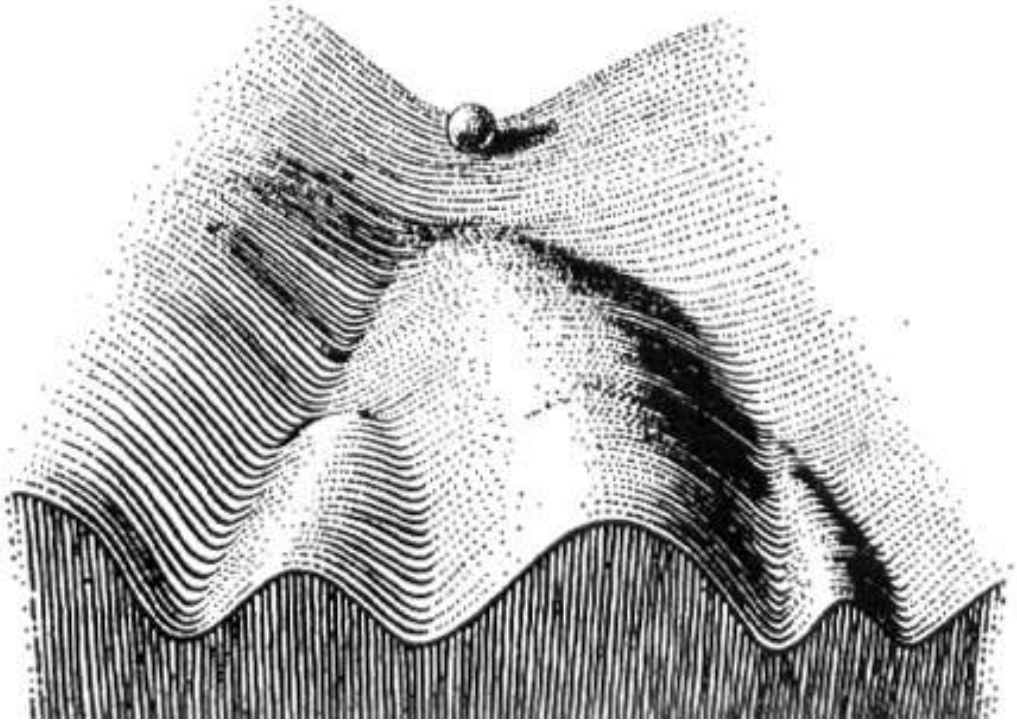
L'épigénétique: au-delà du code génétique

Edith Heard



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The Genetic Code

Human Genome Project:

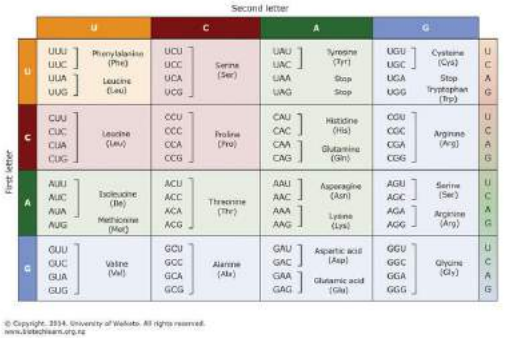
~20,000-25,000 genes

3 billion chemical base pairs of DNA

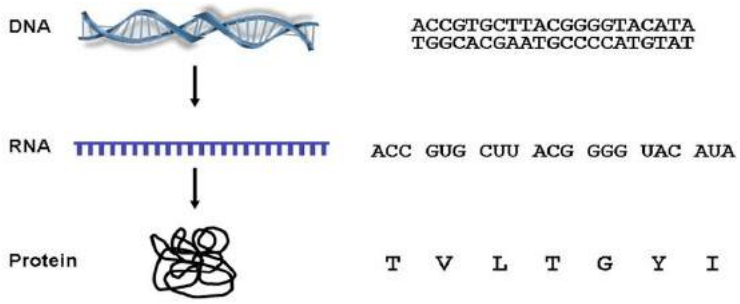
Central Dogma of Molecular Biology

by
FRANCIS CRICK
MRC Laboratory of Molecular Biology,
Hills Road,
Cambridge CB2 2QH

The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that such information cannot be transferred from protein to either protein or nucleic acid.



The Genetic Code



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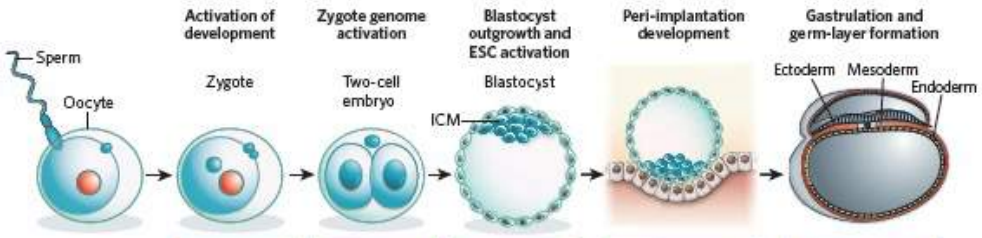
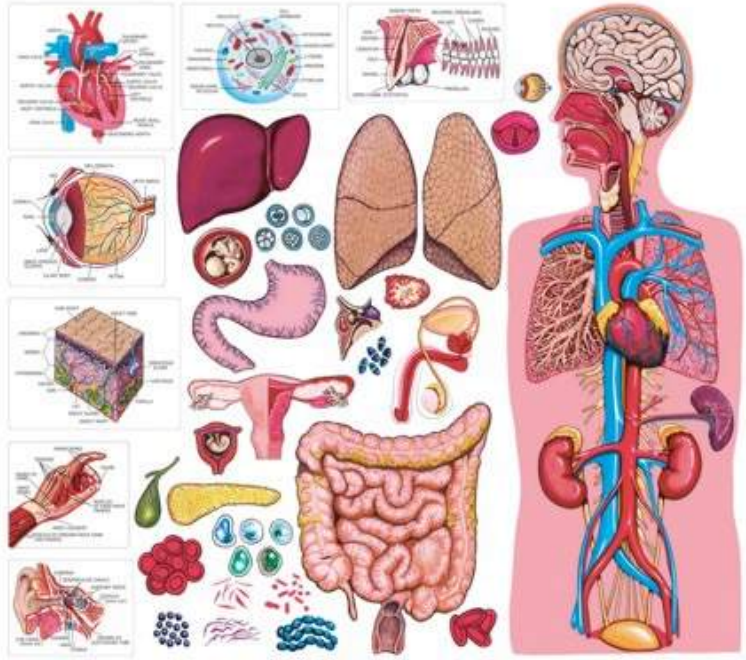
The Genome: one blueprint, multiple interpretations

Epigenesis





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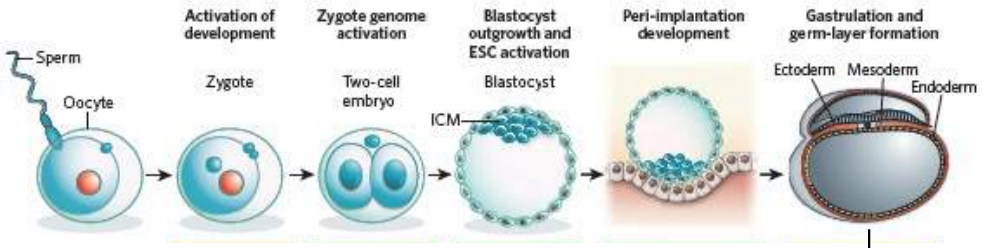
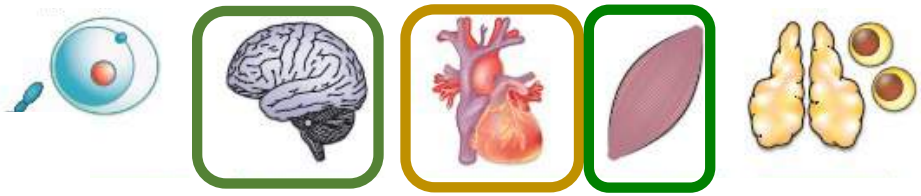
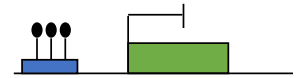
The Genome: one blueprint, multiple interpretations

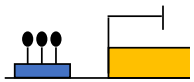
Epigenesis

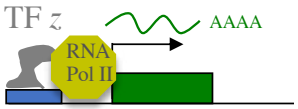


Differential Gene expression

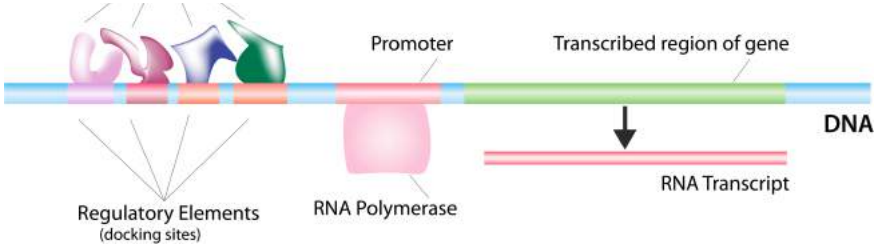








Transcription Factors
Or Repressors



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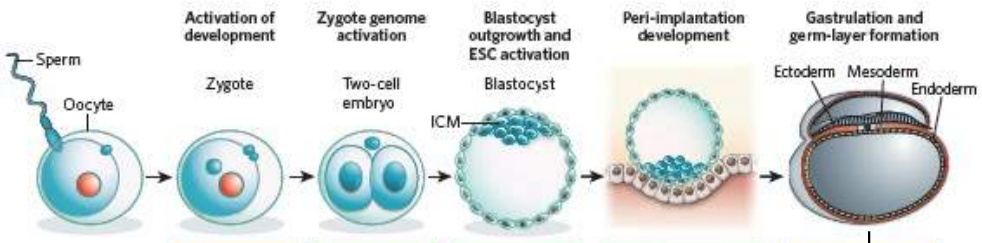
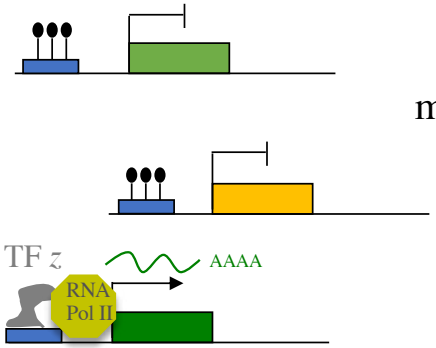
1530

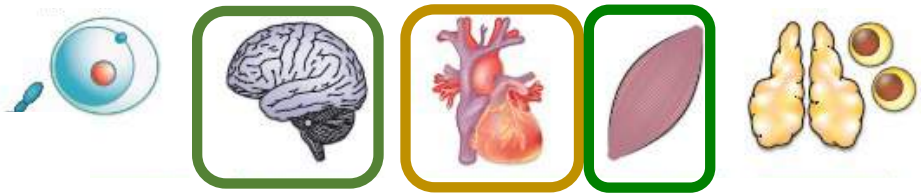
The Genome: one blueprint, multiple interpretations

Epigenesis



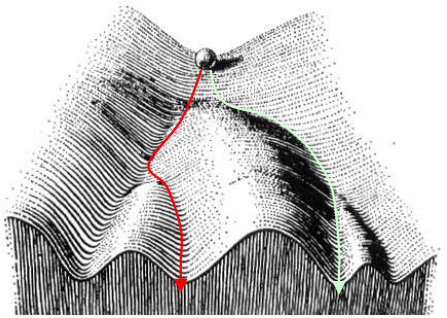
Differential Gene expression



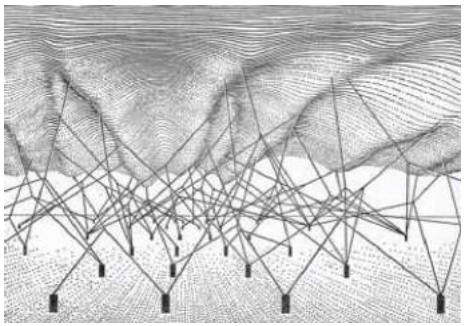


Different cell fates are adopted and have to be memorized as the fertilized egg forms a complex organism

Waddington proposed that **networks of genes** must be involved in defining an epigenetic landscape



Different cell fates



Genes

Conrad H. Waddington (1957)
The strategy of the genes

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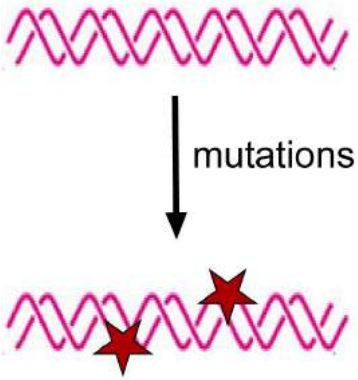
Epigenetics

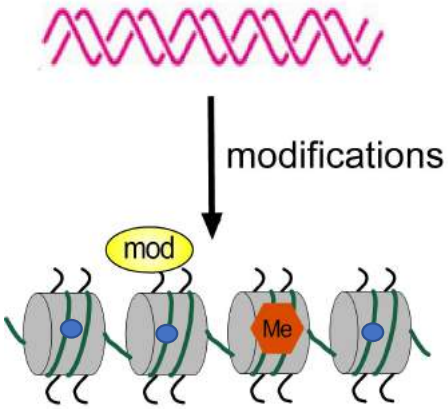
Heritable changes in gene function
that cannot be explained by changes in DNA sequence.

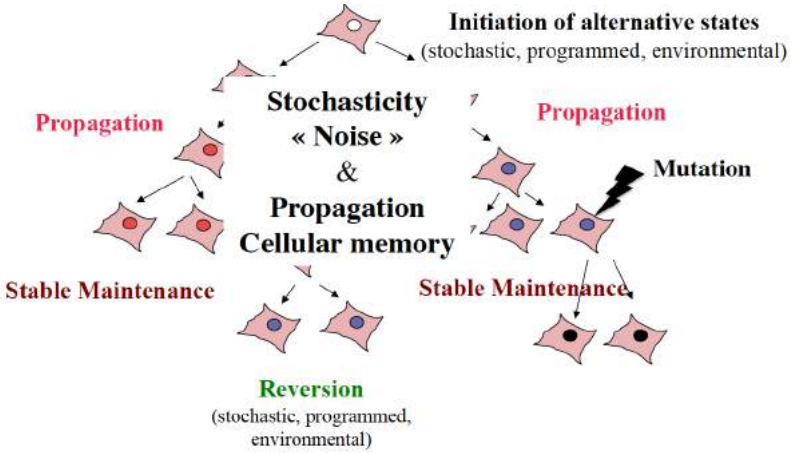
Russo, V.E.A., R.A. Martienssen & A.D. Riggs Eds. (1996) "Epigenetic mechanisms of gene regulation." *CSHL Press*.

