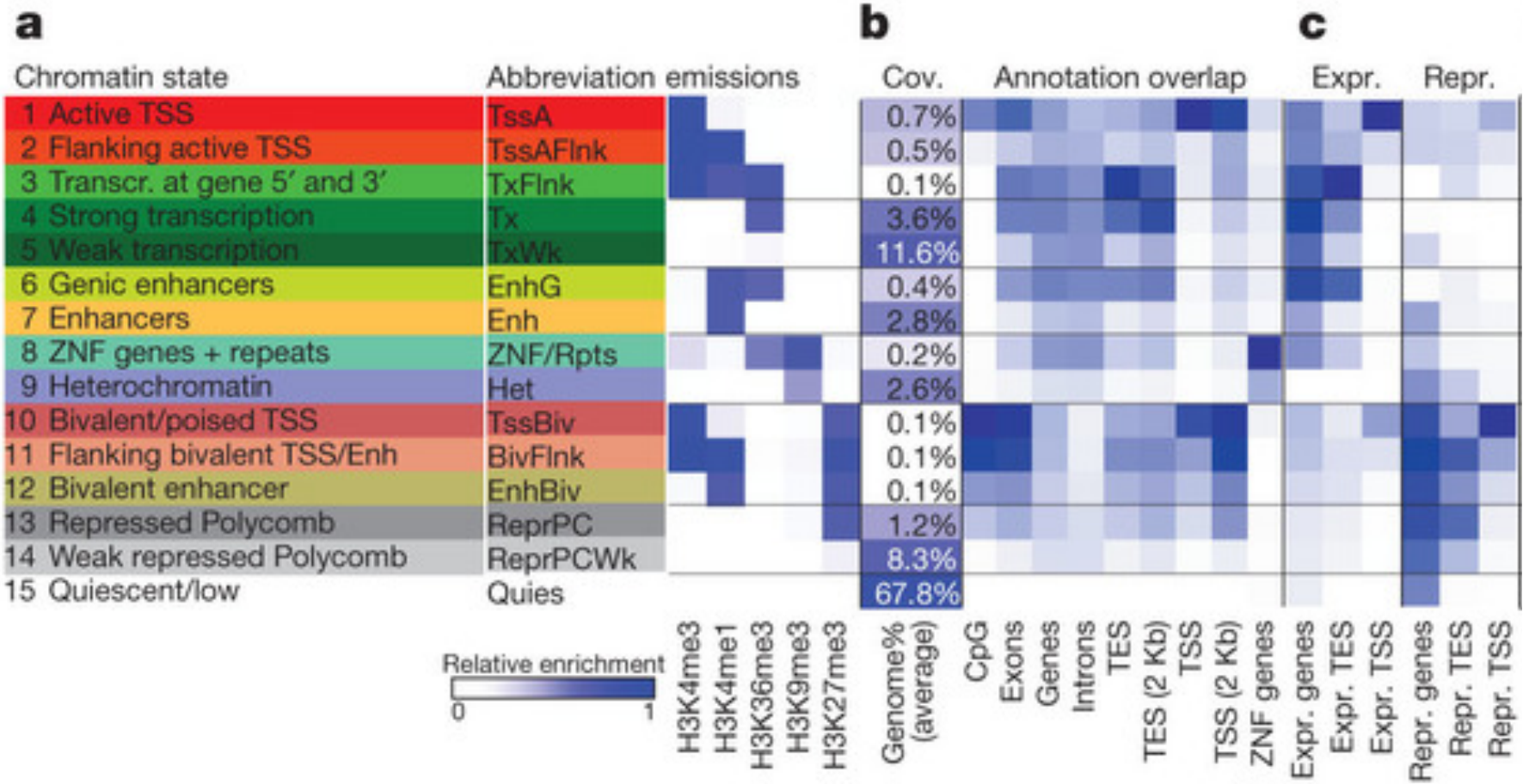
Training of recurring 15-states chromatin model



