National Institute of Environmental Health Sciences
Your Environment. Your Health.

Reality Check – How far has science brought us?

Trevor Archer | U.S. NIH/ National Institute of Environmental Health Sciences

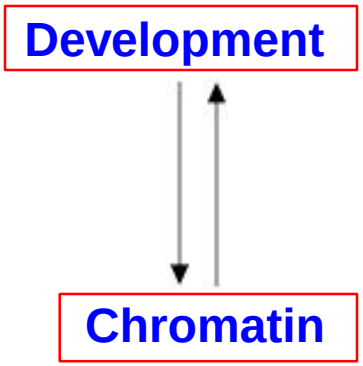
U.S. Environmental Protection Agency
Epigenetics and Cumulative Risk Workshop
Arlington, VA
September 2 – 3, 2015

What is epigenetics?

•'Epigenetics' was coined by Waddington as an English equivalent of Entwicklungsmechanik –the branch of science that undertakes a causal analysis of embryonic development by experimental means.

In modern biology, *epigenetics* has two closely related meanings:

The study of the processes involved in the unfolding development of an organism. This includes phenomena such as X-chromosome inactivation in mammalian females, and gene silencing within an organism.



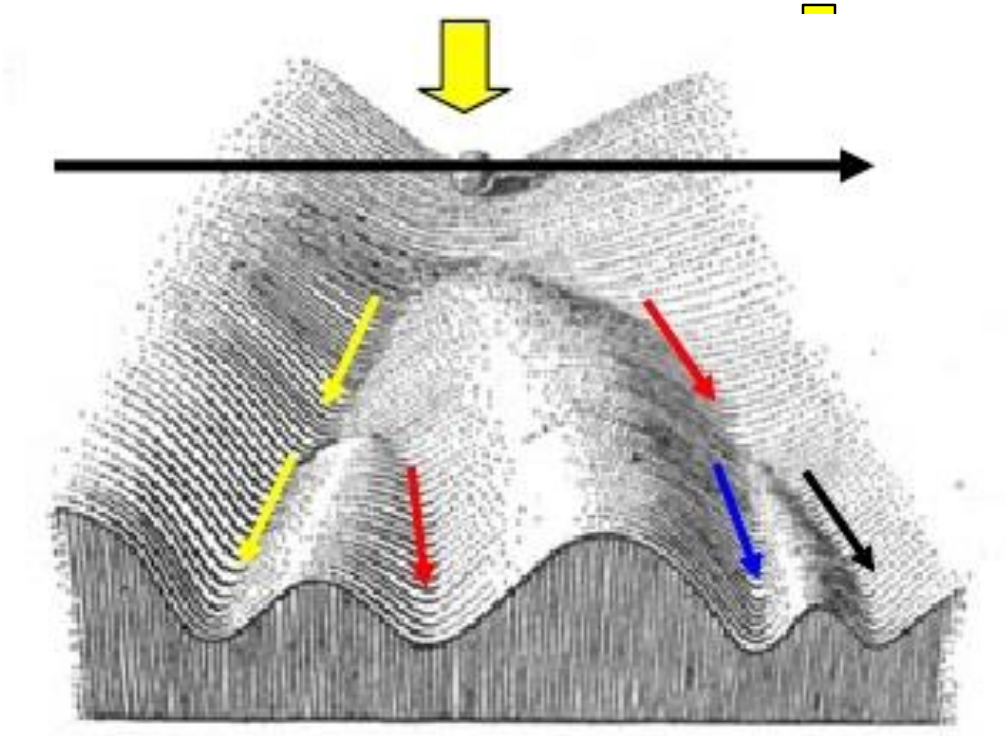
The study of *heritable* changes in gene function that occur without a change in the sequence of nuclear DNA

Medawar & Medawar (1983): "Genetics proposes: epigenetics disposes.'"

Epigenetics

The study of the processes involved in the unfolding development of an organism

Epigenetic landscapes

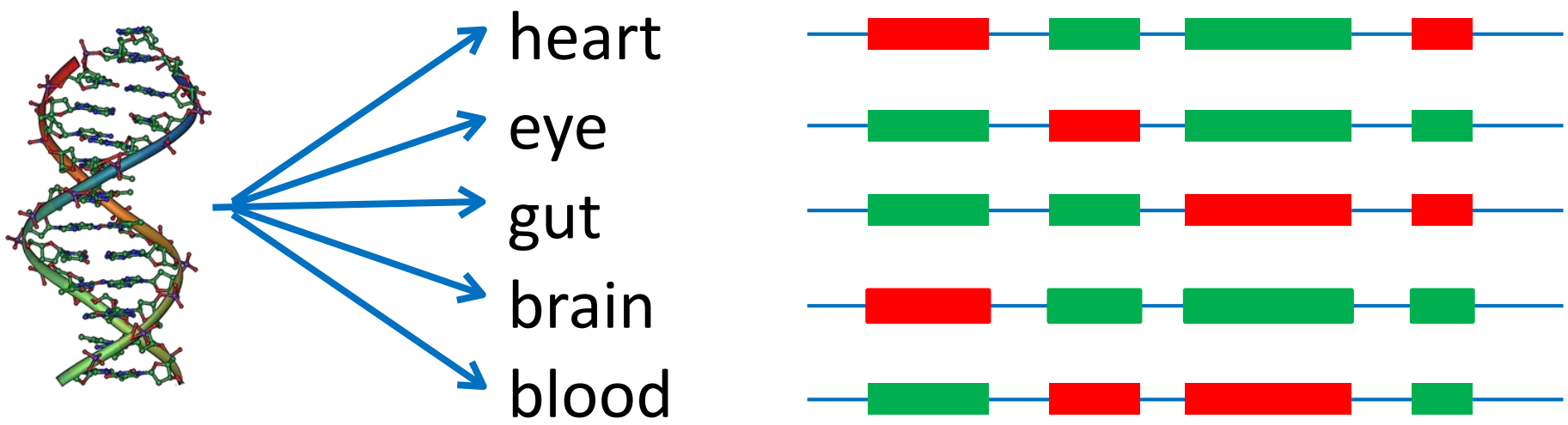


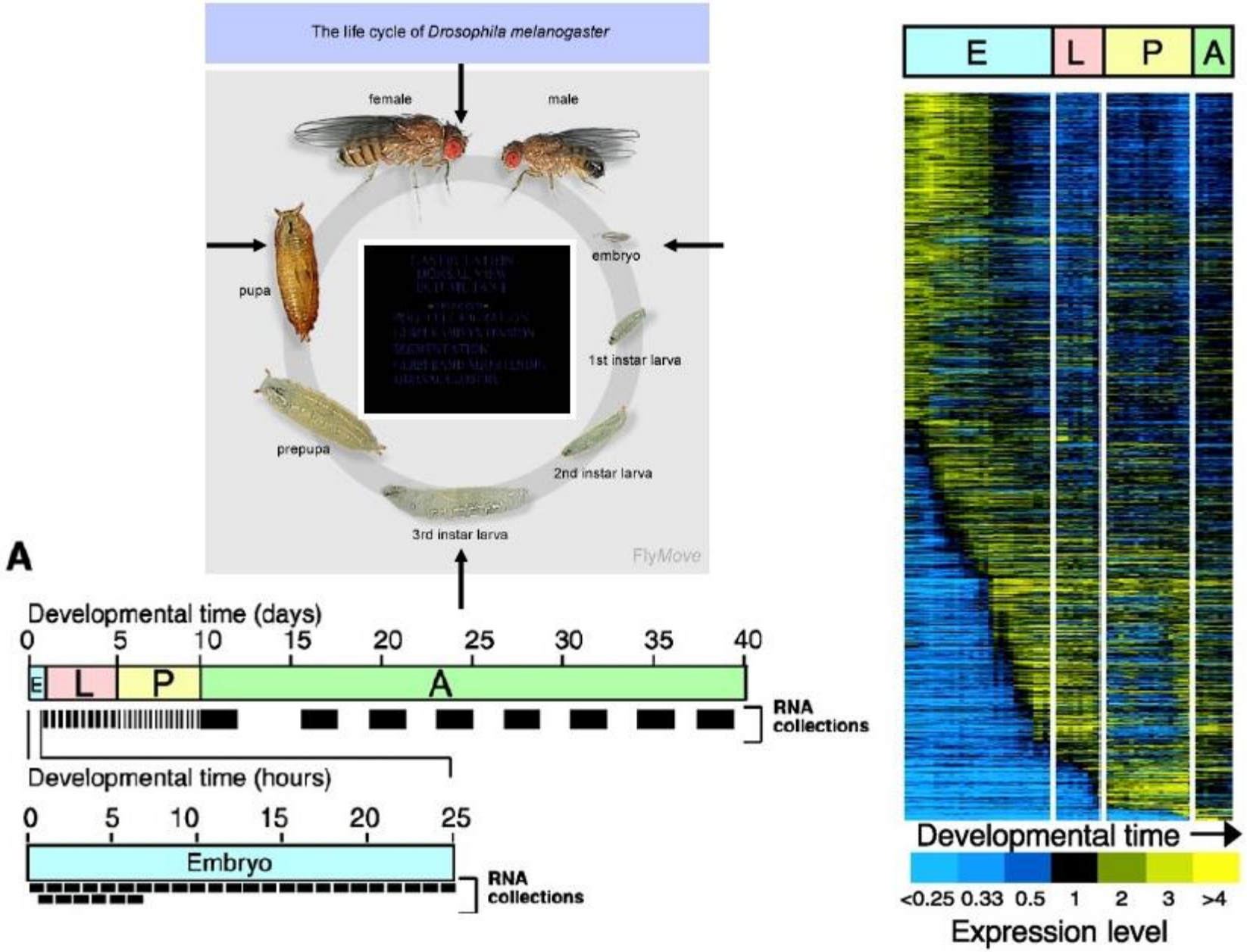
Inherited at each generation

A depiction of the epigenetic landscape. The ball represents a cell, and the bifurcating system of valleys represents the 'chreodes' or bundles of trajectories in state space.