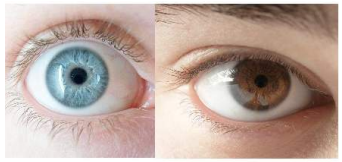
GENETICS



EPIGENETICS



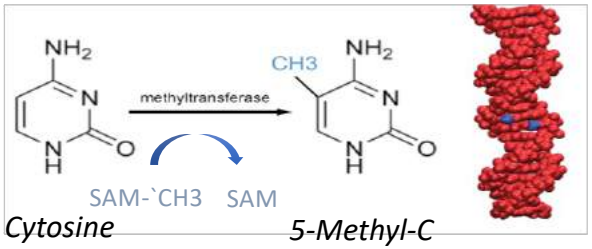




inherited

germ line

species



Cytosine *5-Methyl-C*

Epigenetics

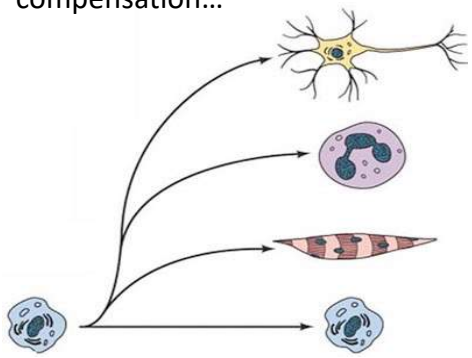
Heritable changes in gene function
that cannot be explained by changes in DNA sequence.

Russo, V.E.A., R.A. Martienssen & A.D. Riggs Eds. (1996) "Epigenetic mechanisms of gene regulation." *CSHL Press*.

Same Genome different Epigenomes

Developmental epigenetics:

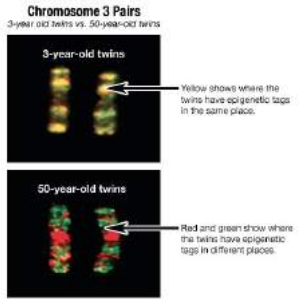
Development, sex chromosome dosage compensation…



Stochastic or age-dependent epigenetics:

Differences in twins, clones…

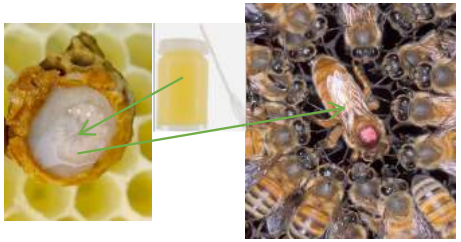
Disease « epimutations »

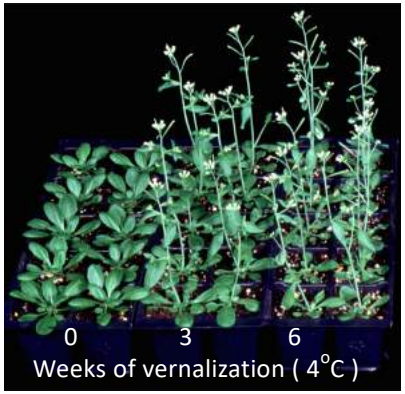


Exogenously or environmentally programmed epigenetics :

Bees, ants - nutrition

Vernalisation in plants - climate





Measure human aging from DNA Methylomes

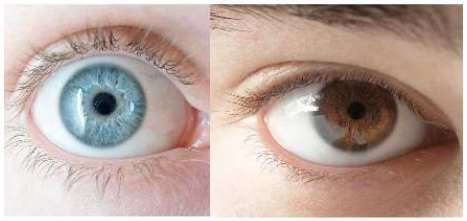
Gender differences

Correlate with gene expression differences

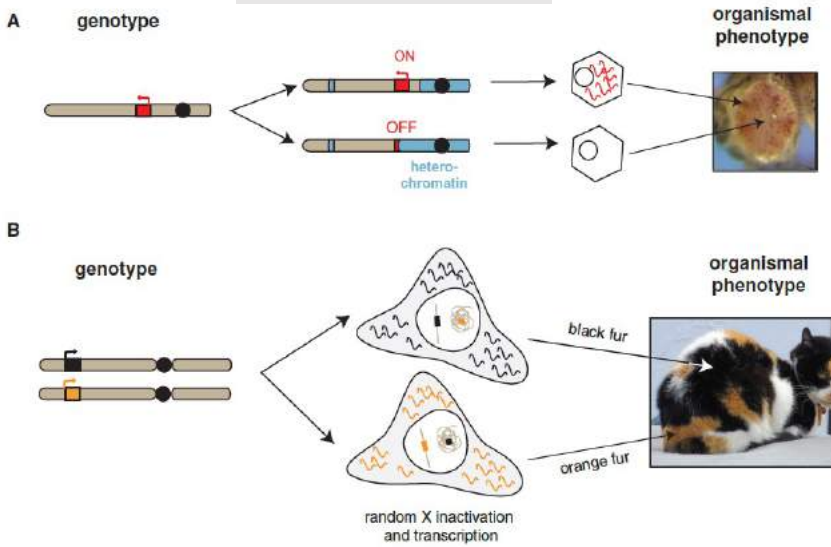
Tumors show faster aging

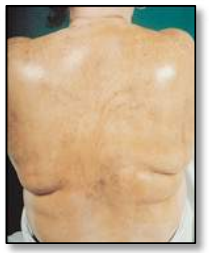
How to understand the basis of differences between and within individuals with the same genotype but different phenotypes

Different genotypes



Same genotypes





Inactivation du chromosome X chez les femelles

Genetic, stochastic and environmental factors give rise to variability between individuals

Armadillos (Tatous)

Symmons and Raj 2016
http://dx.doi.org/10.1016/j.molcel.2016.05.023

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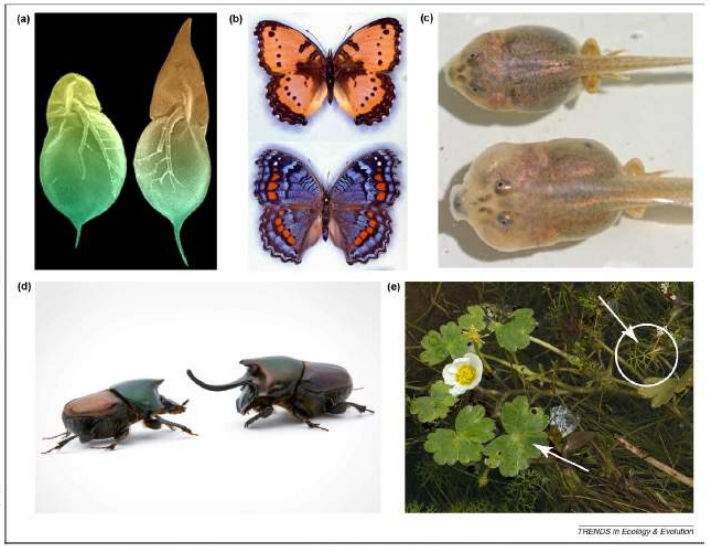
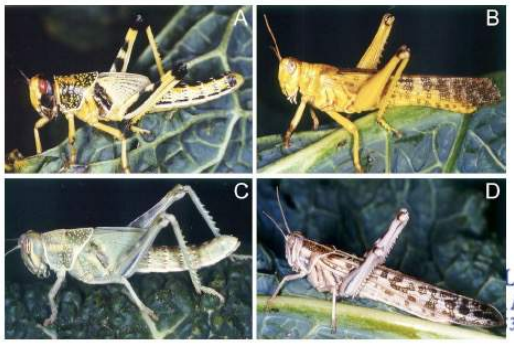
How to understand the basis of differences between and within individuals with the same genotype but different phenotypes

Developmental and Phenotypic Plasticity, Polyphenism

- Most species can display some degree of phenotypic plasticity – either distinctly stable « morphs » - or continuum of traits
- It can be functional (and potentially adaptive), inevitable (neutral or deleterious)
- It can an be restricted to a few minutes, to a whole life time, or to many generations
- How one genotype can give rise to different phenotypes through environmental effects is clearly an EPIGENETICS question
- Back to Waddington's original definition – but actual mechanisms are still elusive





Epigenetic and Phenotypic Plasticity in Locusts

Two animals packed within the same genome

Reared alone:

phase *solitaria*

- Phase transition induces a broad range of differences in **anatomy** (size, colour), **physiology** (lifespan, metabolism, immune responses, endocrinology and reproduction) & **behaviour** (solitary vs gregarious with population density increase)
- Gregarious morphs exhibit a wider dietary range, display increased locomotory activity, and fly during daytime, in contrast to isolated locusts, which generally fly at night

Simpson, S.J., McCaffery, A.R., Hägele, B. (1999 *Biological Reviews* 74: 461-480.

Courtesy of Stephen Simpson

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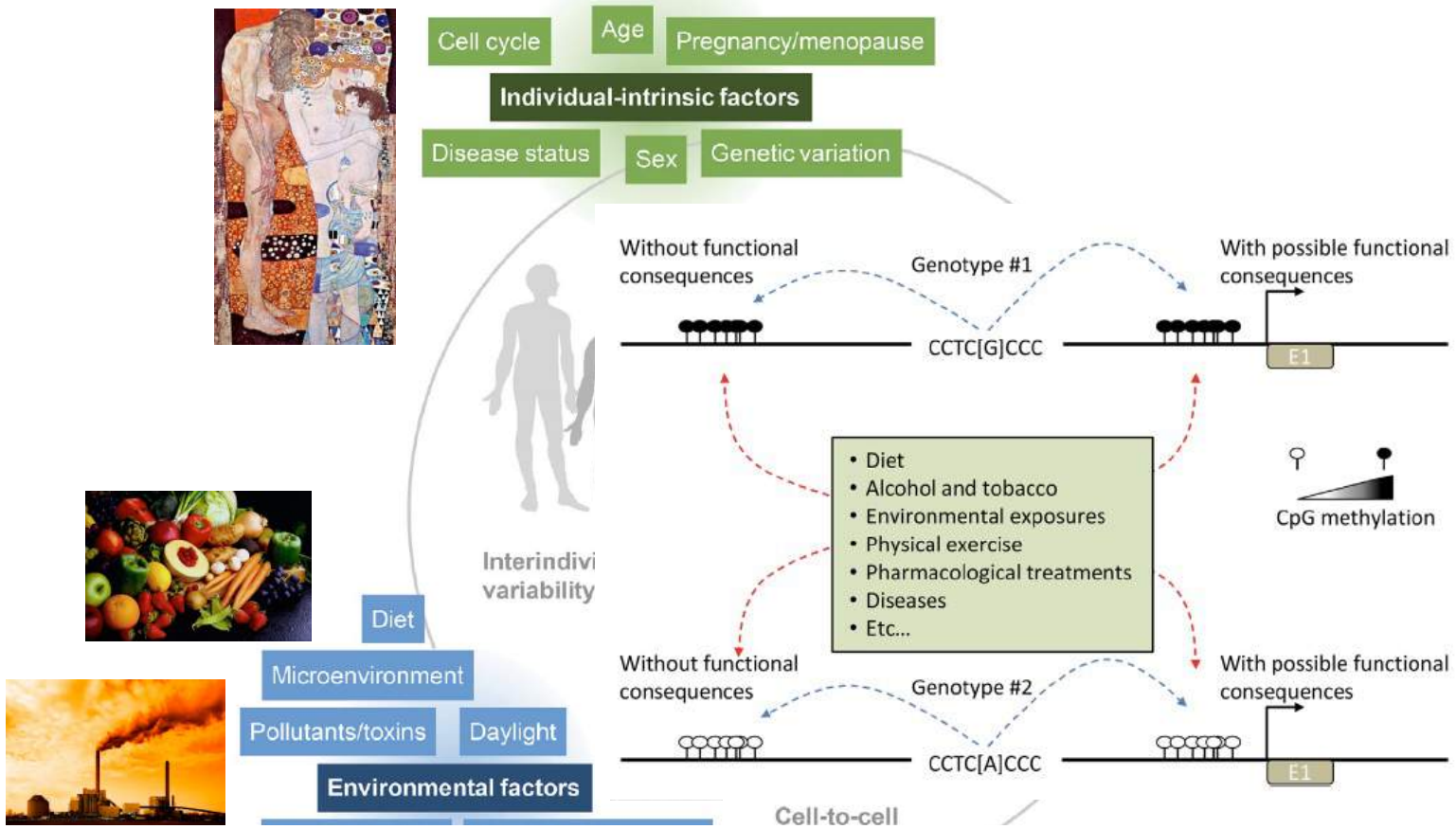
Deciphering How Genotype x Environment leads to Phenotypes



Can we decipher any logic by looking at chromatin and epigenetic marks?

Chromatin is the Physiological Template of the Genome

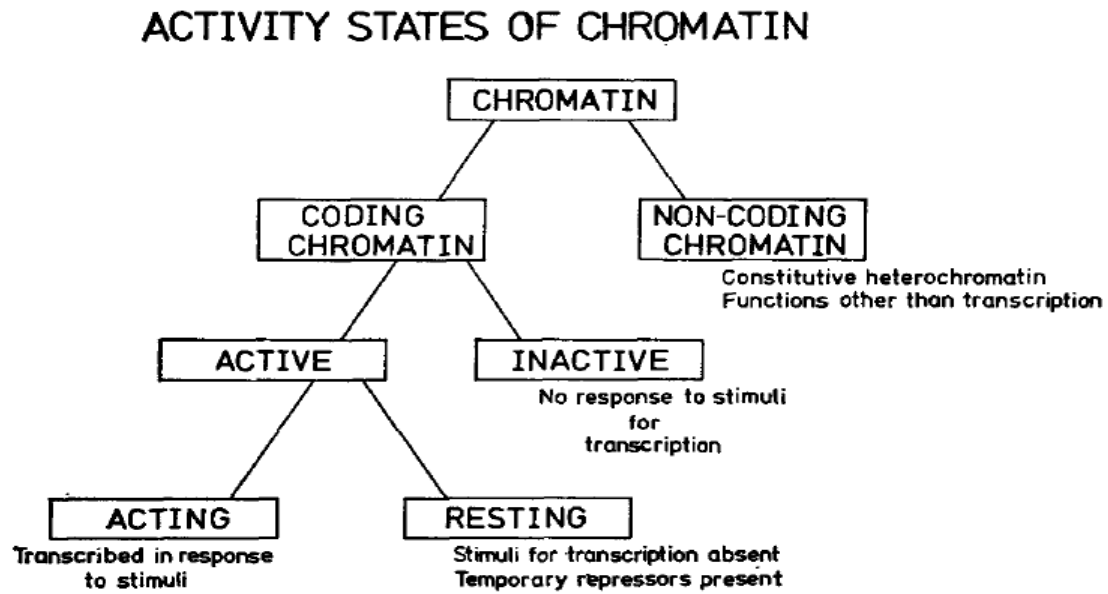
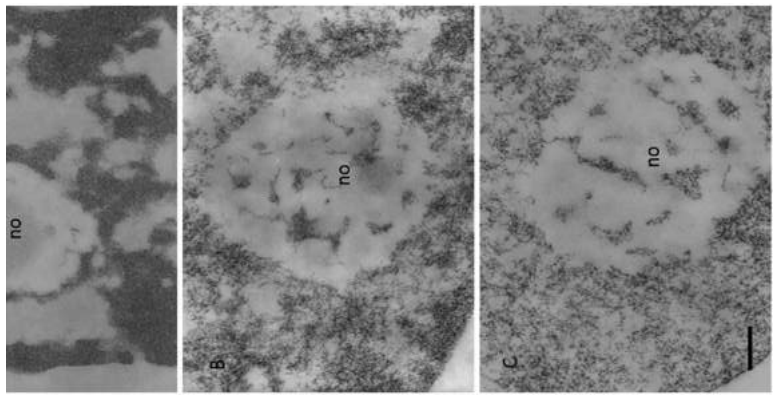


FIGURE 1.—Classification of eukaryote chromatin according to its functional state.