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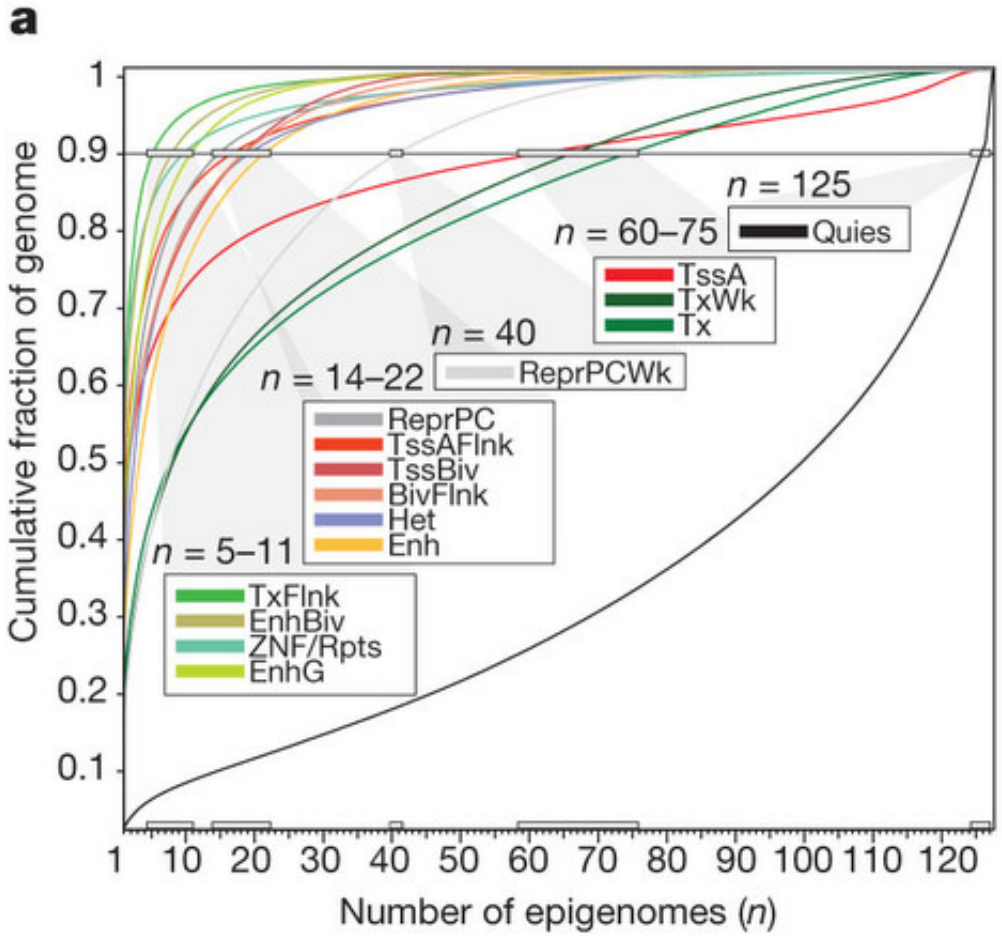
Consisteny of chromatin states across genomic positions



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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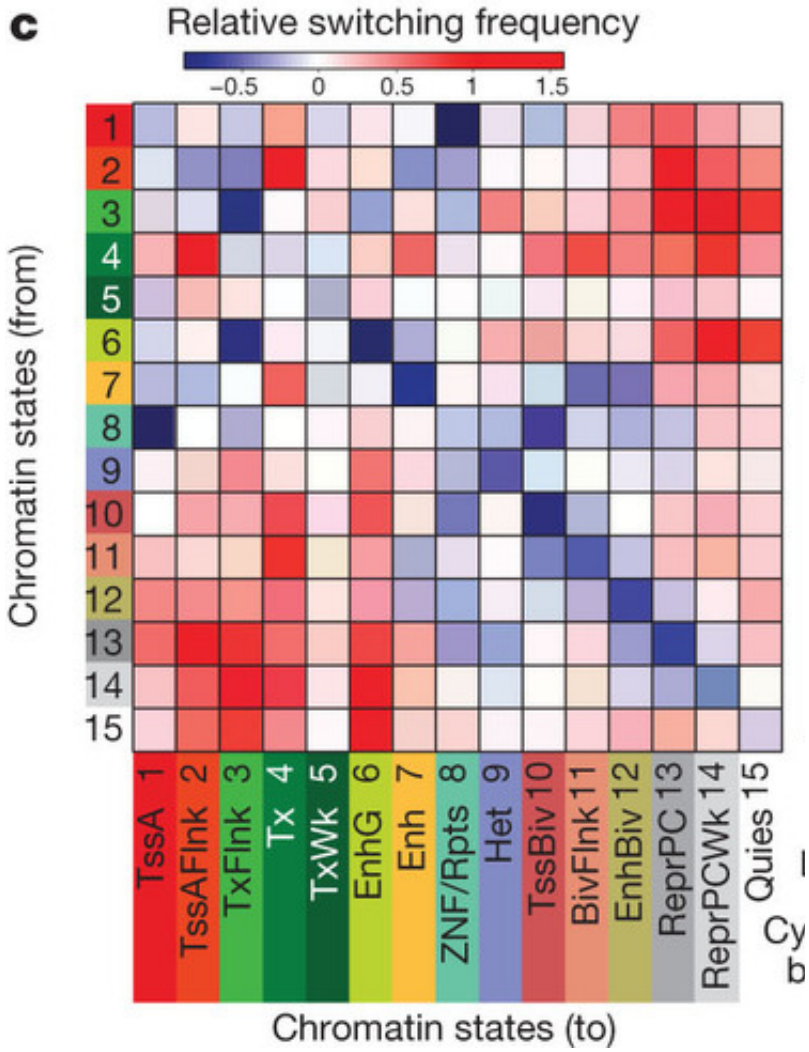
Relative switching between states



More frequent switching found between active states and repressed states.

This is consistent with activation and repression of regulatory regions.

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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.

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