Waddington, C. H. *The Strategy of the Genes* (Geo Allen & Unwin, London, 1957).

One genome, many cell types, many epigenetic programs





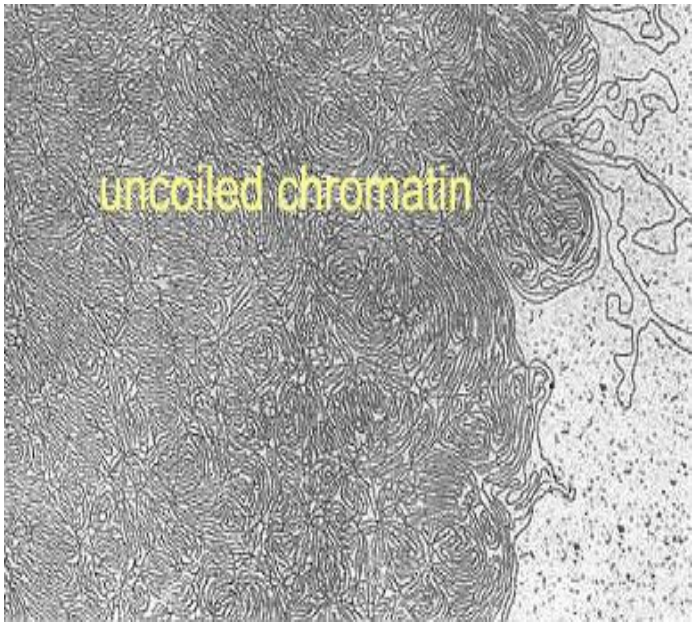
Arbeitman MN, Furlong EE, Imam F, Johnson E, Null BH, Baker BS, Krasnow MA, Scott MP, Davis RW, White KP. Gene expression during the life cycle of Drosophila melanogaster. Science. 2002 Sep 27;297(5590):2270-5

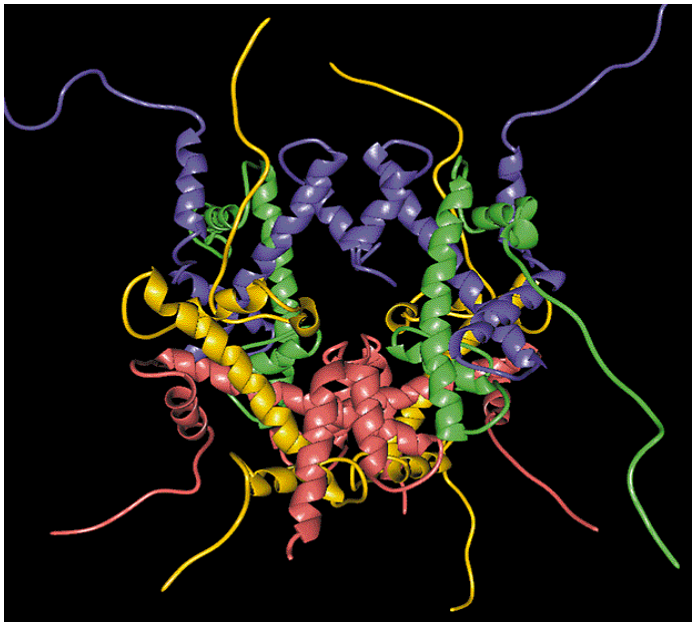
Epigenetics

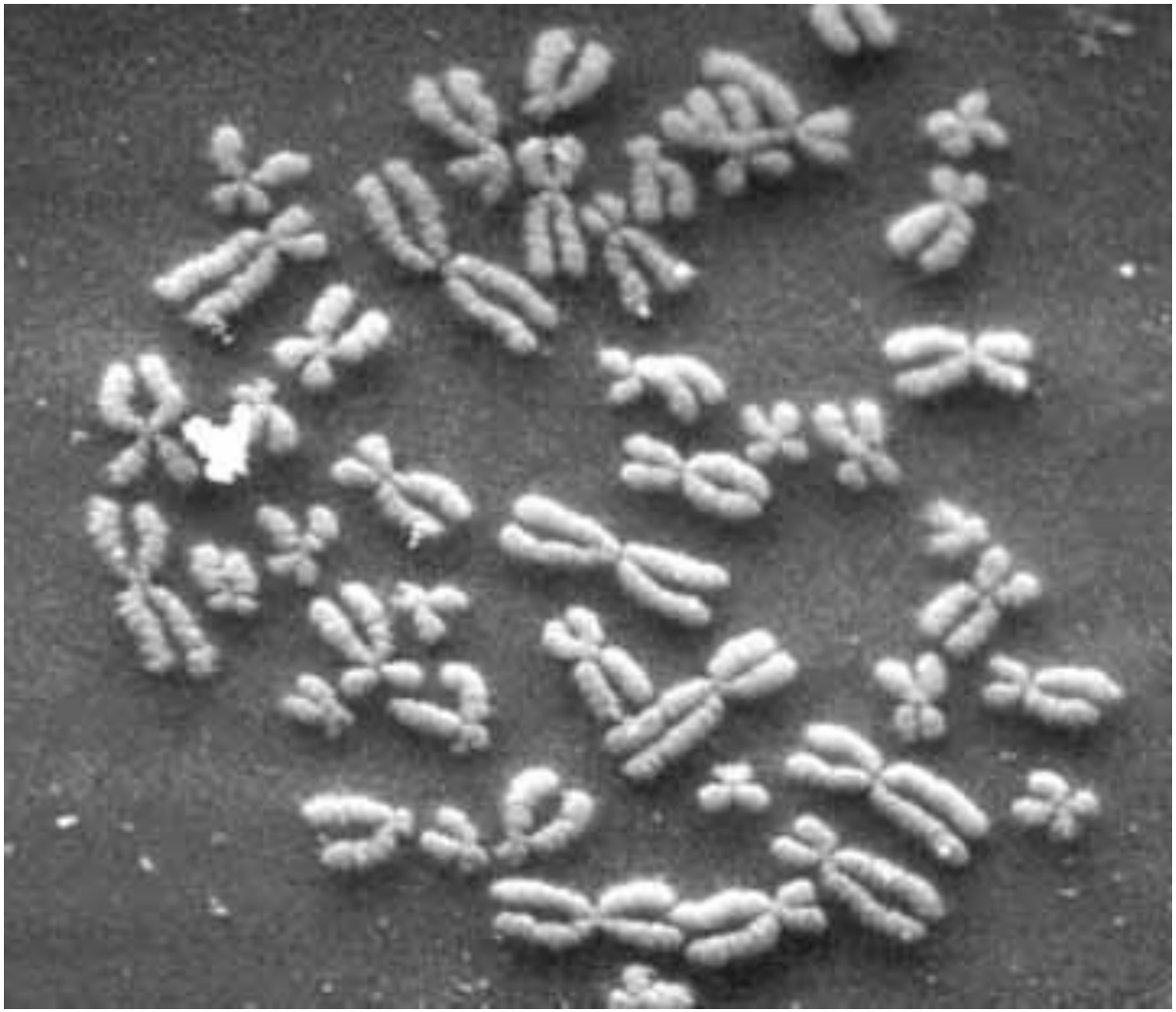
The study of *heritable* changes in gene function that occur without a change in the sequence of nuclear DNA

- Changes in gene expression without changes to DNA sequence passed on through cell division
- Cellular “memory”
- Reversible
- Encoded in chromatin