Genetics 78: 305–309 September, 1974. *From M. Lyon, 1974*



lymphocyte **Lymphocyte stimulated to proliferate and increase RNA PolII Activity**

24 h or 48 h phytohemagglutinin exposure

Derenzini et al, 2014

Chromosomes during interphase are highly plastic structures.

The relationship between chromatin and the interchromatin space is highly variable depending upon RNA transcription, cell cycle phases

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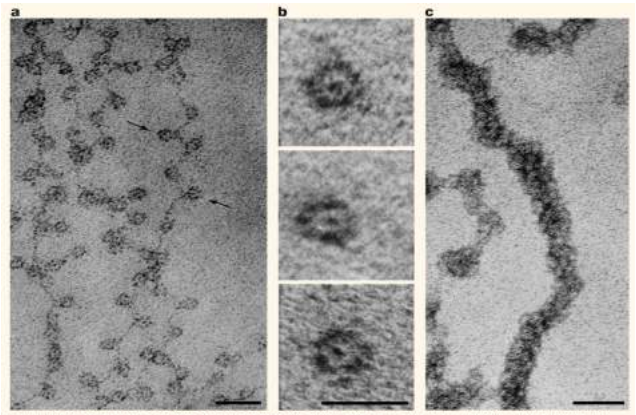
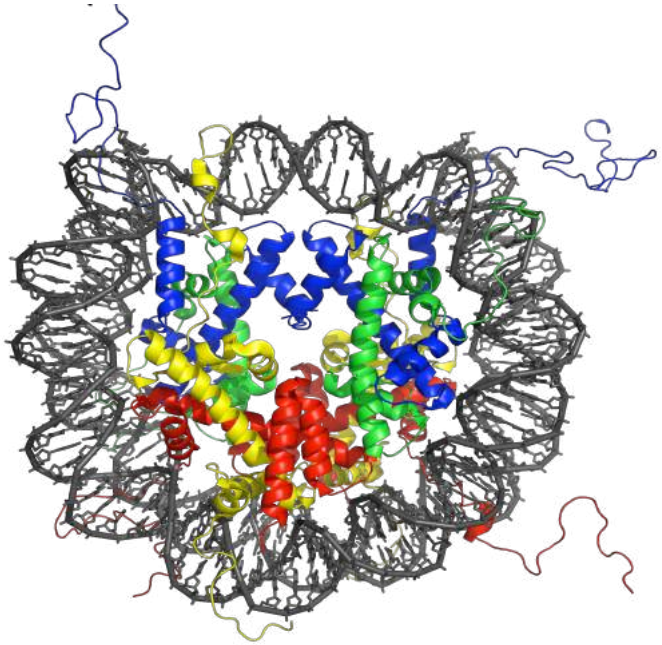
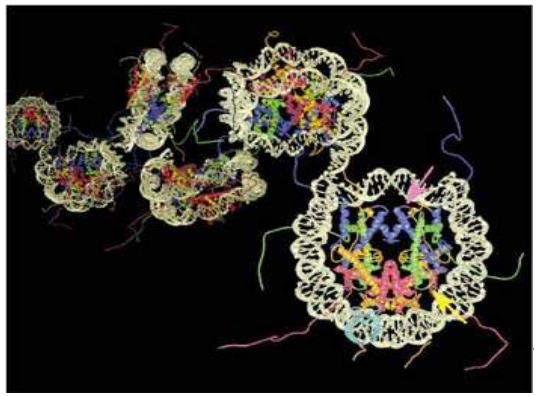
Chromatin is the Physiological Template of the Genome

The Nucleosome: Basic Repeat Unit of Chromatin



Ultrastructurally, nucleosomes are flat cylinders with a diameter of 11 nm &with a height of 5.5 nm. (Feulgen-like osmium-ammine staining - only DNA is stained) (review Olins & Olins, 2003)





DNA is wrapped around an octamer of Histones

Histones are small basic proteins consisting of a globular domain and a more flexible and charged NH2-terminus (histone "tail") that protrudes from the nucleosome.

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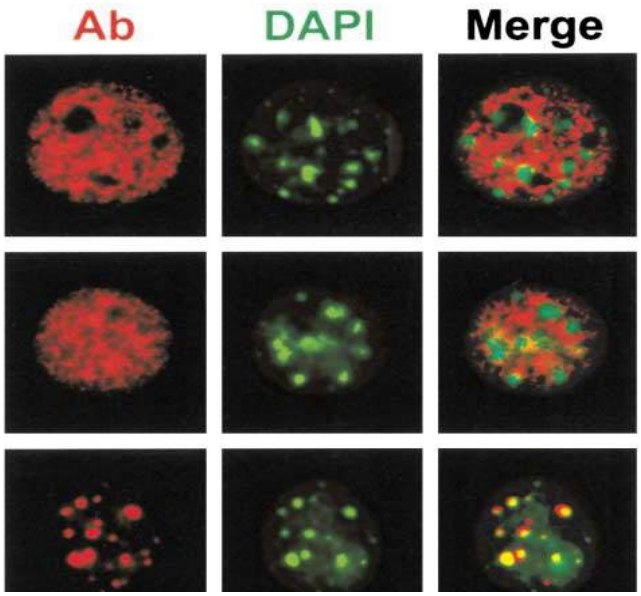
The 1990's: New tools for detecting Histone Modifications

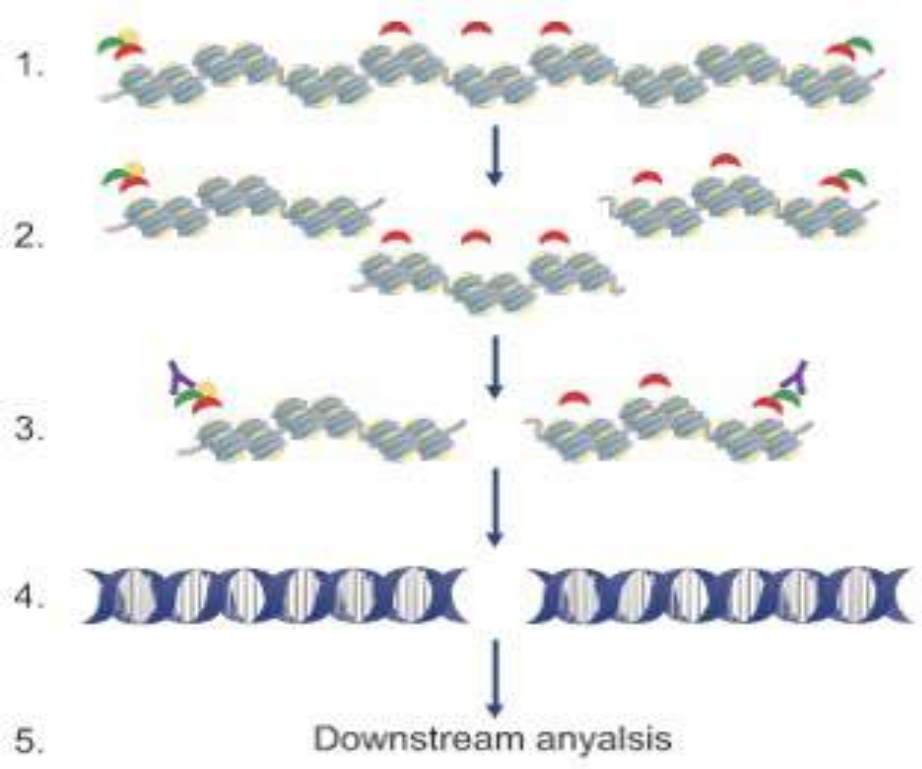
Highly specific **antibodies** raised - discriminating between chemically modified histones at specific amino acids, => histone modifications could be detected by **immunofluorescence** (IF) and **chromatin immunoprecitipation (ChIP)**



α-H3 Lys9-monomethyl

α-H3 Lys9-dimethyl

α-H3 Lys9-trimethyl



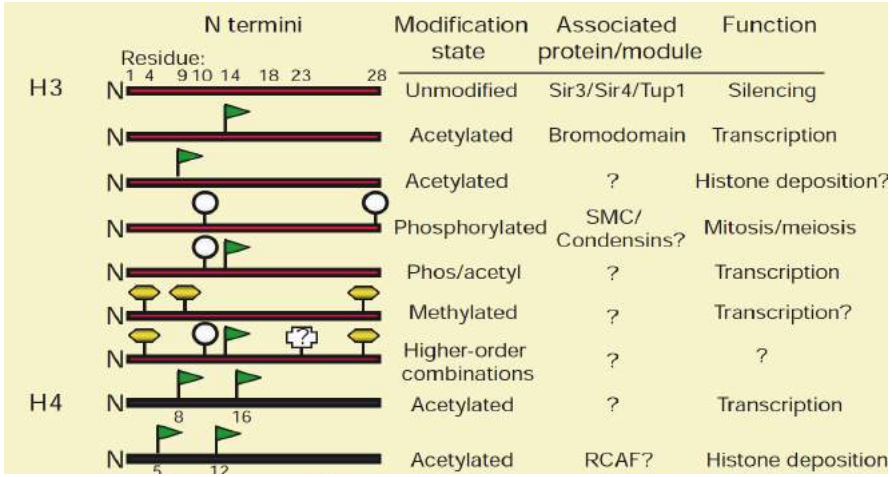
Unique tools to explore the differential states of chromatin by immunofluorescence and by chromatin immunoprecipitation

The Histone "Code" Hypothesis (2000-2001)

The language of covalent histone modifications

Brian D. Strahl & C. David Allis

Department of Biochemistry and Molecular Genetics, University of Virginia Health Science Center, Charlottesville, Virginia 22908, USA

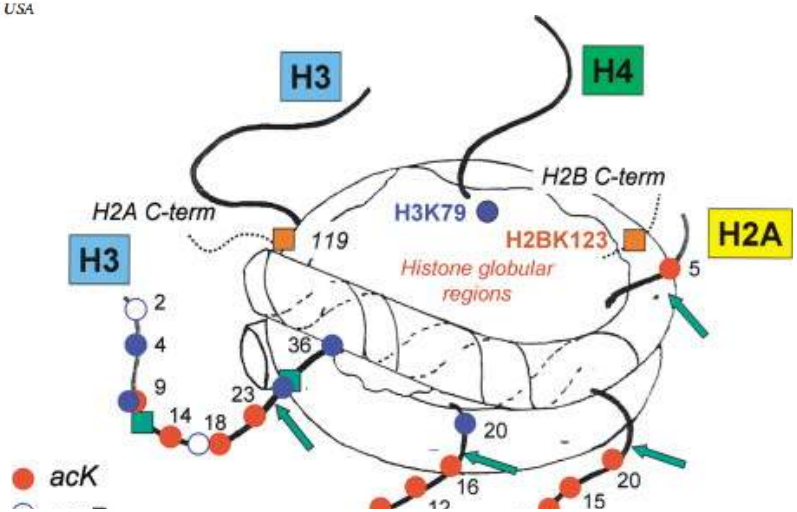


	N termini	Modification state	Associated protein/module	Function
H3	Residue: 1 4 9 10 14 18 23 28	Unmodified	Sir3/Sir4/Tup1	Silencing
		Acetylated	Bromodomain	Transcription
		Acetylated	?	Histone deposition?
		Phosphorylated	SMC/ Condensins?	Mitosis/meiosis
		Phos/acetyl	?	Transcription
		Methylated	?	Transcription?
		Higher-order combinations	?	?
H4	8 16	Acetylated	?	Transcription
		Acetylated	RCAF?	Histone deposition

Bryan Turner

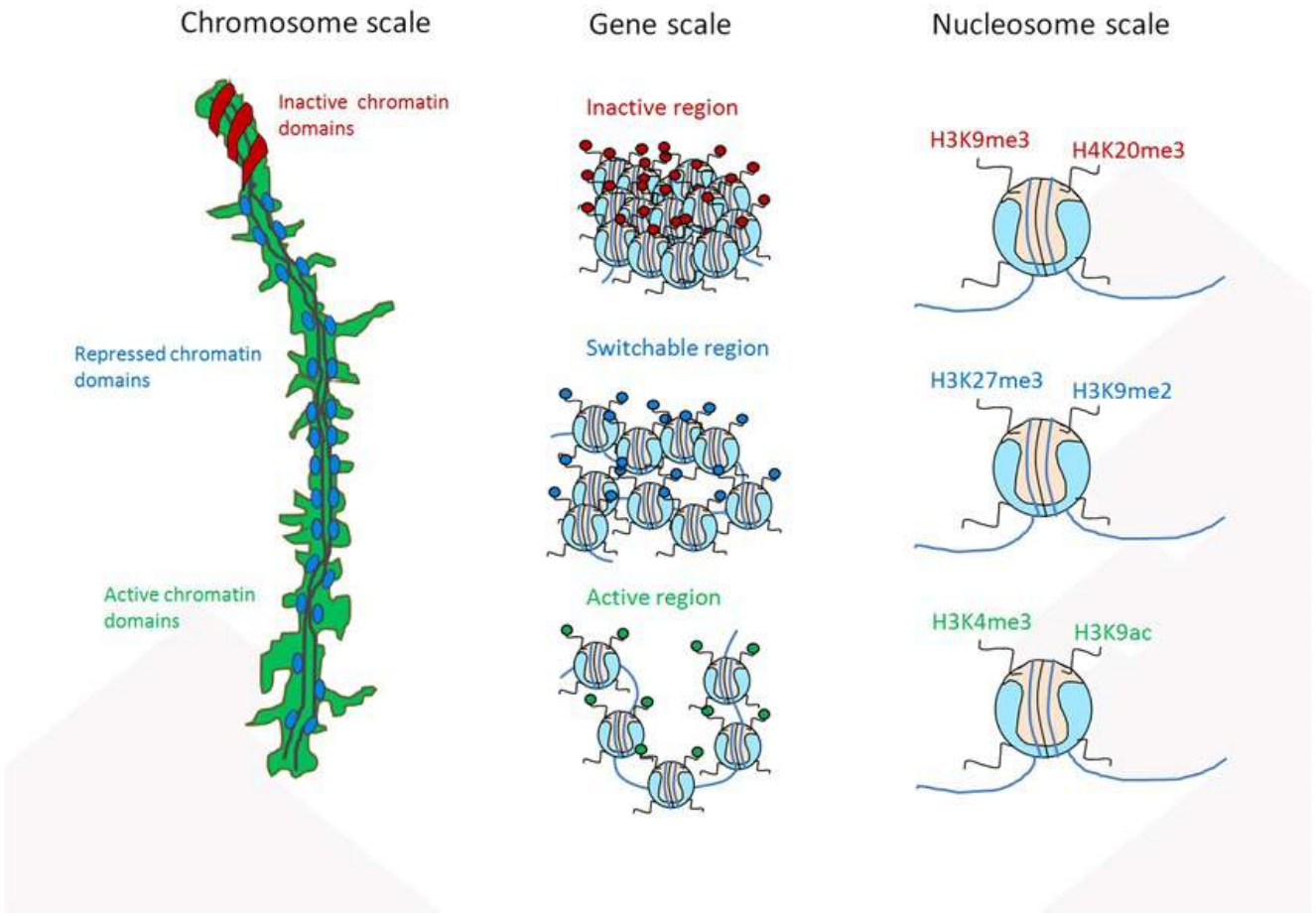
C. David Allis

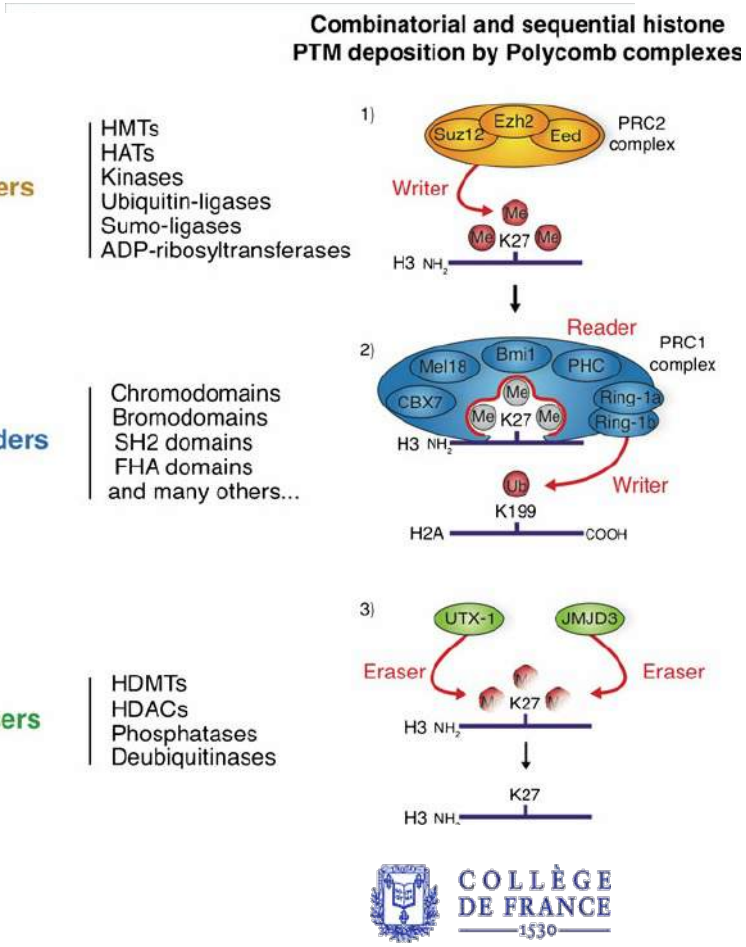
and others…



Can histone modifications function in combinations to recruit factors or facilitate / mediate their roles?

2002: The Histone “Code” Hypothesis...is still a Hypothesis





The molecular characterisitcs of heterochromatin and euchromatin

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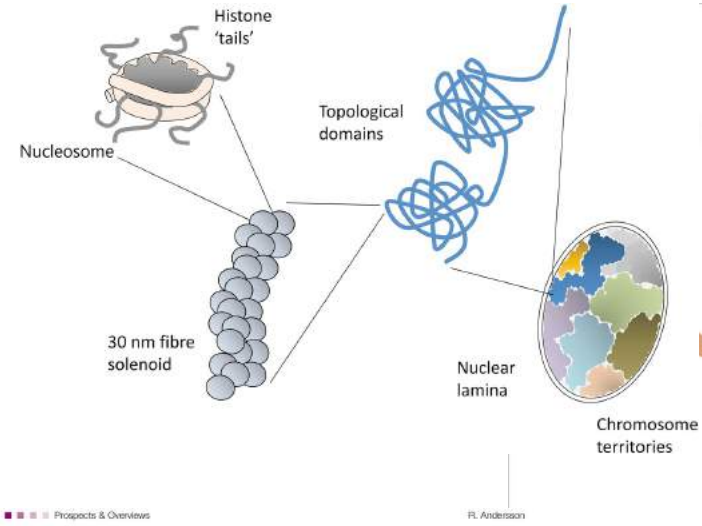
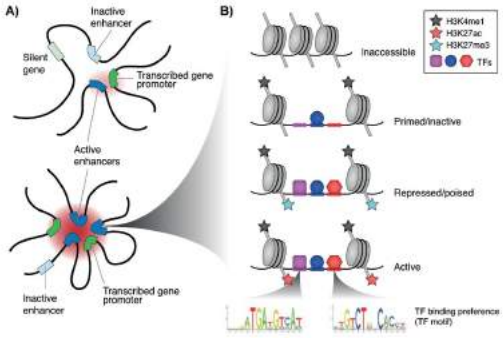
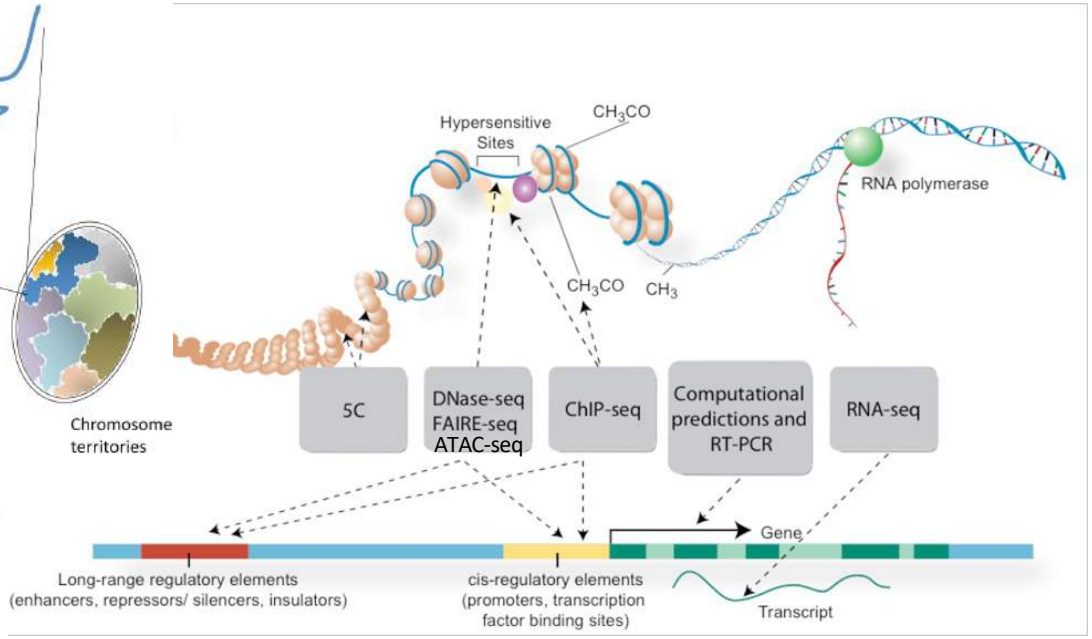
Technologies to understand Epigenomes and 3D Genome Folding







Gene expression
Regulatory DNA sequence elements of genes
Transcription Factor DNA binding sites
DNA methylation
Chromatin accessibility
Chromatin modifications
3D organization

COLLÈGE DE FRANCE 1530

Deciphering an Epigenetic logic using Epigenomics?

Arabidopsis

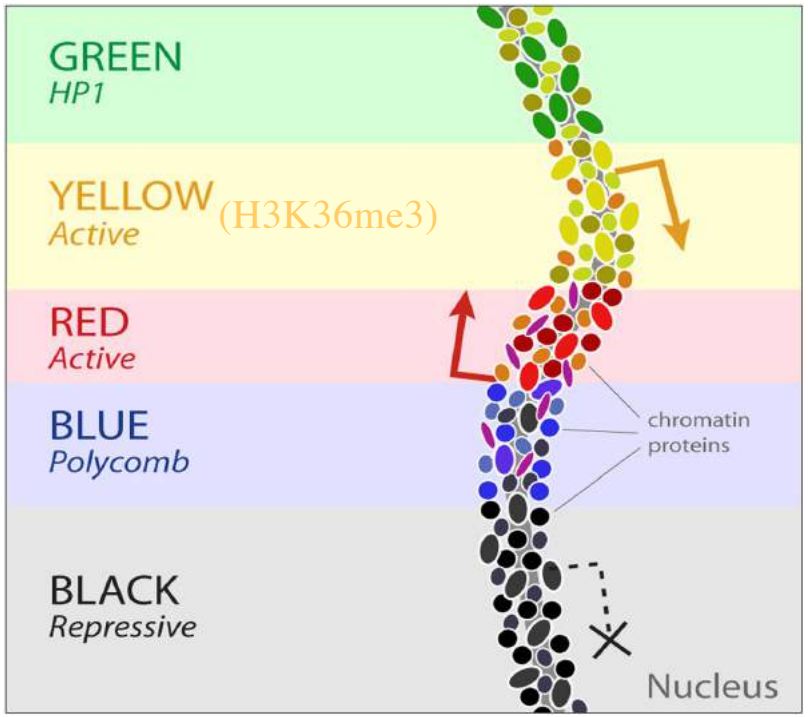
Drosophila

CS3
Heterochromatin

CS1
Expressed genes

CS2
PcG-mediated gene repression

CS4
Silent euchromatin



Roudier et al., *EMBO J* (2011)

Filion et al., *Cell* (2010)

Systematic Protein Location Mapping Reveals Principal Chromatin Types

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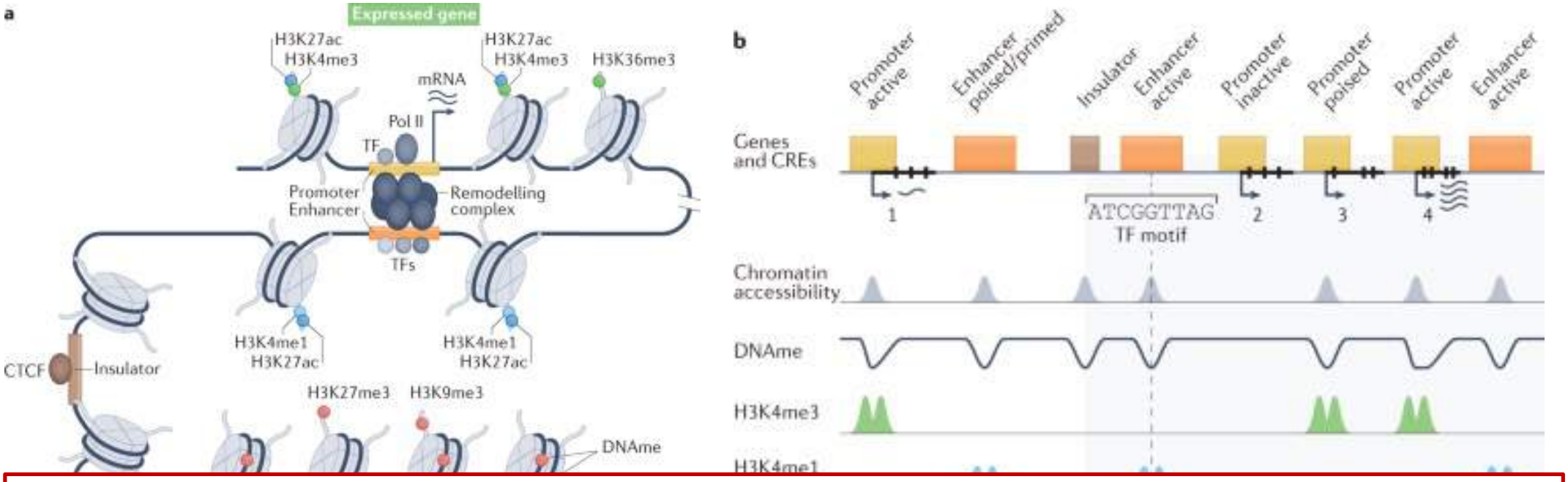
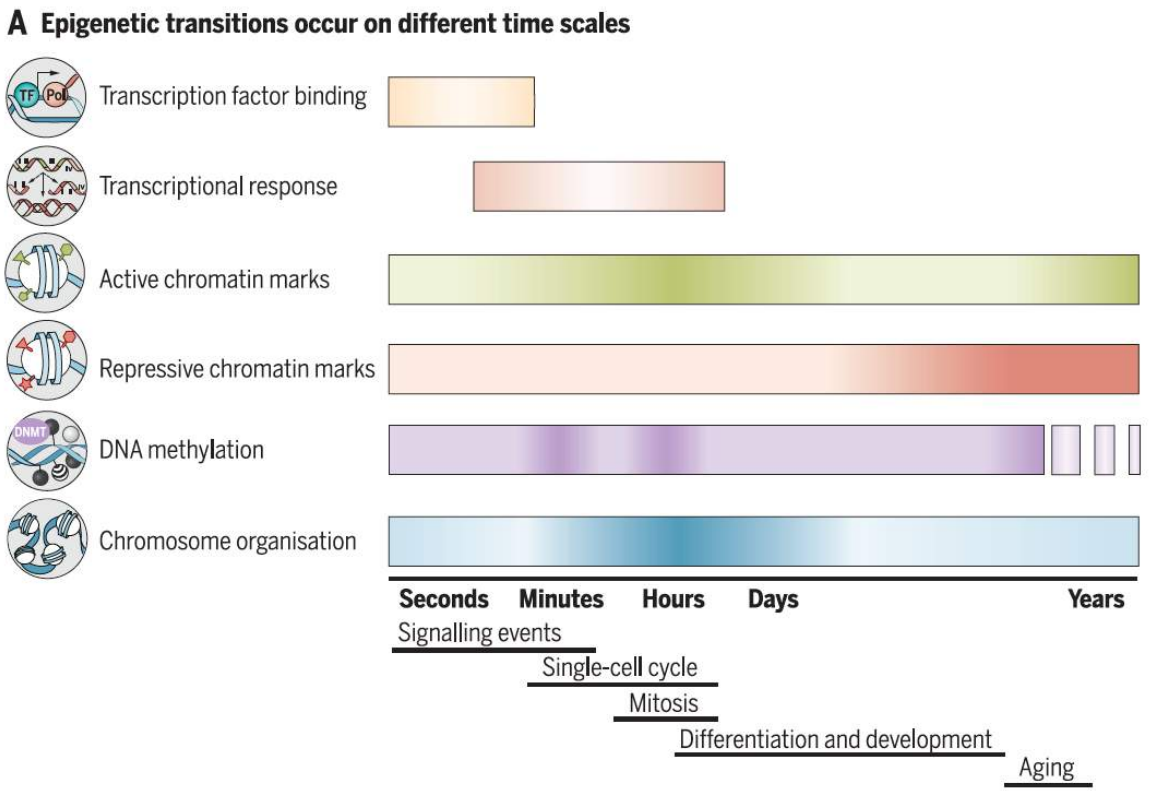
Deciphering an Epigenetic logic using Epigenomics?