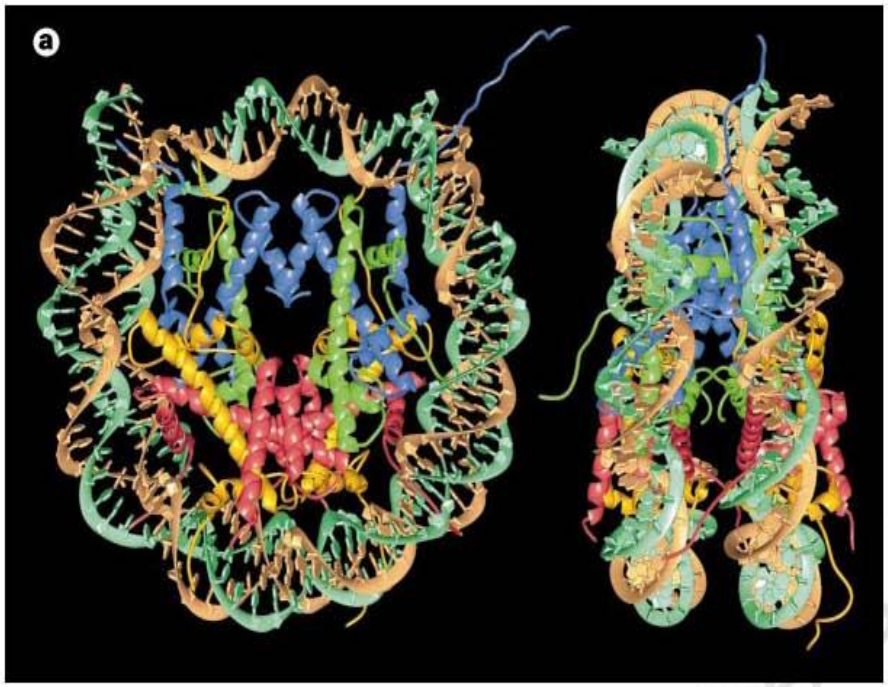
Chromatin Compacts DNA





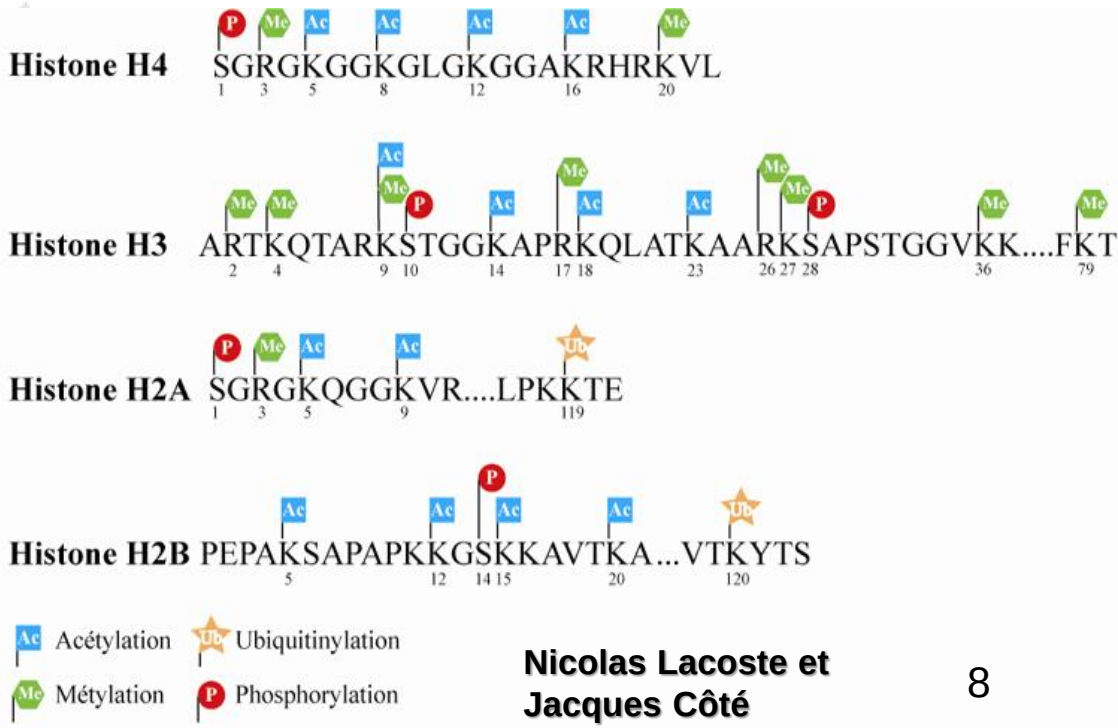


The structure of DNA in the nucleosome



Luger et al., Nature 1997

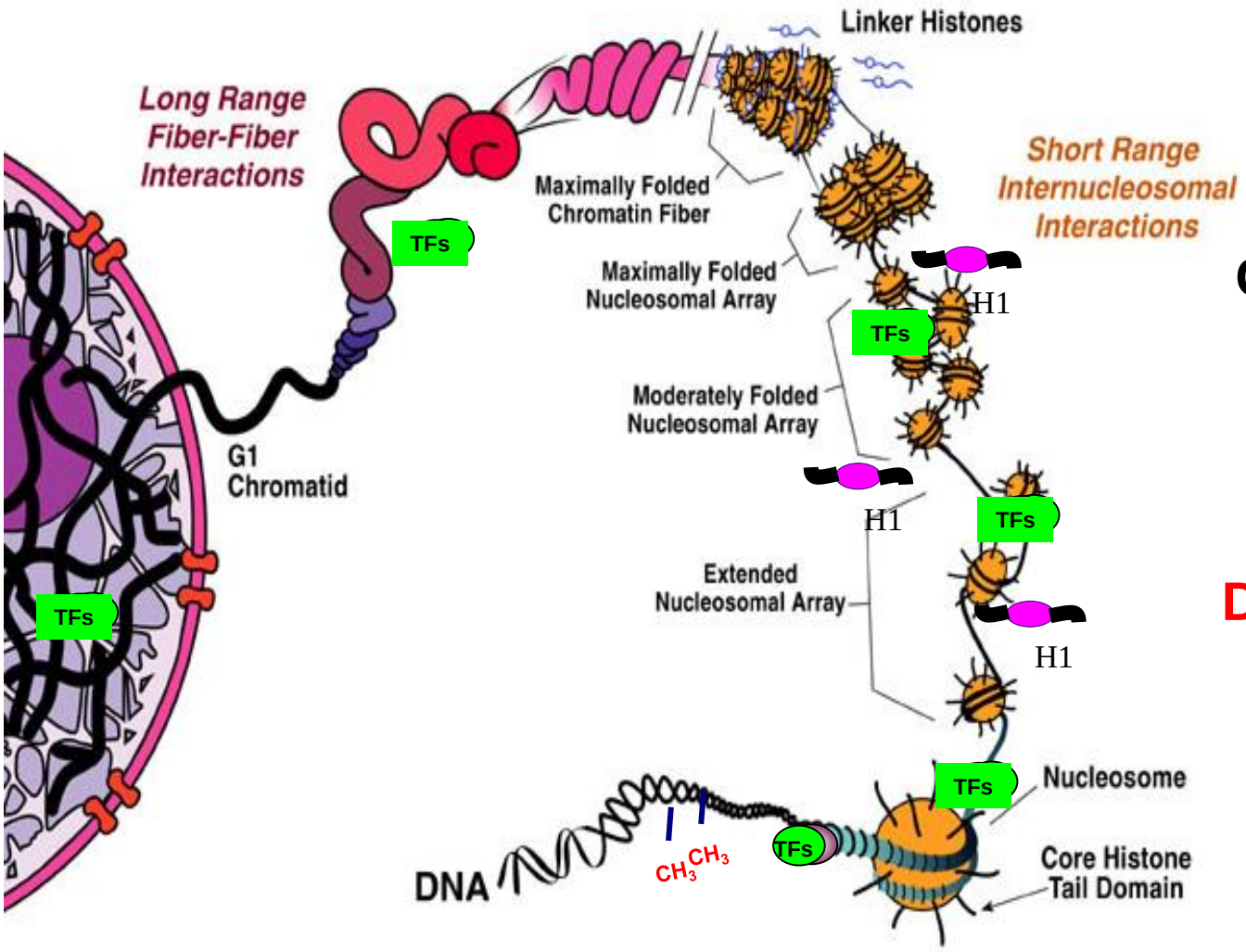
Histone tail modifications



Nicolas Lacoste et Jacques Côté

Chromatin Compacts and Regulates DNA

Multiple chromatin enzymes are required for regulated transcription



Chromatin Remodeling

Histone modifications

DNA Methyltransferases

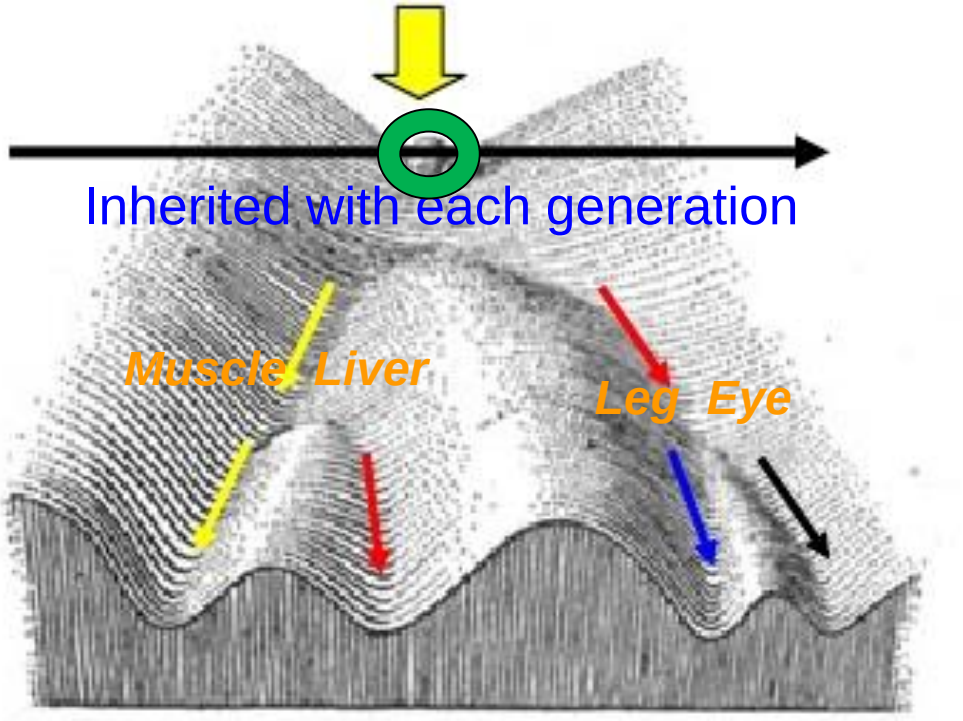
Regulatory RNAs

J Hansen

Epigenetics

Multiple chromatin enzymes are required for Epigenetic control.

Epigenetic landscapes



Chromatin Remodeling

Histone Modifications

DNA Methyltransferases

26S Proteosome

Regulatory RNAs

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Molecular Mechanisms for setting up and reinforcing (epigenetic memory) alternative chromatin states.

DNA methylation-modification machinery:

- DNA methyltransferases
- Methyl-CpG Binding proteins
- Associated chromatin remodeling machinery (MeCP1/NuRD, MeCP2/Sin3a/Lsh)

Histone-modification machinery:

- Histone acetyltransferases
- Histone deacetylases
- Histone methyltransferases
- Histone demethylases
- Histone phosphorylases
- Non-Histone proteins (HP1 etc)
- Associated chromatin remodeling machinery

Function:

Imprinting

X inactivation

Developmental gene expression

Germ cell specific gene expression

Repeat gene silencing

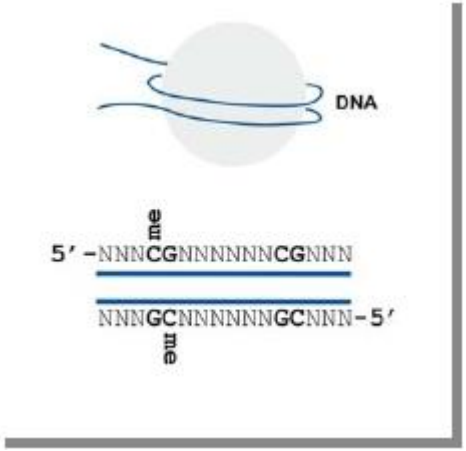
Centromeric heterochromatin

Non-coding RNAs and heterochromatin (RNAi machinery)

RNAi (inhibition) was initially defined by Andrew Fire and colleagues as a process that is triggered by double strand RNA (dsRNA) and silences the expression of genes complementary to the dsRNA in Caenorhabditis elegans.

DNA methylation

- DNA methylation is:
  - a heritable epigenetic mark
  - essential for normal development
- Is a key repressive mark critical to:
  - Imprinting
  - X-inactivation
  - Suppression of repetitive elements
  - Regulation of gene expression (lineage-spec genes)
- Is a powerful mutagen
  - CpG suppression in mammalian genomes
- CpG Islands are normally free of this mark



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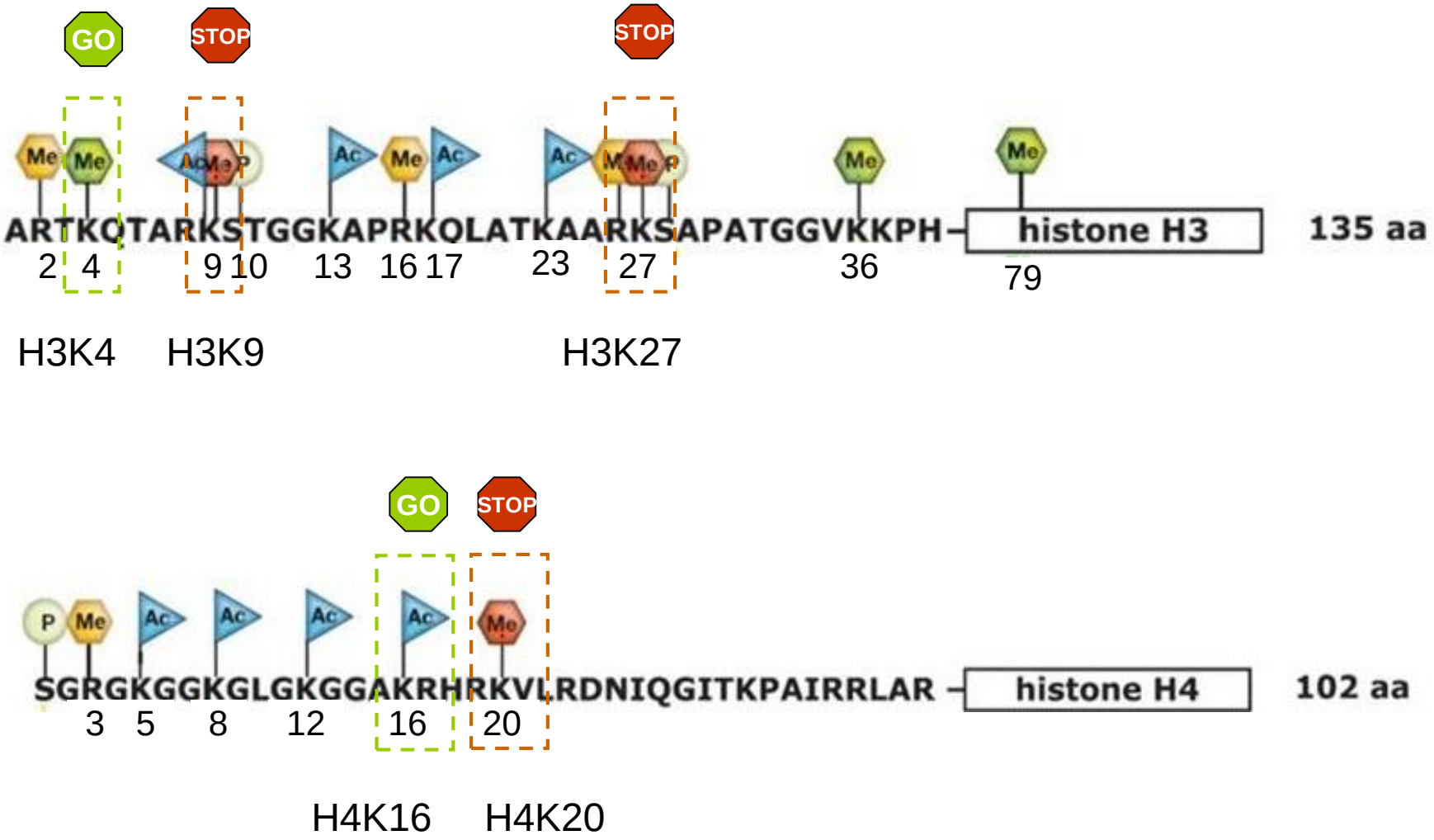
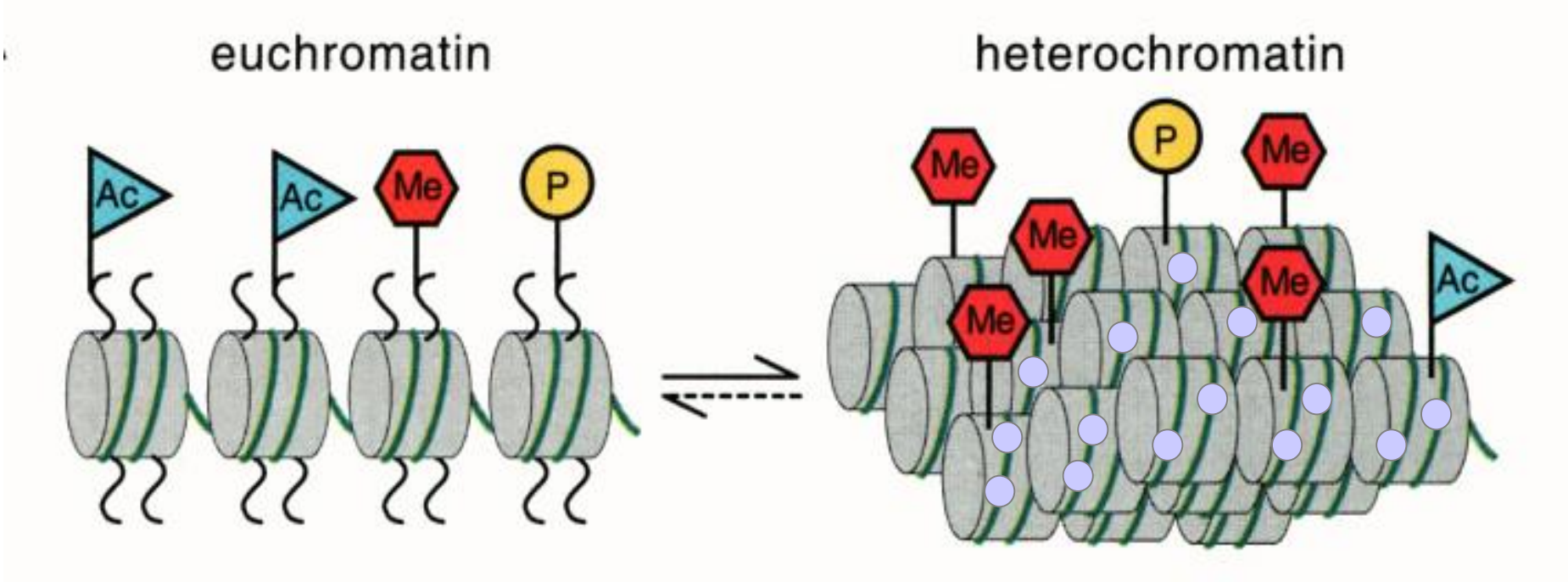
Histone Code