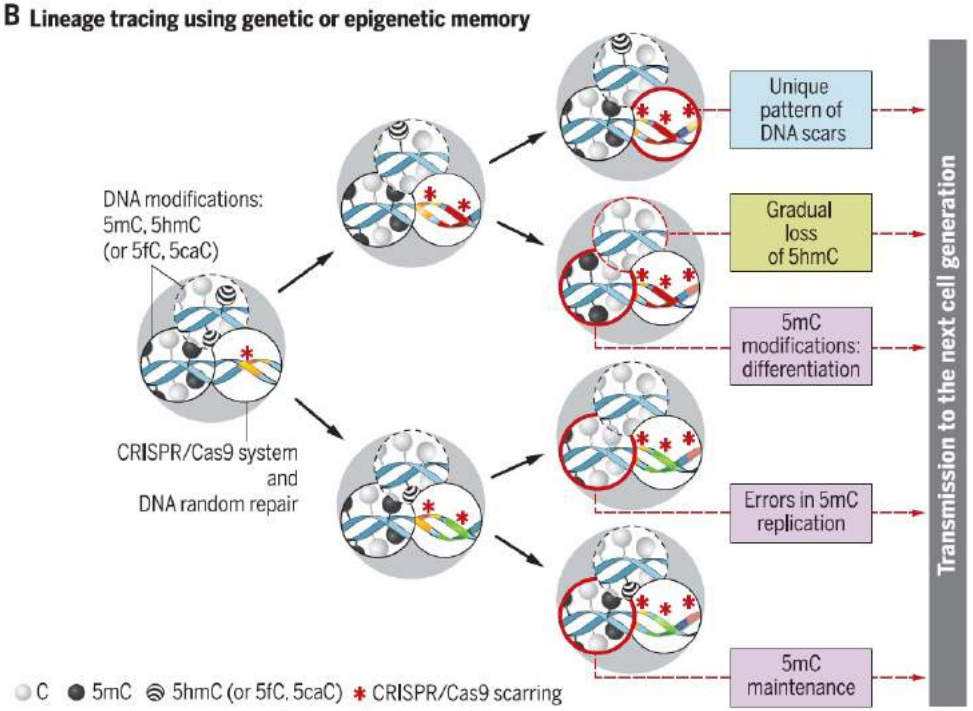


Understanding the relationship between DNA sequence-specific binding of transcription factors, changes in chromatin modifications and 3D structure <u>in gene rgulation and its variability</u>

Time dimension… Genetic analyses…

Following Gene expression and Epigenomic changes over Time





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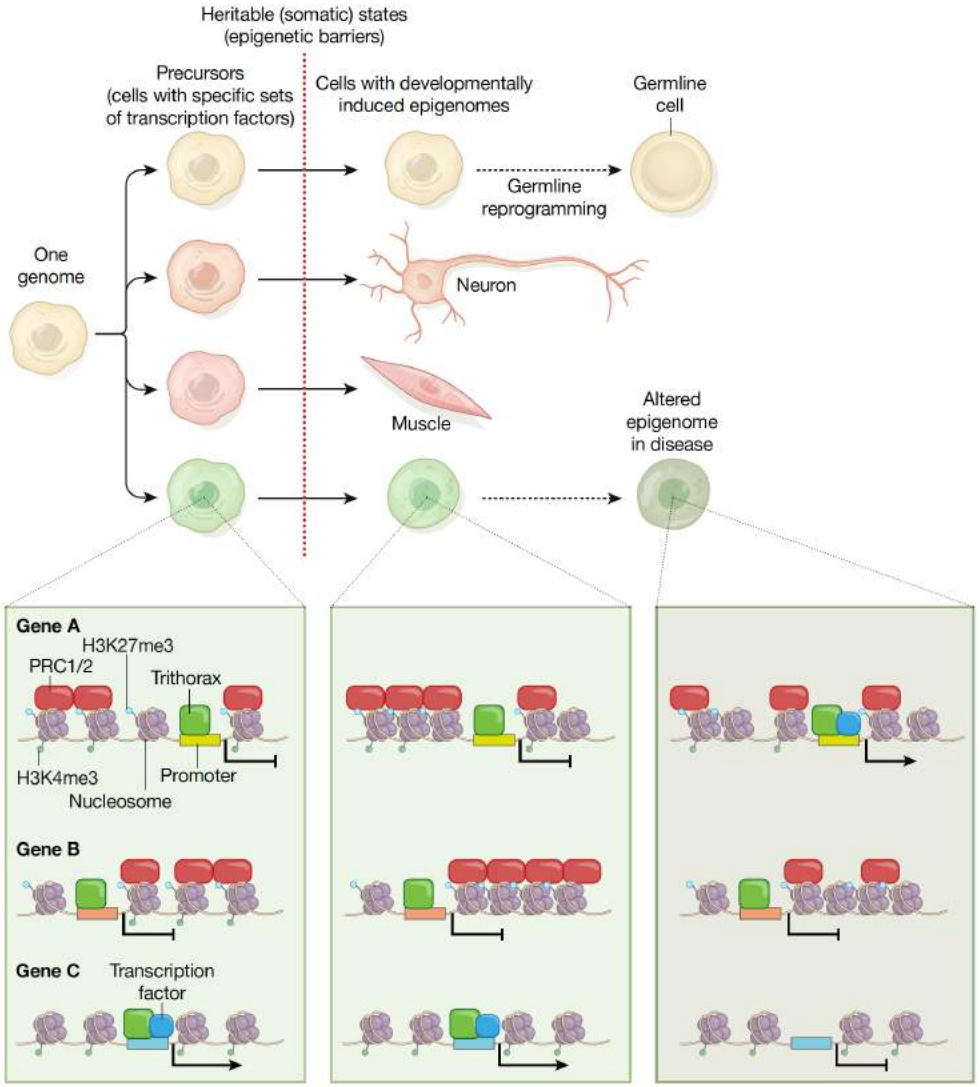
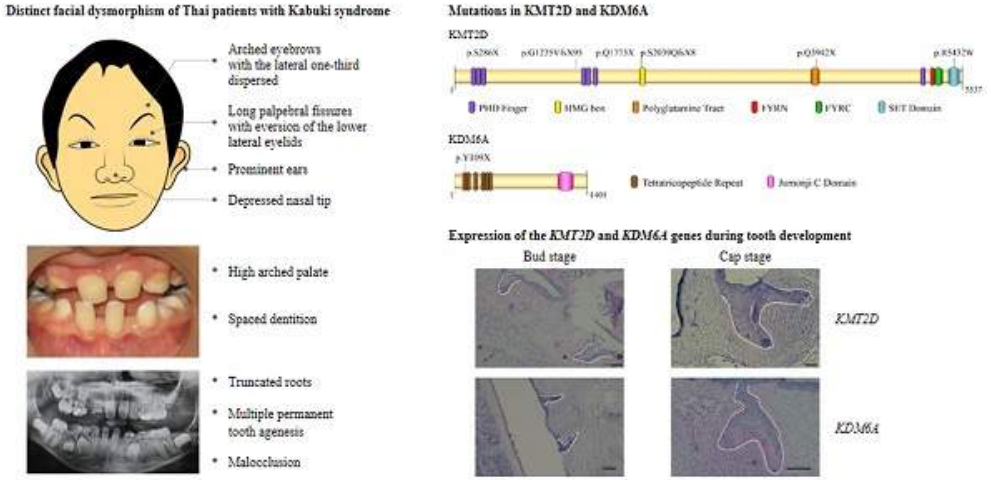
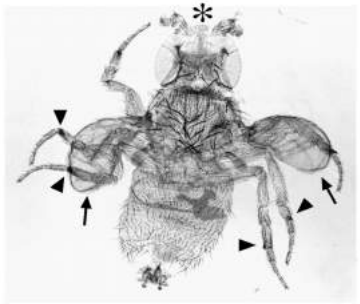
Deciphering the Logic of Development



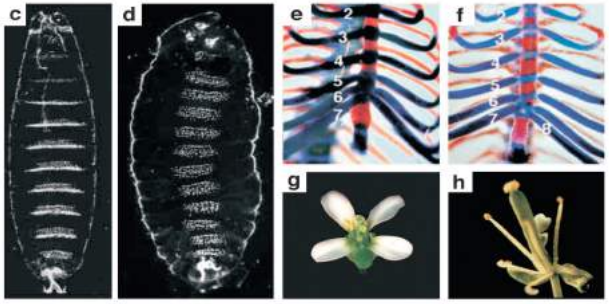
Mutations in epigenetic factors disrupt development



Porntaveetud et al, Int J Biol Sci 2018



$Su(z)12^{5}/Su(z)12^{3}$



Deciphering how Genotype x Environment leads to Phenotypes

Cell cycle | Age | Pregnancy/menopause

Individual-intrinsic factors

Disease status | Sex | Genetic variation

Interindividual variability

Diet

Microenvironment

Pollutants/toxins | Daylight

Environmental factors

Physical activity | Prenatal environment

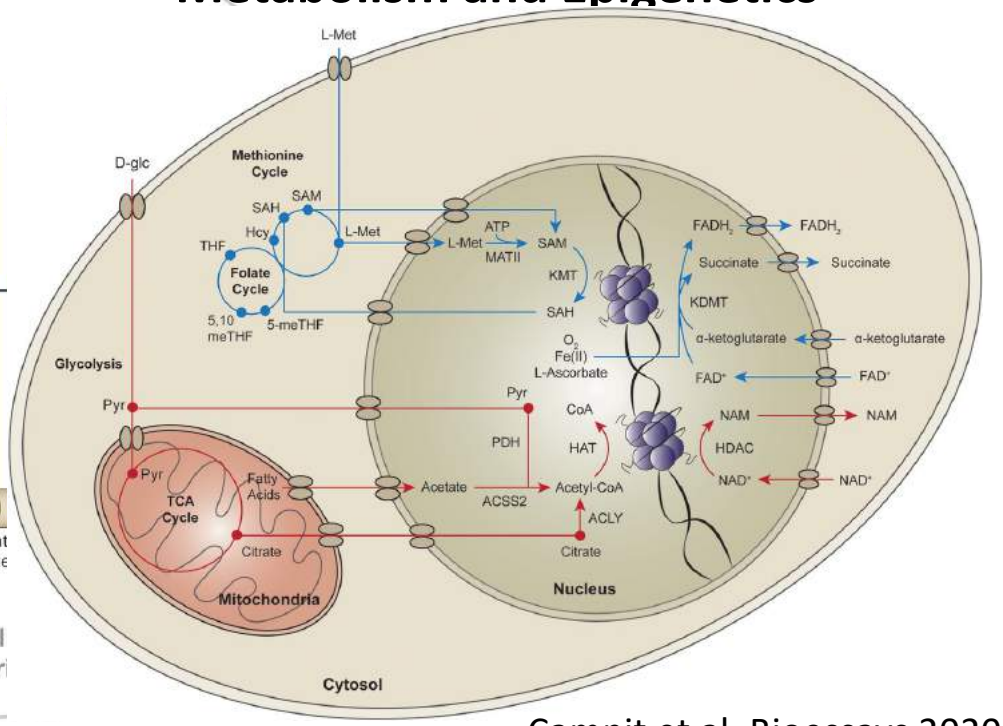
Smoking | Climate | Stress

Nutrient transporte

Cel vari

Adapted from Ecker et al, Bioessays 2018

Cross-talk between Metabolism and Epigenetics



Campit et al, Bioessays 2020

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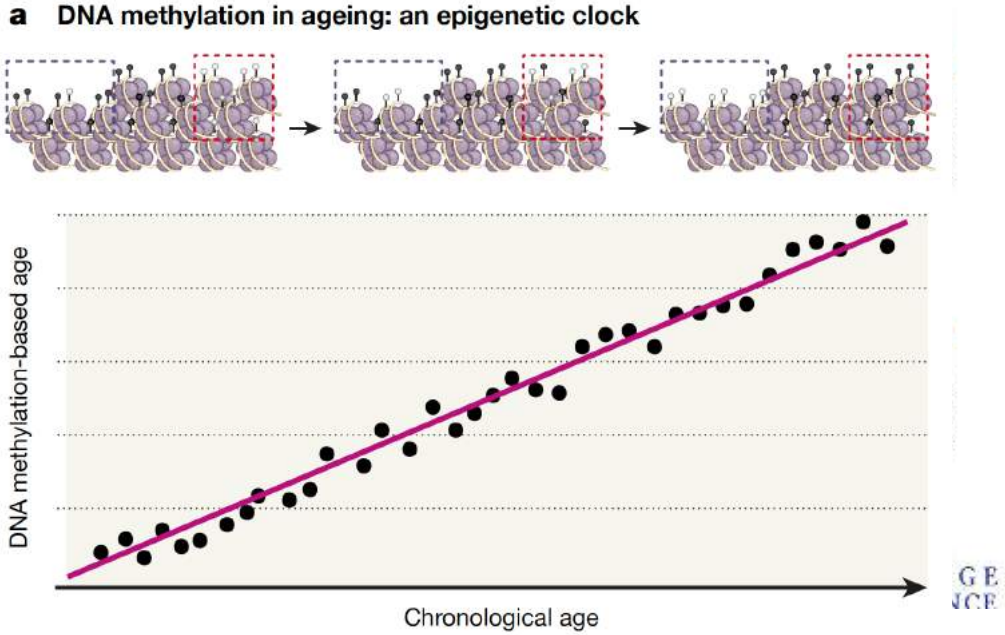
Measuring Ageing through Epigenetic Changes?

Just how similar are two supposedly genetically identical individuals as they age…

The term 'epigenetic clock' is used to denote two distinct but related things:

- synonym of a highly accurate age estimator based on DNA methylation levels
- concept of an innate process in the body that continues inexorably, resulting in ageing

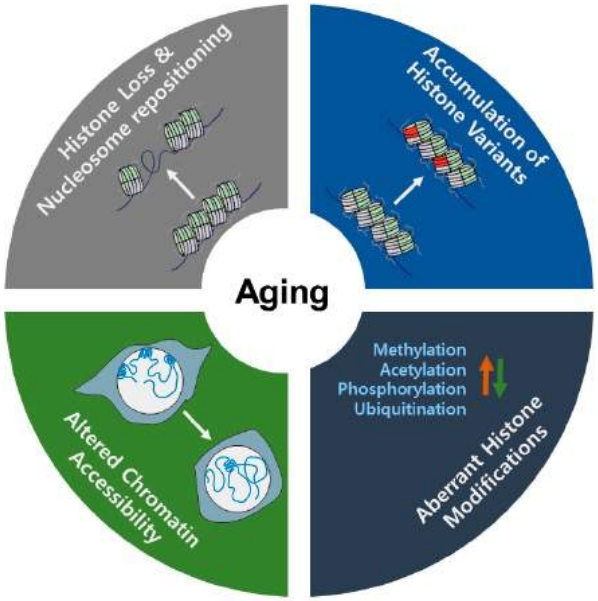
Horvath and Raj, NRG 2018



Ageing and Epigenetic Changes

Evidence that epigenetic alterations may play a major part in the ageing process?