DNA Methylation and Histone Modifications help to compartmentalize the genome into domains of different transcriptional potentials

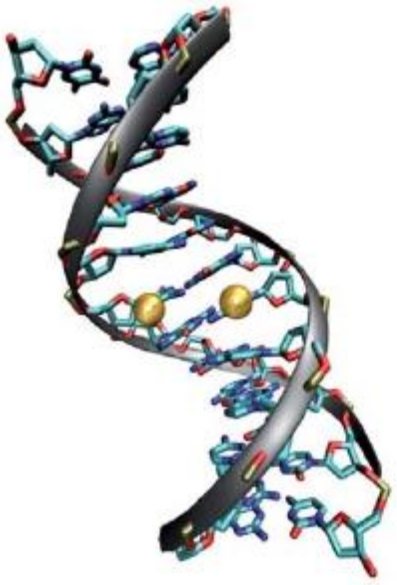


hyperacetylated histones
Low DNA methylation
H3-K4 methylation
H4-K16 acetylation

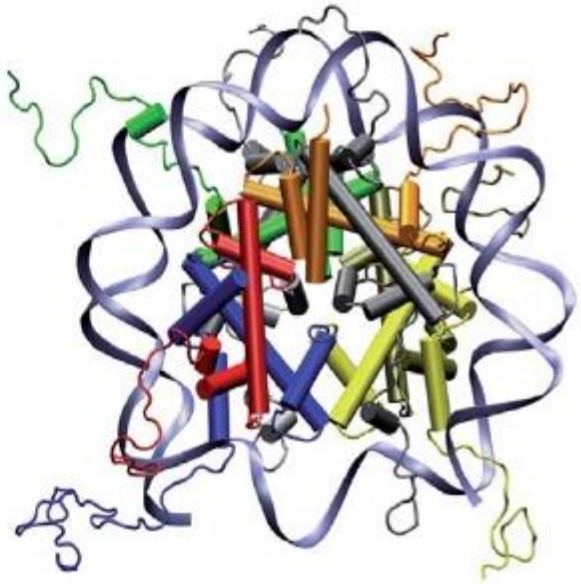
hypoacetylated histones
Dense DNA methylation
H3-K9 methylation
H4-K20 methylation

Epigenetic Significance for Long Term Health

Understanding how epigenetic information is deposited, maintained and processed is key to understanding gene regulation during development and disease

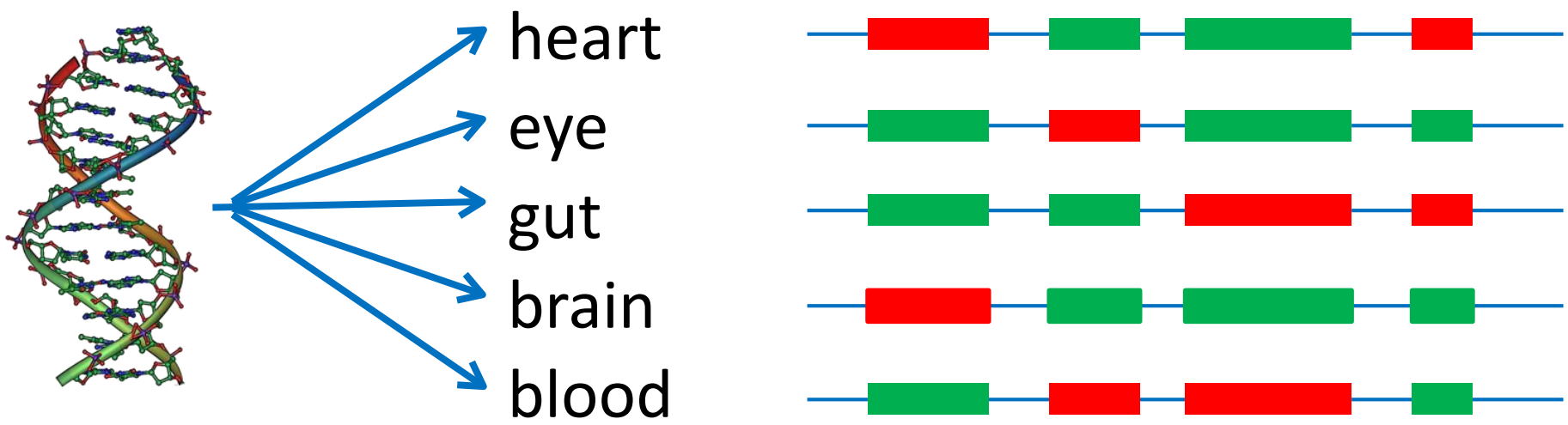


DNA methylation



Histone modification

One genome, many cell types, many epigenetic programs

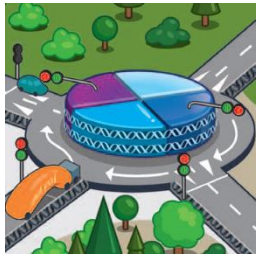


ARTICLE OPEN

Integrative analysis of 111 reference human epigenomes

Roadmap Epigenomics Consortium, *et al.*
Nature Volume: 518, Pages:317–330 Date published:(19 February 2015)
DOI:doi:10.1038/nature14248

- The reference human genome sequence set the stage for studies of genetic variation and its association with human disease, but epigenomic studies lack a similar reference. To address this need, the NIH Roadmap Epigenomics Consortium generated the largest collection so far of human epigenomes for primary cells and tissues. Here we describe the integrative analysis of 111 reference human epigenomes generated as part of the programme, profiled for histone modification patterns, DNA accessibility, DNA methylation and RNA expression. We establish global maps of regulatory elements, define regulatory modules of coordinated activity, and their likely activators and repressors. We show that disease- and trait-associated genetic variants are enriched in tissue-specific epigenomic marks, revealing biologically relevant cell types for diverse human traits, and providing a resource for interpreting the molecular basis of human disease. Our results demonstrate the central role of epigenomic information for understanding gene regulation, cellular differentiation and human disease.



EPIGENOME ROADMAP
A Nature special issue
nature.com/epigenomeroadmap

Tissues and cell types profiled in the Roadmap Epigenomics Consortium.

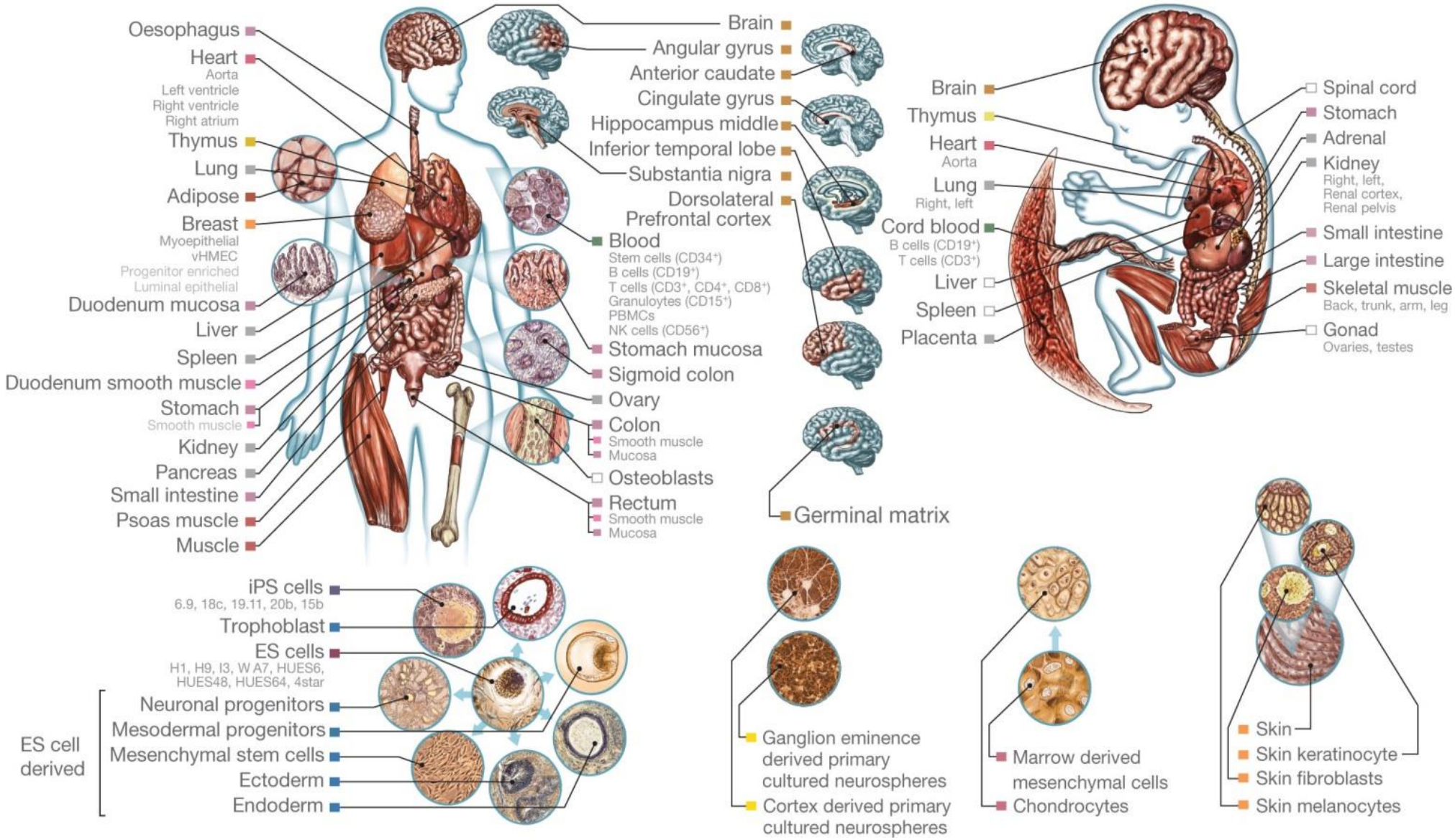


Figure 1 | Tissues and cell types profiled in the Roadmap Epigenomics Consortium. Primary tissues and cell types representative of all major lineages in the human body were profiled, including multiple brain, heart, muscle, gastrointestinal tract, adipose, skin and reproductive samples, as well as immune lineages, ES cells and iPS cells, and differentiated lineages derived from ES cells.

Roadmap Epigenomics Consortium *et al. Nature* **518**, 317-330 (2015) doi:10.1038/nature14248

nature

Data sets available for each reference epigenome.

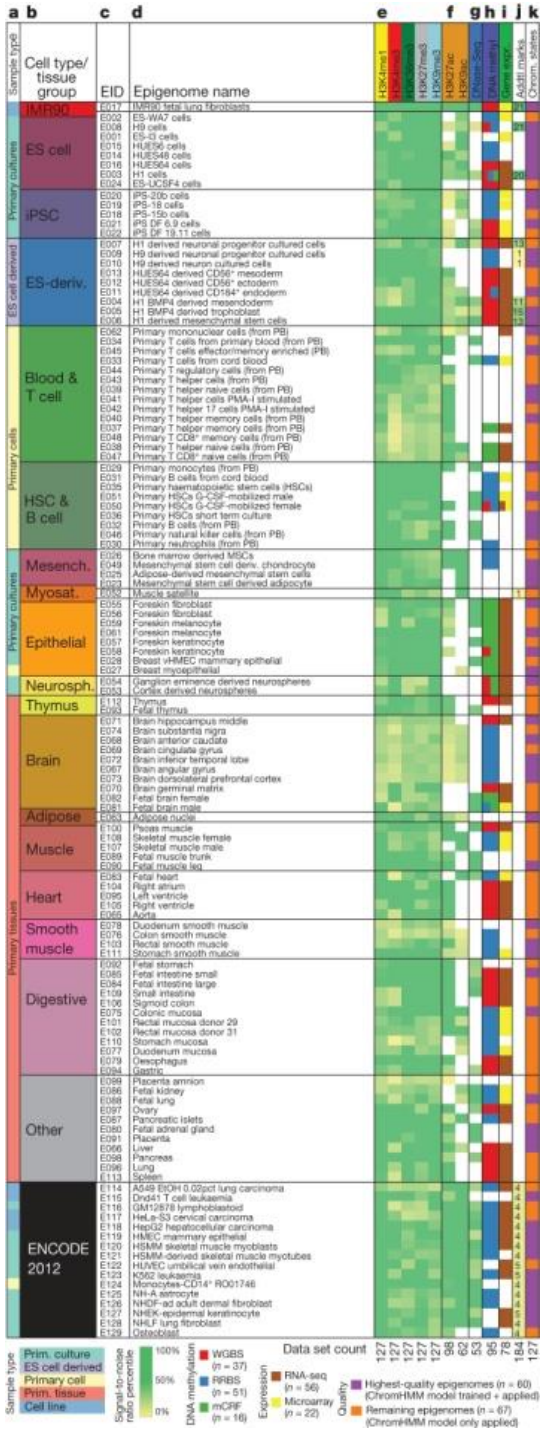


Figure 2 | Data sets available for each reference epigenome. List of 127epigenomes including 111 by the Roadmap Epigenomics program (E001 – E113) and 16 by ENCODE (E114 – E129). See Supplementary Table 1 for a full list of names and quality scores. **a** – **d**, Tissue and cell types grouped by type of biological material (**a**), anatomical location (**b**), reference epigenome identifier (EID, **c**) and abbreviated name (**d**). PB, peripheral blood. ENCODE 2012 reference epigenomes are shown separately. **e** – **g**, Normalized strand cross-correlation quality scores (NSC)[37] for the core set of five histone marks (**e**), additional acetylation marks (**f**) and DNase-seq (**g**). **h**, Methylation data by WGBS (red), RRBS (blue) and mCRF (green). A total of 104 methylation data sets available in 95 distinct reference epigenomes. **i**, Gene expression data using RNA-seq (brown) and microarray expression (yellow). **j**, A total of 26 epigenomes contain 184 additional histone modification marks. **k**, Sixty highest-quality epigenomes (purple) were used for training the core chromatin state model, which was then applied to the full set of epigenomes (purple and orange).

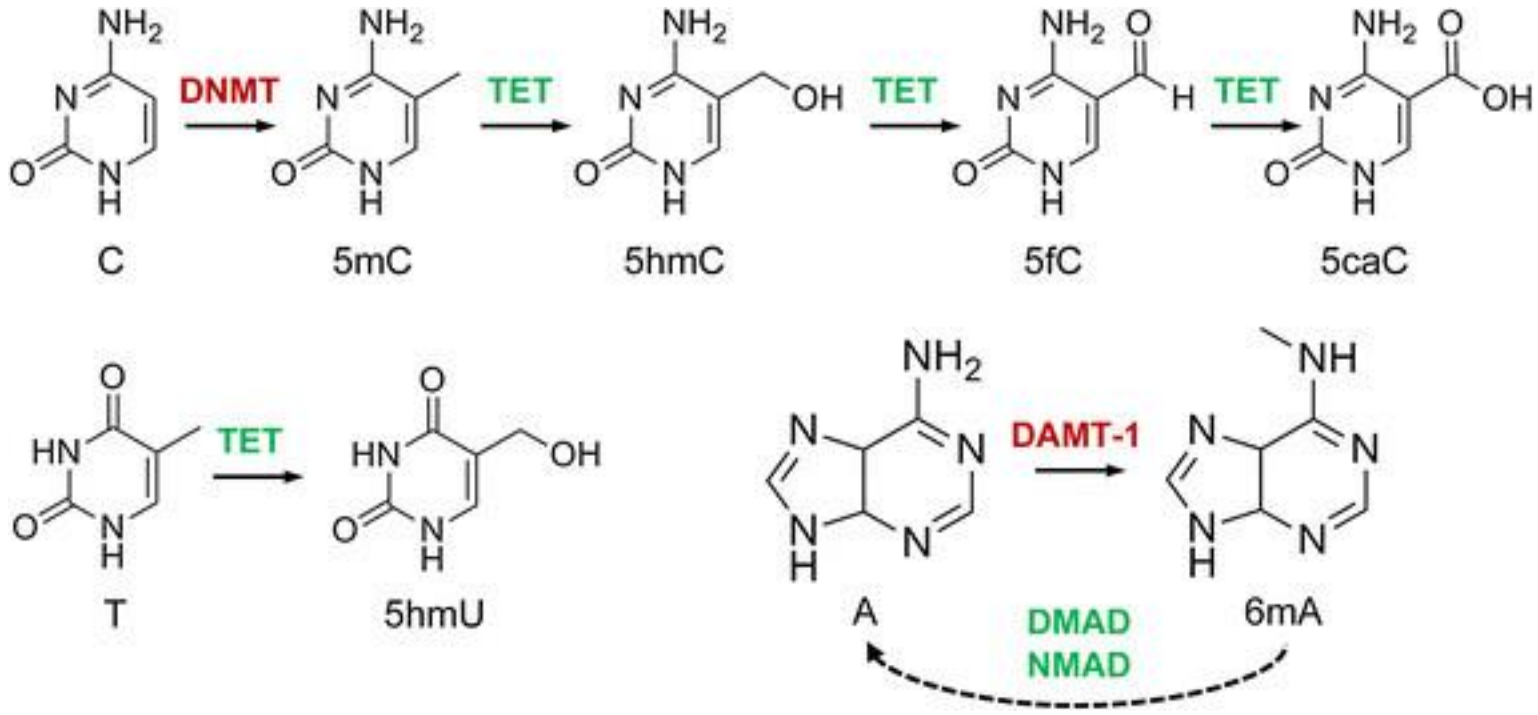
Roadmap Epigenomics Consortium *et al. Nature* **518**, 317-330 (2015) doi:10.1038/nature14248

nature

So…. do we have a Reference Epigenome?