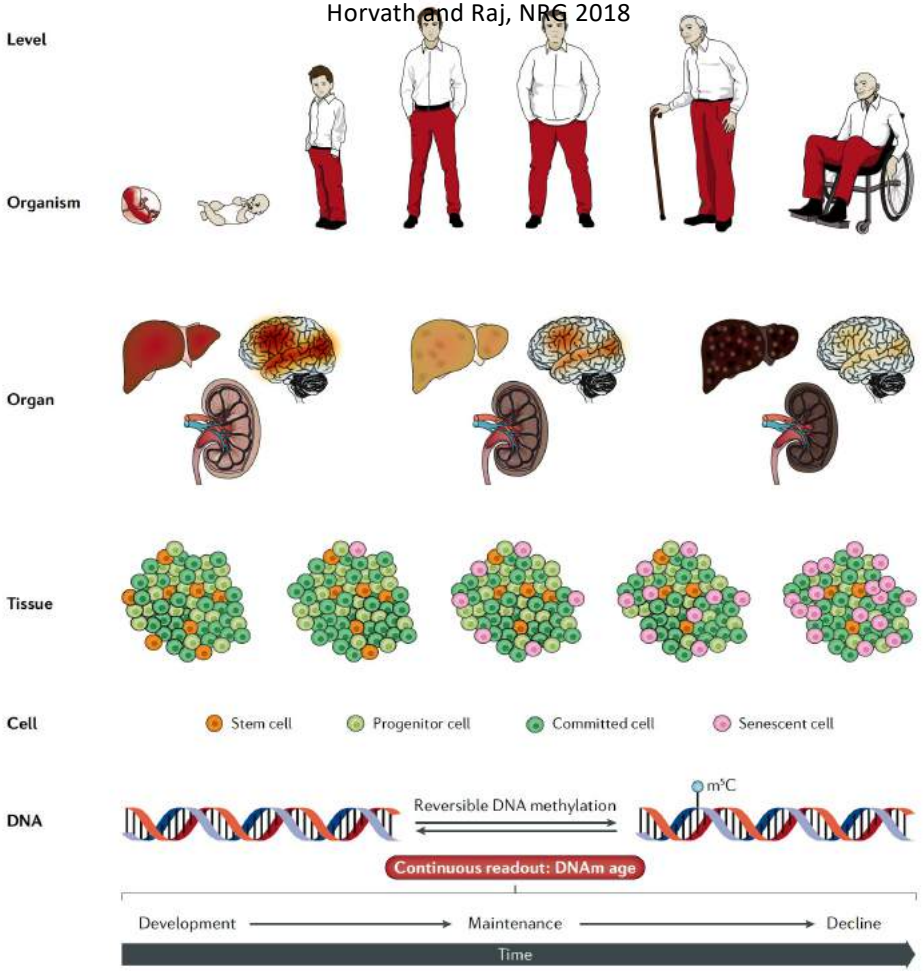
Epigenetic changes during ageing can contribute to changes in genomic instability and changes in gene expression profiles that are characterized by an increase in gene expression noise - associated with ageing process?



Zhang… Izpisua Belmonte 2020 “The ageing epigenome and its rejuvenation”

DNA methylation-based biomarkers and the epigenetic clock theory of ageing

Horvath and Raj, NRG 2018



Ageing and Epigenetic Changes: Rejuvenation Potential?

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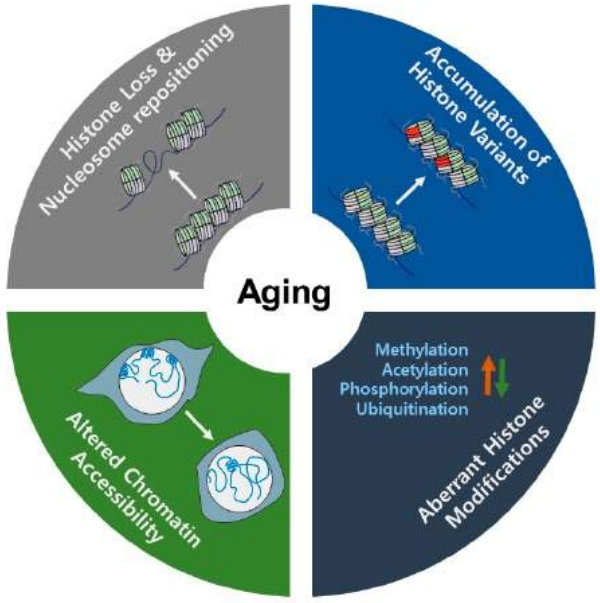

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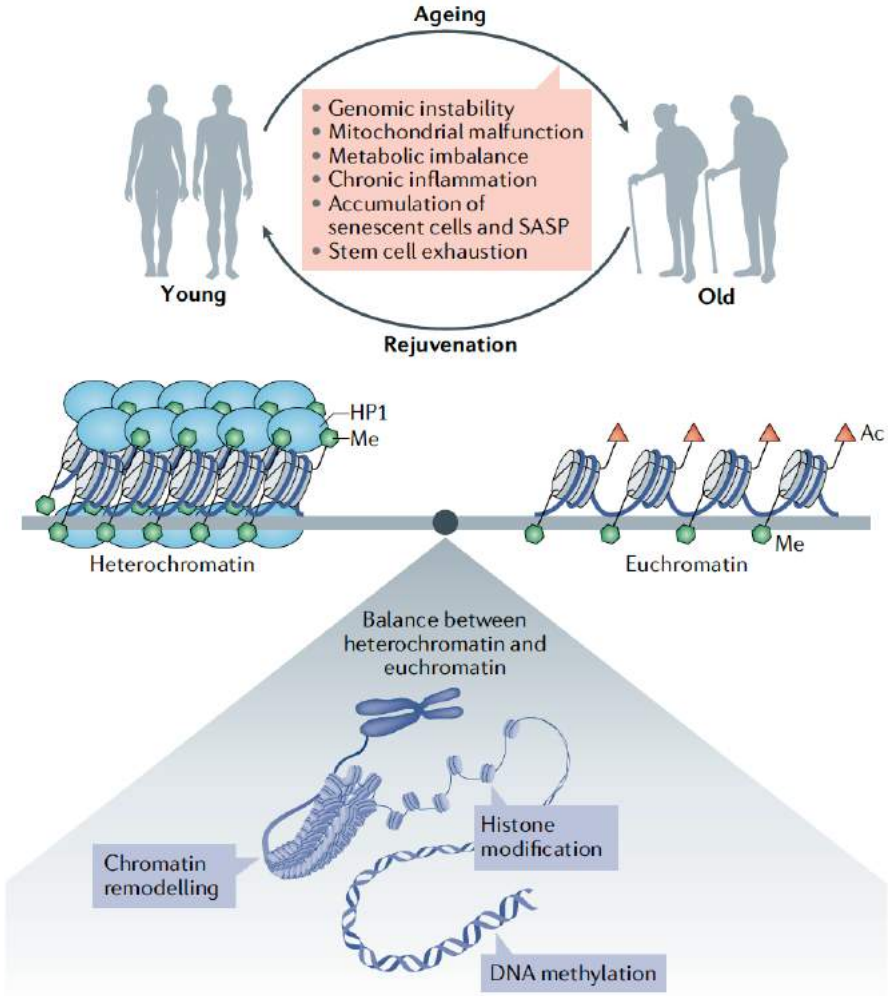

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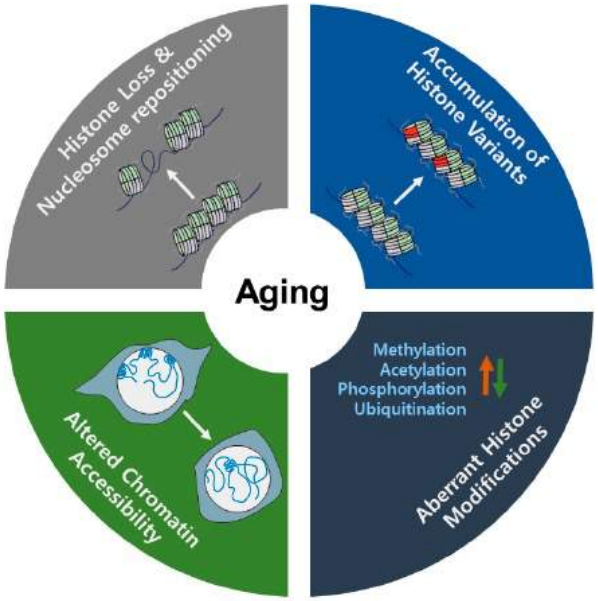

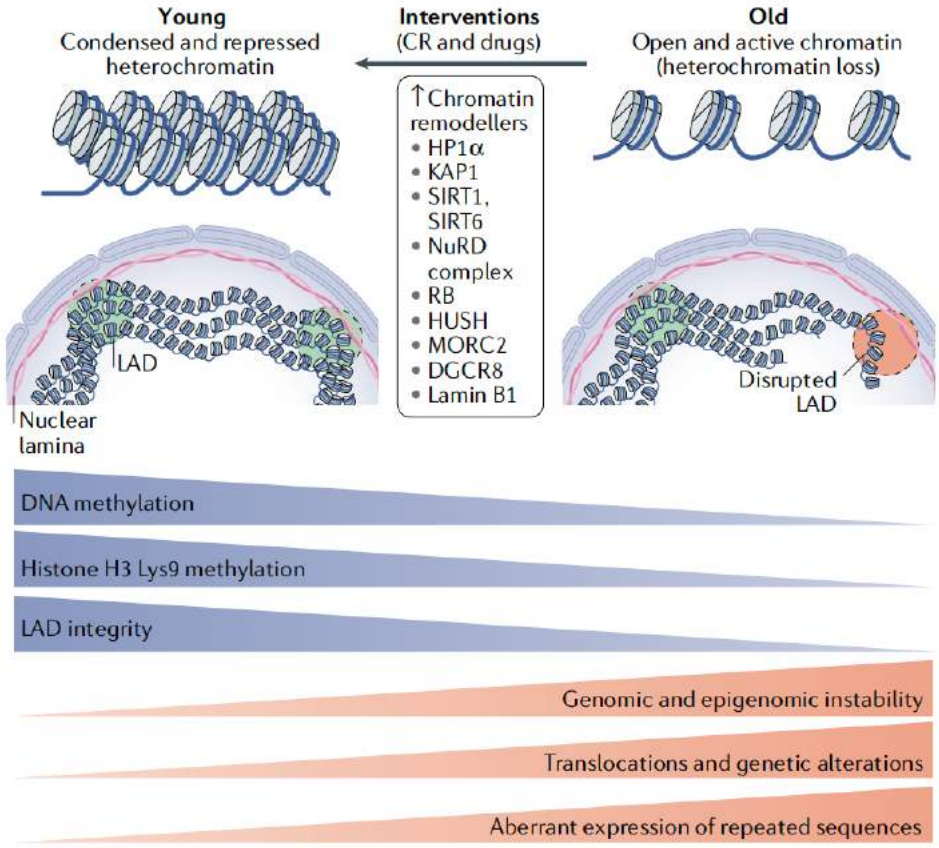

Zhang... Izpisua Belmonte 2020 "The ageing epigenome and its rejuvenation"

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Where are we today in “decoding” the logic of life beyond the genome?

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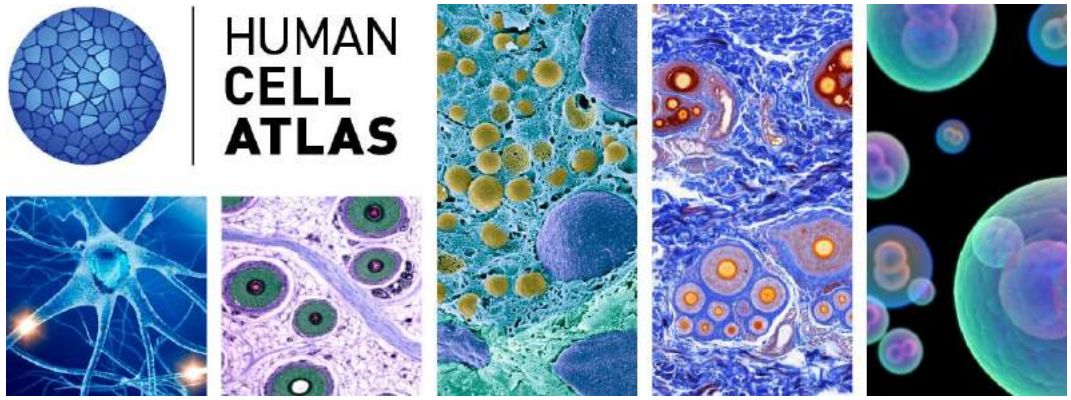
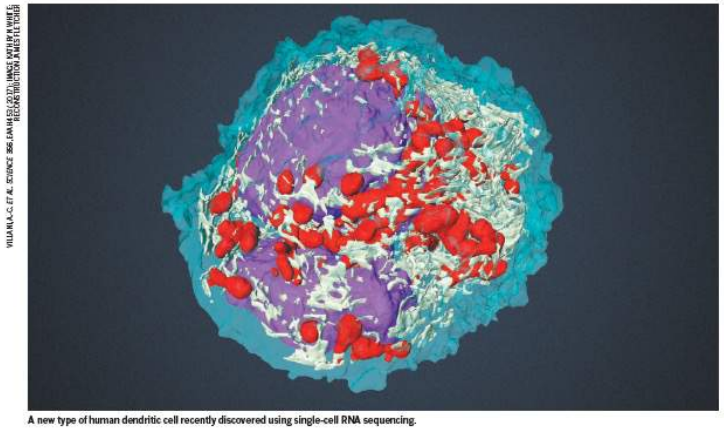
Defining Cell Type, Cell Identity and Variation at the Single-Cell Level



https://www.humancellatlas.org/



A new type of human dendritic cell recently discovered using single-cell RNA sequencing.

The Human Cell Atlas: from vision to reality

The human body at cellular resolution: the NIH Human Biomolecular Atlas Program

HuBMAP Consortium*

Transformative technologies are enabling the construction of three-dimensional maps of tissues with unprecedented spatial and molecular resolution. Over the next seven years, the NIH Common Fund Human Biomolecular Atlas Program (HuBMAP) intends to develop a widely accessible framework for comprehensively mapping the human body at single-cell resolution by supporting technology development, data acquisition, and detailed spatial mapping. HuBMAP will integrate its efforts with other funding agencies, programs, consortia, and the biomedical research community at large towards the shared vision of a comprehensive, accessible three-dimensional molecular and cellular atlas of the human body, in health and under various disease conditions.