DNA methylation, typically associated with repressed regulatory regions or active gene transcripts and profiled using whole-genome bisulfite sequencing (WGBS), reduced-representation bisulfite sequencing (RRBS), and mCRF-combined31 methylation-sensitive restriction enzyme (MRE) and immunoprecipitation based assays (Fig. 2h)

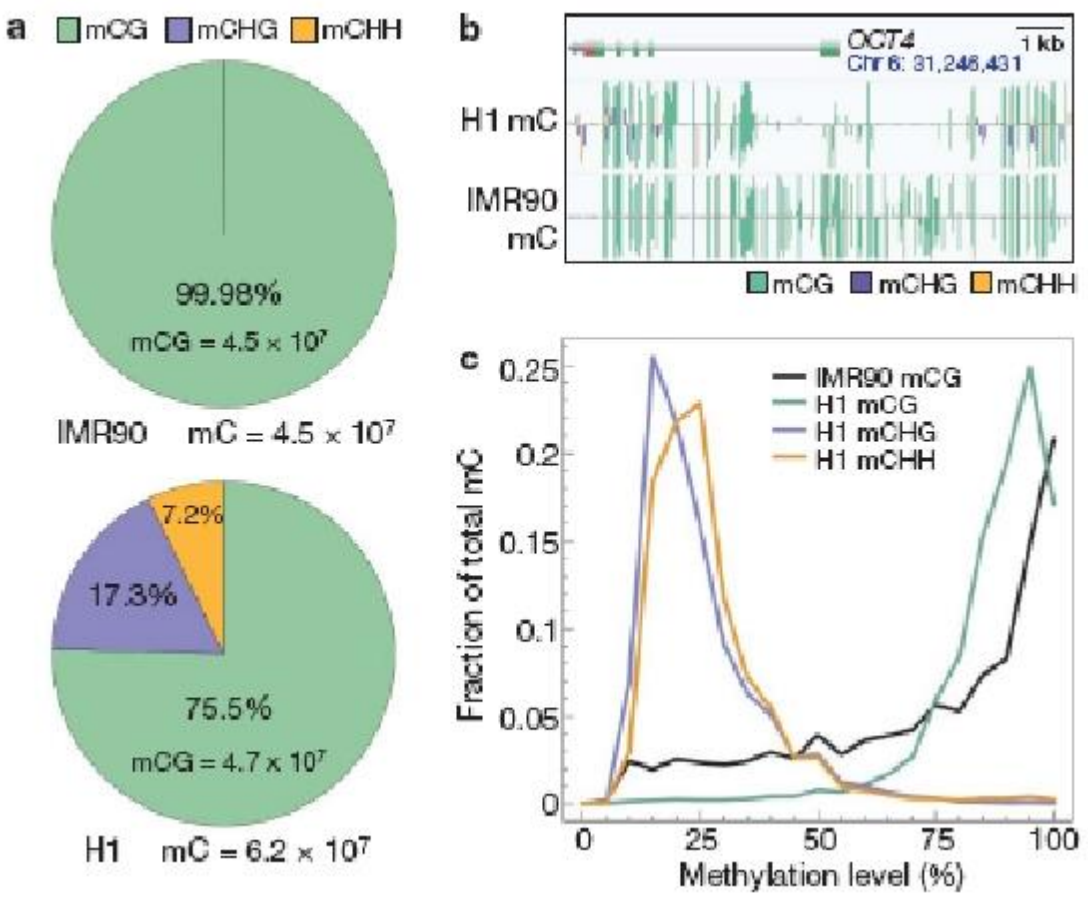
2h, Methylation data by WGBS (red), RRBS (blue) and mCRF (green). A total of 104 methylation data sets available in 95 distinct reference epigenomes.



DNA modifications with epigenetic regulatory functions and their interdependencies. Cytosine (C) is methylated to 5-methylcytosine (5mC) by DNA methyltransferases (DNMT) and then further oxidised to 5hmC, 5fC and 5caC by Tet dioxygenases. 5-Hydroxyuracil (5hmU) is produced by Tet-catalysed oxidation of thymine (T). N6-methyladenine (6mA) is likely catalysed by DNA N6 adenine methyltransferases (DAMT-1 in C. elegans), even though the biochemical activity of these enzymes remains to be characterized. The Tet-like ALKB enzymes NMAD (N6-methyl adenine demethylase 1) and DMAD (DNA 6mA demethylase) have been shown to be involved in 6mA demethylation in C. elegans and in Drosophila, respectively, possibly by using a conserved dioxygenase mechanism.

Breiling and Lyko Epigenetics & Chromatin 2015 8:24 doi:10.1186/s13072-015-0016-6

Human DNA methylomes at base resolution show widespread epigenomic differences. Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo QM, Edsall L, Antosiewicz-Bourget J, Stewart R, Ruotti V, Millar AH, Thomson JA, Ren B, Ecker JR Nature. 2009 Oct 14.



High levels of non-CpG methylation in ES cells

So…. do we have a Reference Epigenome?

Histone H3 lysine 4 trimethylation **(H3K4me3**), associated with promoterregions10,24;
H3 lysine 4 monomethylation **(H3K4me1**), associated with enhancer regions10;
H3 lysine 36 trimethylation **(H3K36me3**), associated with transcribed regions;
H3 lysine 27 trimethylation **(H3K27me3**), associated with Polycomb repression25;
H3 lysine 9 trimethylation **(H3K9me3**), associated with heterochromatin regions26.

Selected epigenomes also contain a subset of additional epigenomic marks, including: acetylation marks H3K27ac and H3K9ac, associated with increased activation of enhancer and promoter regions27–29 (Fig. 2f);

Cell

Resource

Identification of 67 Histone Marks and Histone Lysine Crotonylation as a New Type of Histone Modification

Minjia Tan,[1,6] Hao Luo,[1,6] Sangkyu Lee,[1,6] Fulai Jin,[2] Jeong Soo Yang,[1] Emilie Montellier,[3] Thierry Buchou,[3] Zhongyi Cheng,[1] Sophie Rousseaux,[3] Nisha Rajagopal,[2] Zhike Lu,[1] Zhen Ye,[2] Qin Zhu,[4] Joanna Wysocka,[5] Yang Ye,[4] Saadi Khochbin,[3] Bing Ren,[2] and Yingming Zhao[1,*]

[1]Ben May Department of Cancer Research, The University of Chicago, Chicago, IL 60637, USA

[2]Ludwig Institute for Cancer Research and Department of Cellular and Molecular Medicine, University of California San Diego School of Medicine, 9500 Gilman Drive, La Jolla, CA 92093, USA

[3]INSERM, U823; Université Joseph Fourier - Grenoble 1; Institut Albert Bonniot, Faculté de Médecine, Domaine de la Merci, 38706 La Tronche Cedex, France

[4]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, P.R. China

[5]Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA 94305, USA

[6]These authors contributed equally to this work

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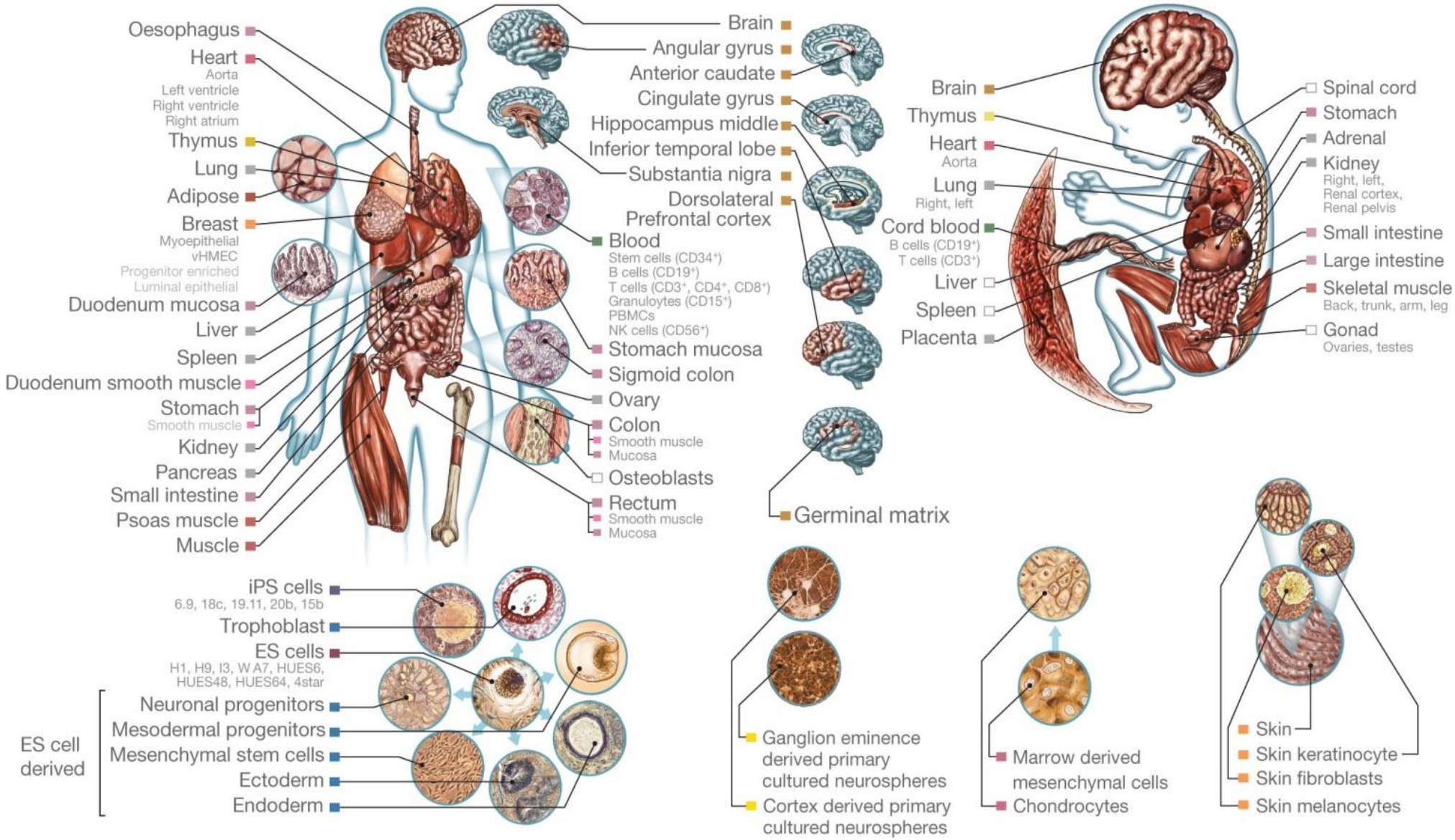
DOI 10.1016/j.cell.2011.08.008

Using this approach, we identified **130 unique PTM sites**, which not only confirmed **63 previously known** histone PTMs, but also revealed 67 novel ones, including 28 Kcr sites, 18 lysine monomethylation (Kme) sites, 1 lysine dimethylation (Kme2) site, 4 lysine formylation (Kfo) sites, 2 lysine acetylation (Kac) sites, 8 arginine monomethylation (Rme) sites, and 6 tyrosine hydroxylation (Yoh) sites (Figure 1C).