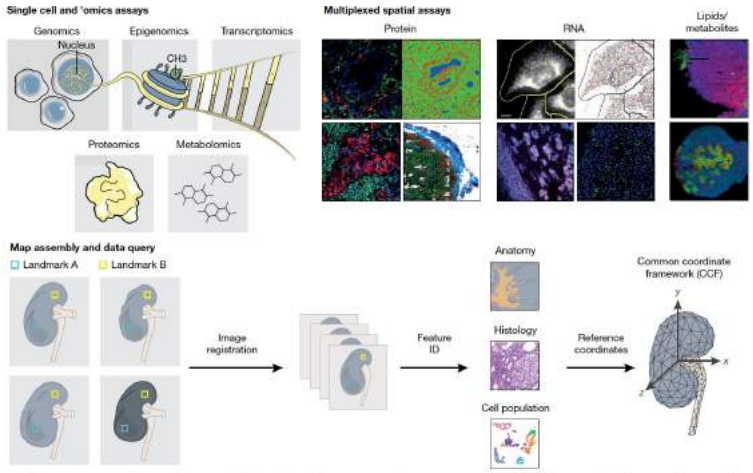


Fig. 3 | Map generation and assembly across cellular and spatial scales. HuBMAP aims to produce an atlas in which users can refer to a histological slide from a specific part of an organ and, in any given cell, understand its contents on multiple 'omic levels—genomic, epigenomic, transcriptomic, proteomic, and/or metabolomic. To achieve these ends, centres will apply a combination of imaging, 'omics and mass spectrometry techniques to specimens collected in a reproducible manner from specific sites in the body. These data will be then be integrated to arrive at a high-resolution, high-content three-dimensional map for any given tissue. To ensure inter-individual differences will not be confounded with collection heterogeneity, a robust CCF will be developed.

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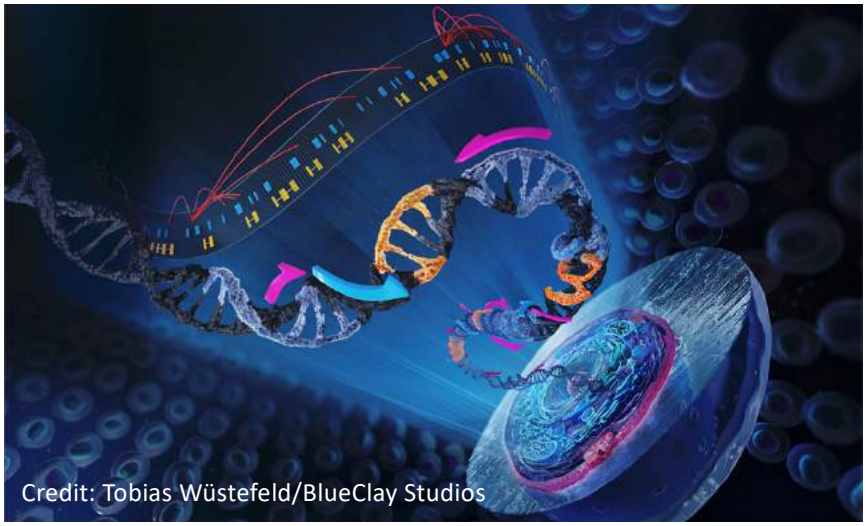
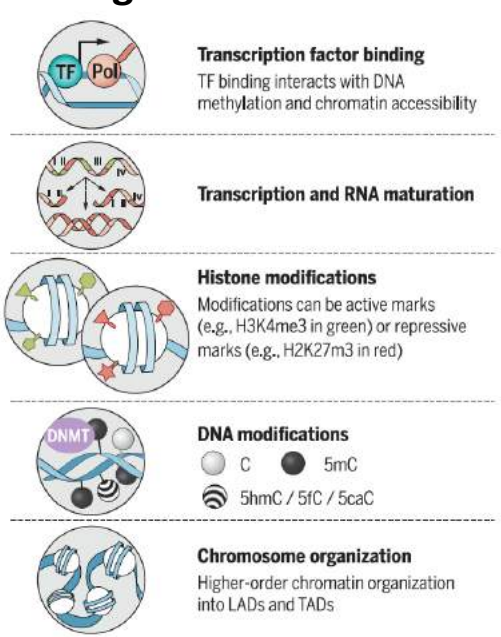
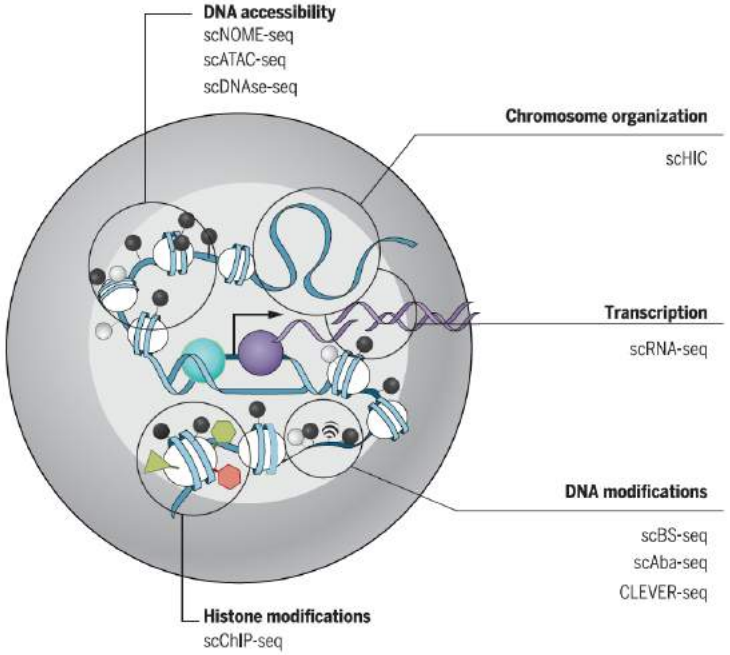
Deciphering phenotypic variation within individuals at the single cell leve over space and time

Single-cell RNA sequencing (scRNA-seq)



Single-cell Multi-omics





Single-cell epigenomics: Recording the past and predicting the future

Gavin Kelsey,[1,2]*† Oliver Stegle,[3,4]*† Wolf Reik[1,2,5]†

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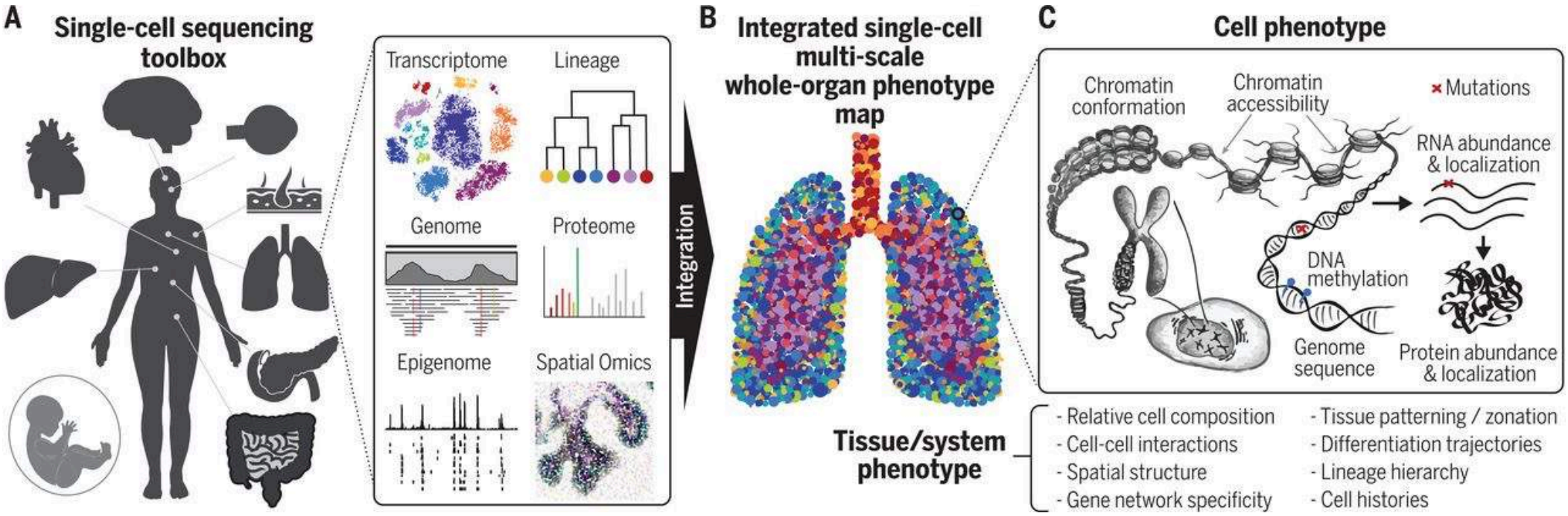
What defines Cell Type or Cell Identity?



Camp et al. 2019 "Mapping human cell phenotypes to genotypes with single-cell genomics". *Science* 365, 1401-1405

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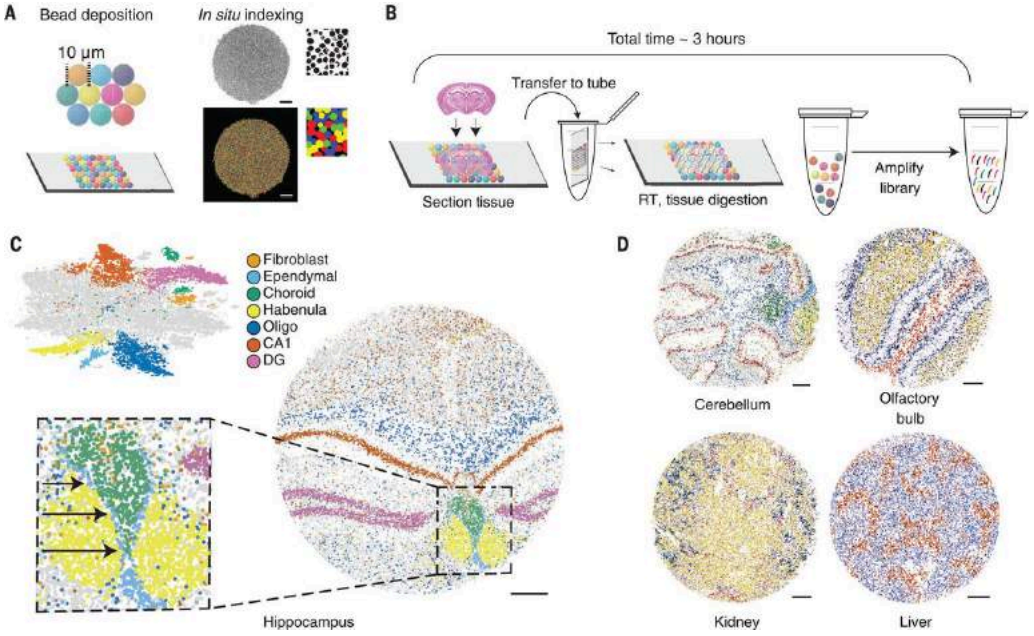
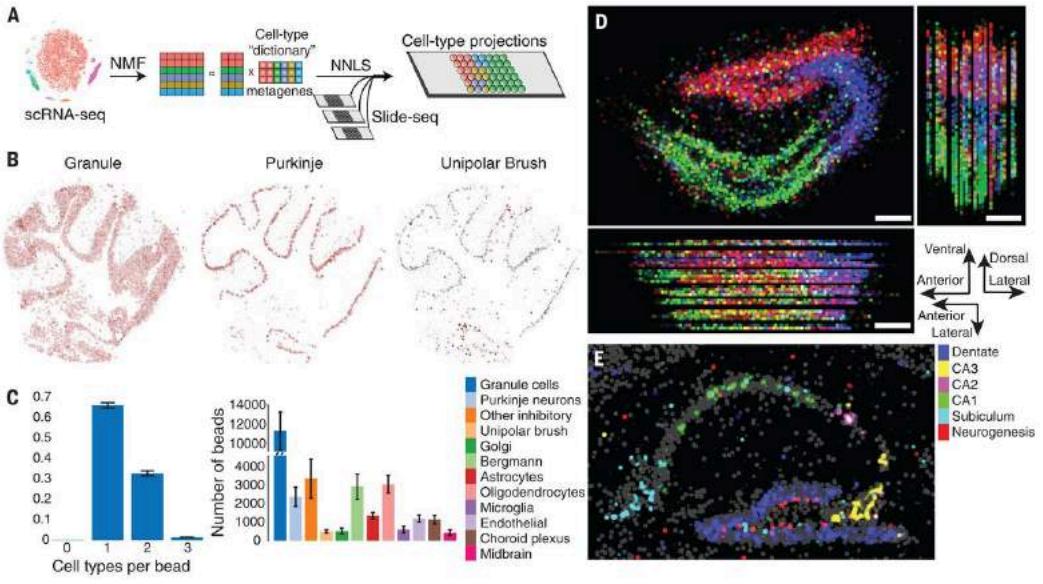
Spatial-omics atlases

High-resolution RNA capture from tissue by Slide-seq.



Localization of cell types in the cerebellum and hippocampus using Slide-seq.



Rodriques et al. Science 2019;363:1463-1467

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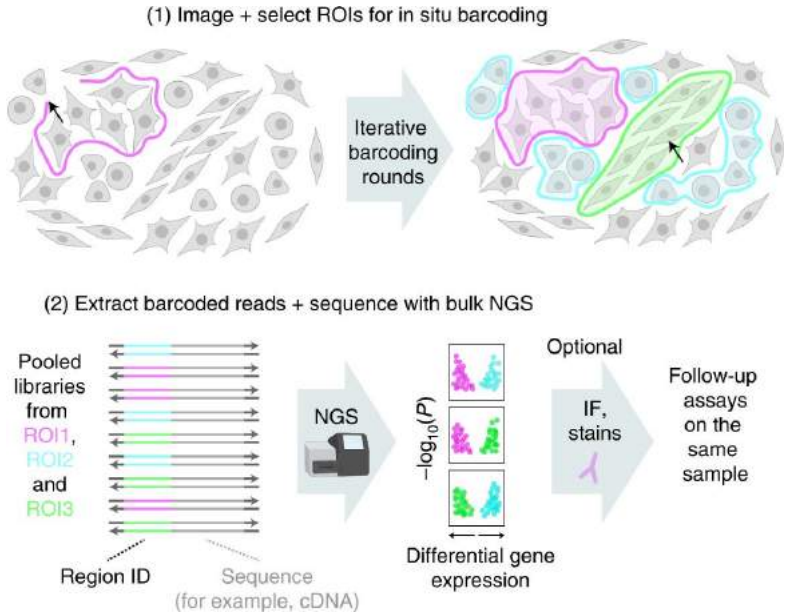
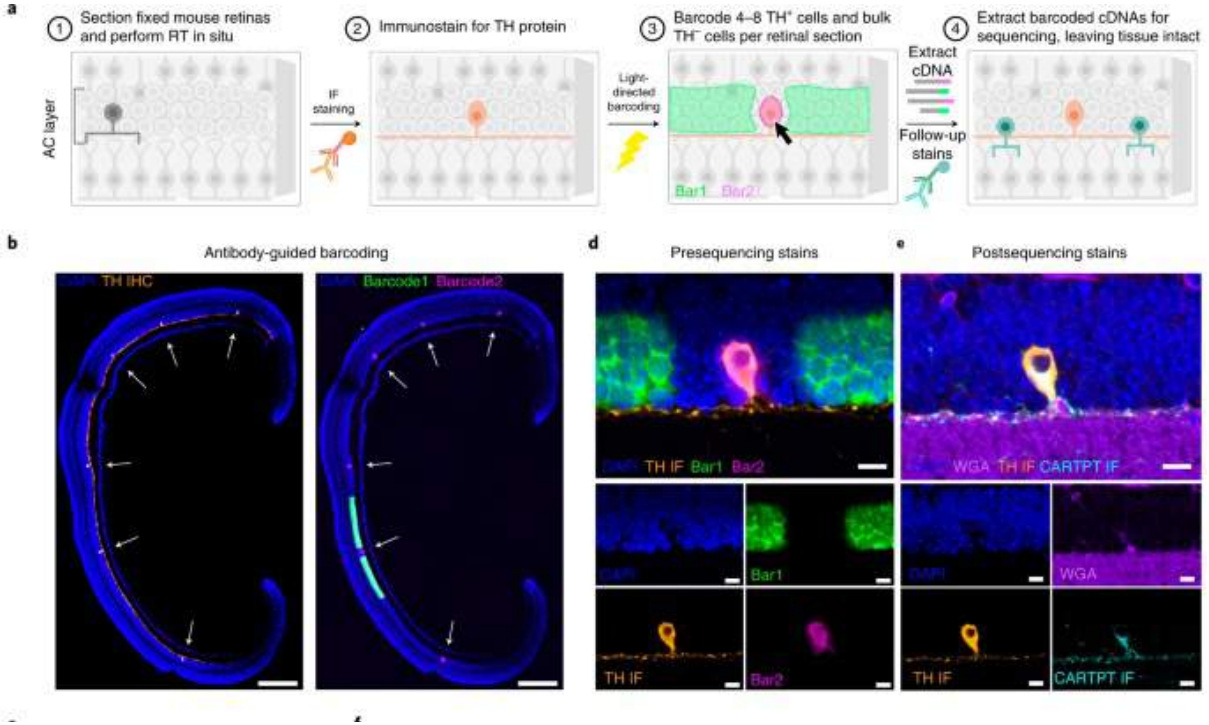
Spatial-omics atlases

Light-Seq: light-directed in situ barcoding of biomolecules in fixed cells and tissues for spatially indexed sequencing





Light-Seq enables selective barcoding of custom selected cells or tissue regions in situ for transcriptomic sequencing

It enables rare cells to be identified and transcriptomics to be performed

Kishi et al, Mature Methods 2022

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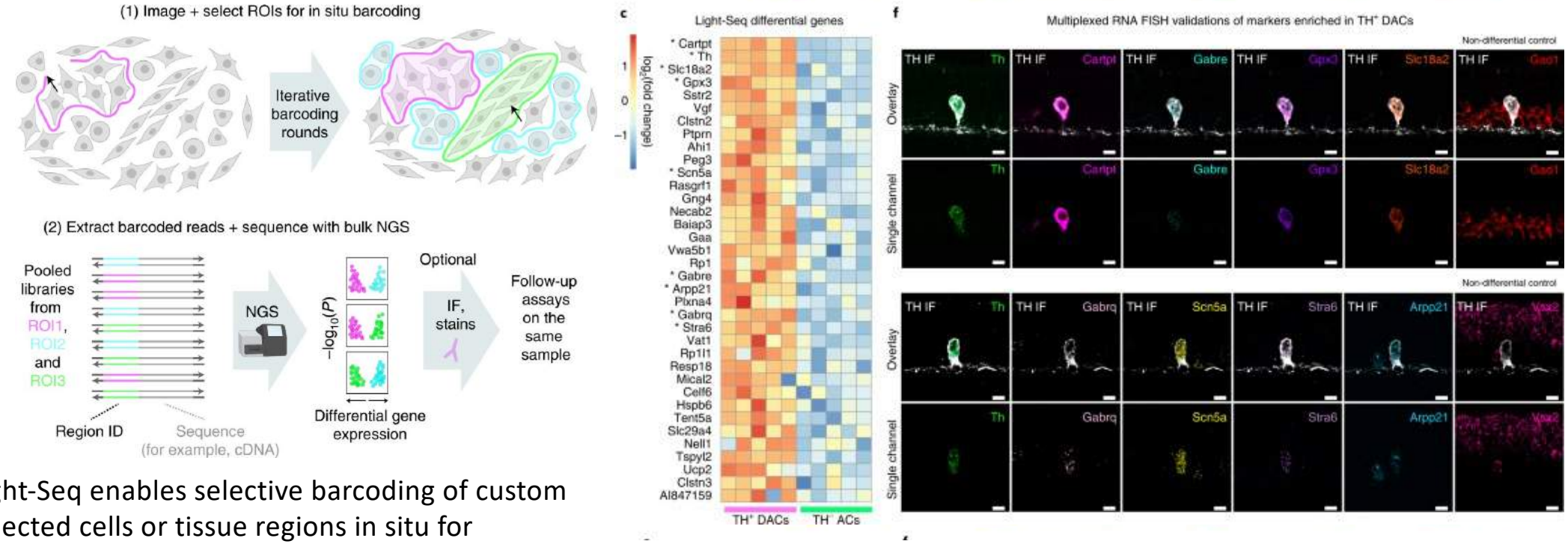

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Kishi et al, Mature Methods 2022

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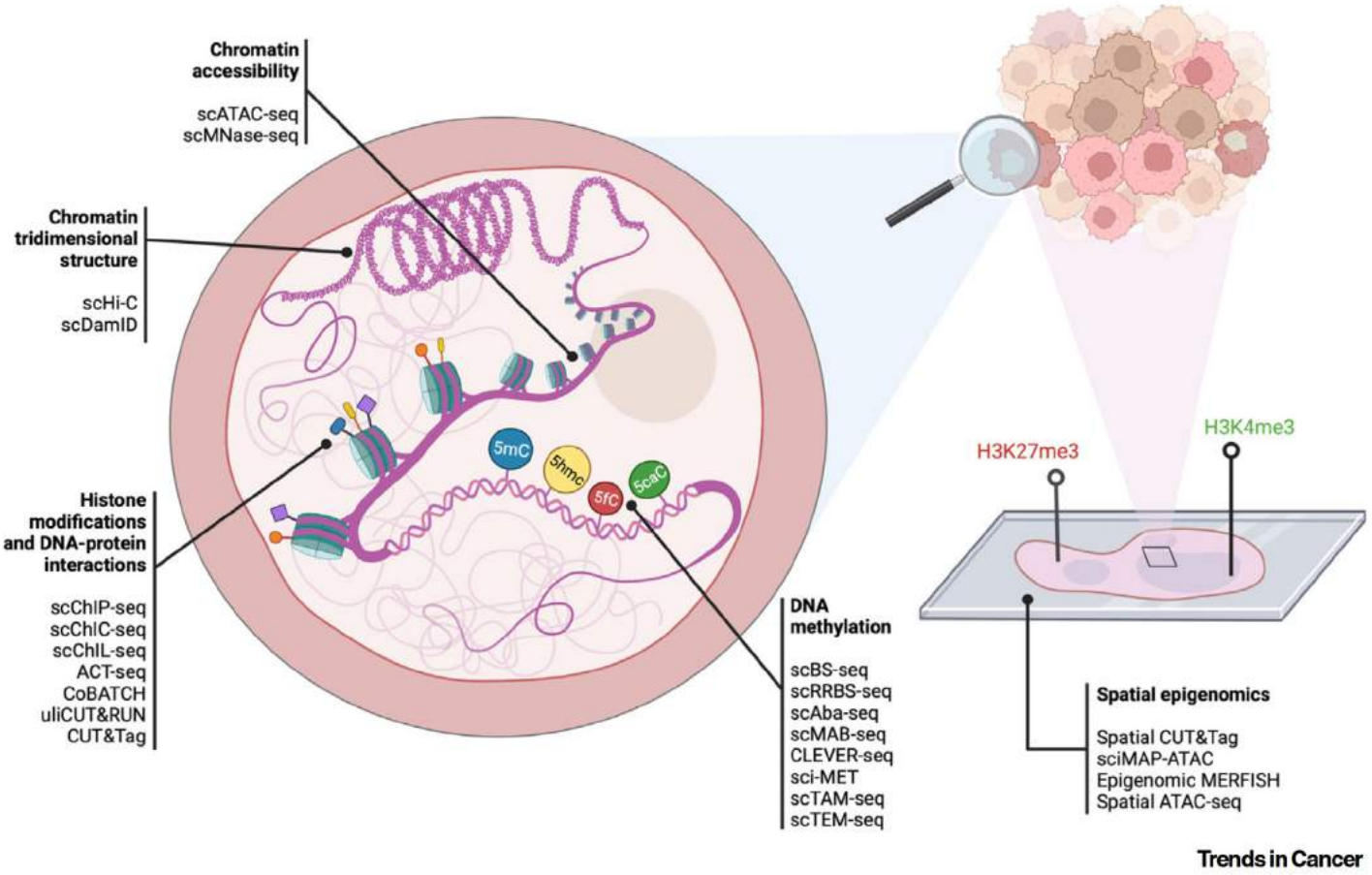
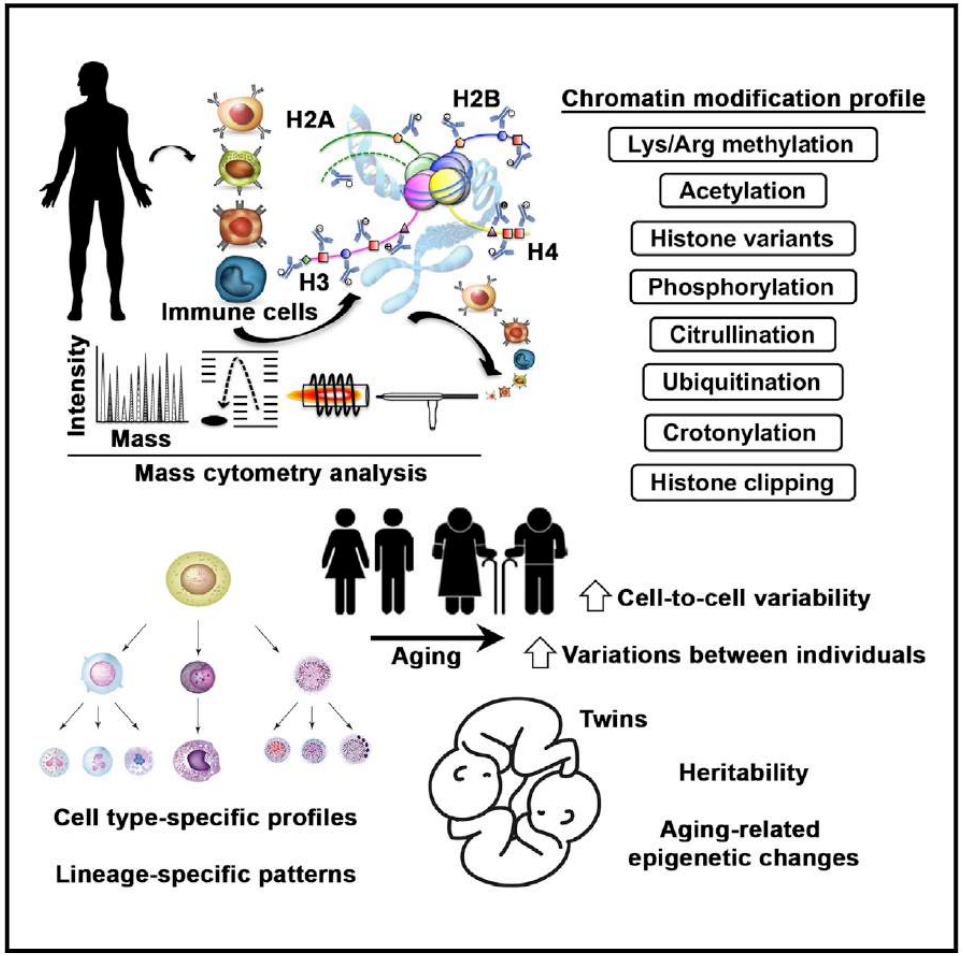
Deciphering Tumor Heterogeneity

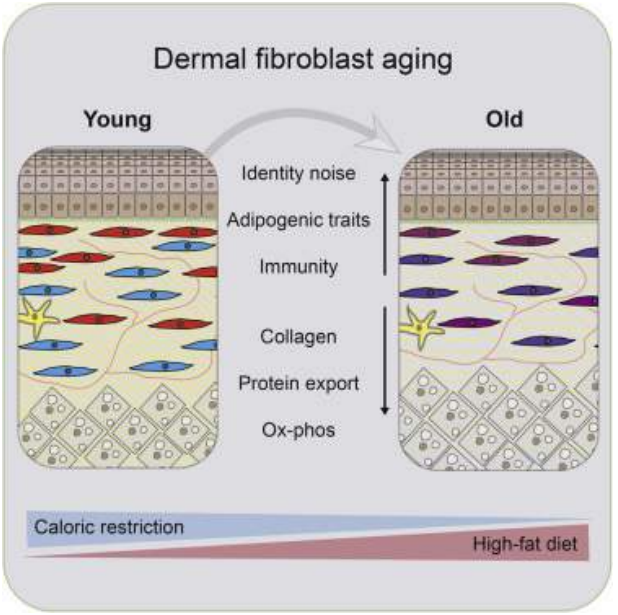


Casado-Pelaez et al, Trends in Cancer, 2022

Aging and Epigenetic changes: importance of the environment

Twin studies





- The identity of old dermal fibroblasts becomes undefined and noisy
- Loss of cell identity is a possible mechanism underlying aging

Salzer et al., 2018, Cell 175, 1575–1590

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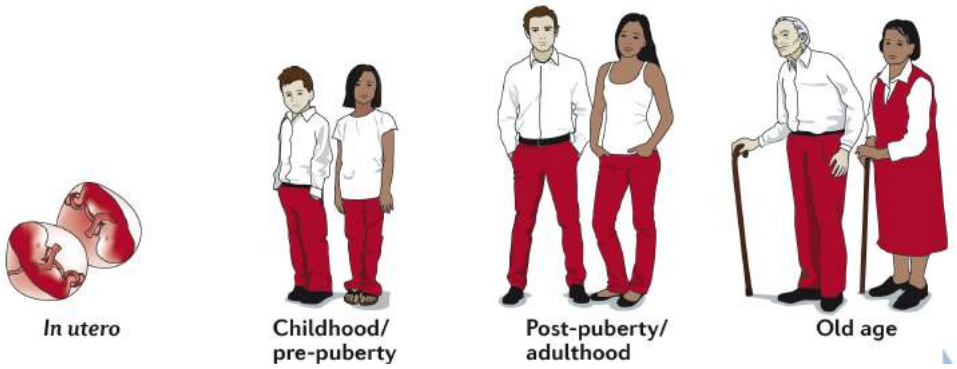
Conclusion

- Life does not happen in Isolation
- Organisms are heterogeneous, due to the environment or to intrinsic biological processes
- Deciphering phenotypic variation requires an understanding of the relationship between DNA sequence variation and epigenetic changes at the cellular and organismal levels over time
- We now have the tools!
- Although we do not yet know if there is a true epigenetic code, we are closer than ever to a molecular understanding of development, phenotypic plasticiy, ageing and pathology
- And to the hope of truly personalised medicine



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Merci!

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