Tissues and cell types profiled in the Roadmap Epigenomics Consortium.



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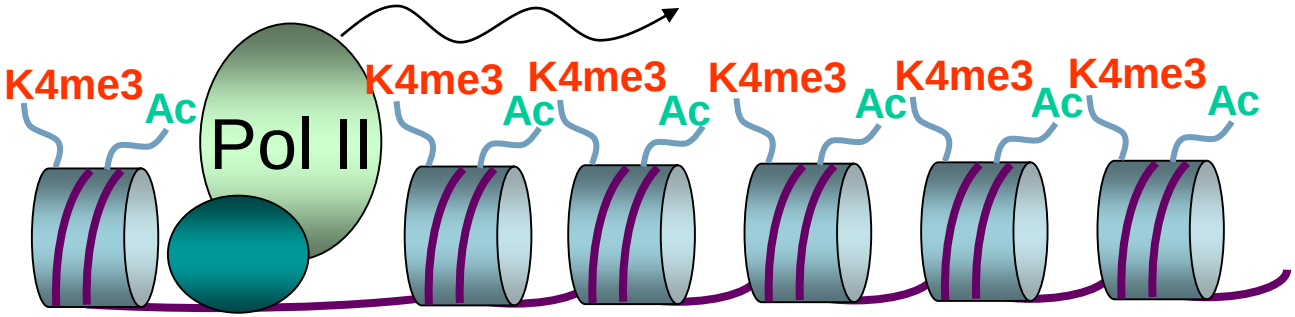
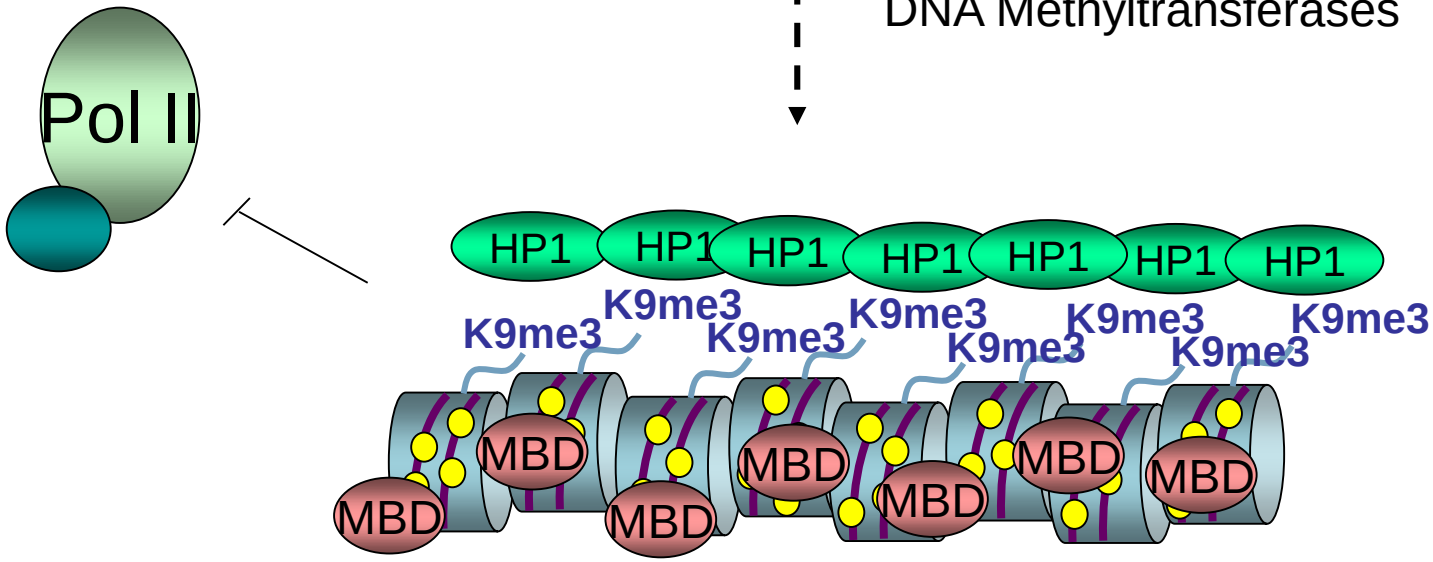
nature

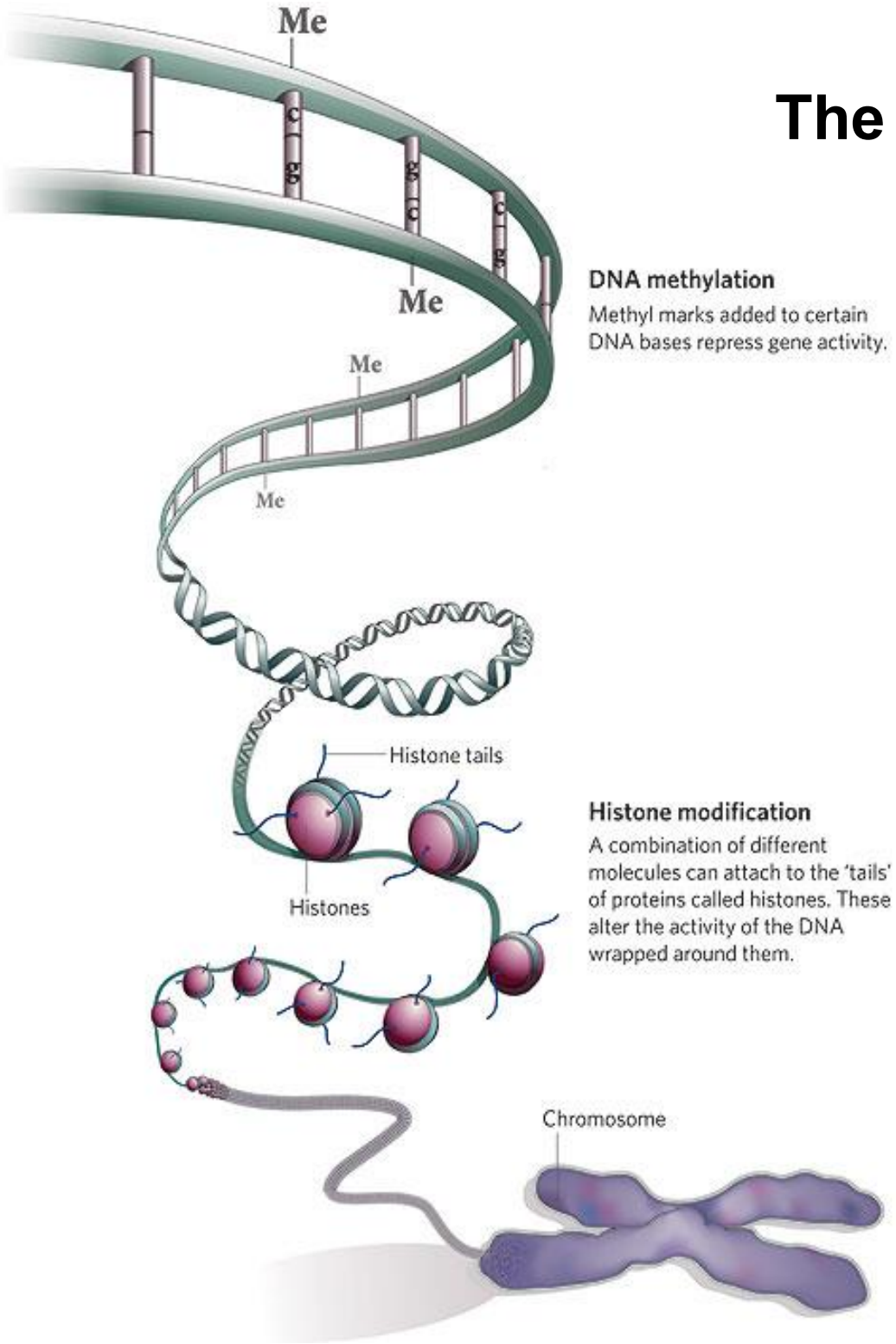
Epigenetic Silencing in Cancer



Histone Deacetylases
Histone Methyltransferases
DNA Methyltransferases



The ‘epigenetic’ code ?



Functions:

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Centromeric heterochromatin

Lee Langer (NIEHS)
Paul Wade (NIEHS)
Richard Meehan (MRC-UK)
Paula Vertino (Emory)
Lisa Chadwick (NIEHS)
Archer Lab Colleagues