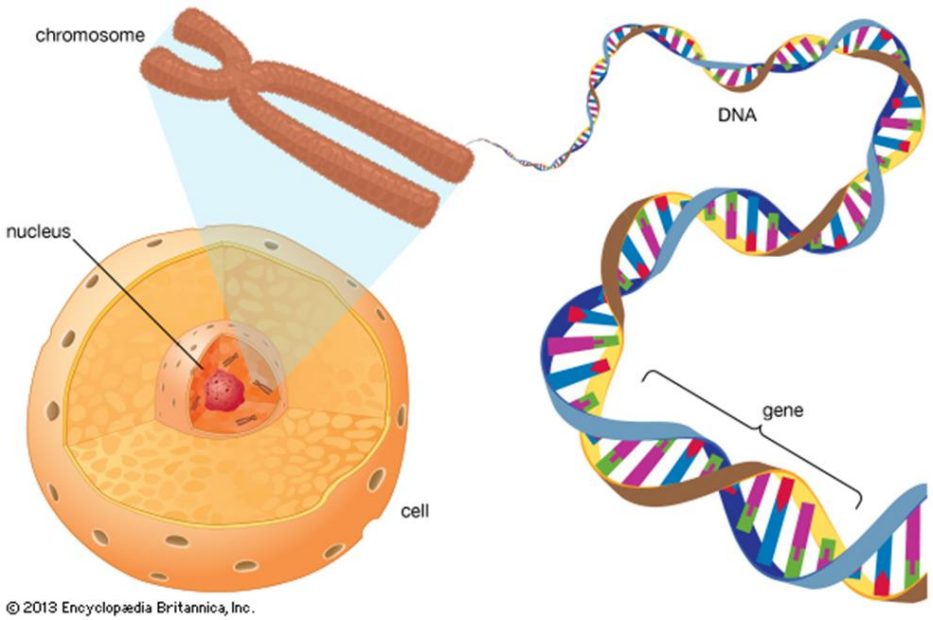
MUNI
MED

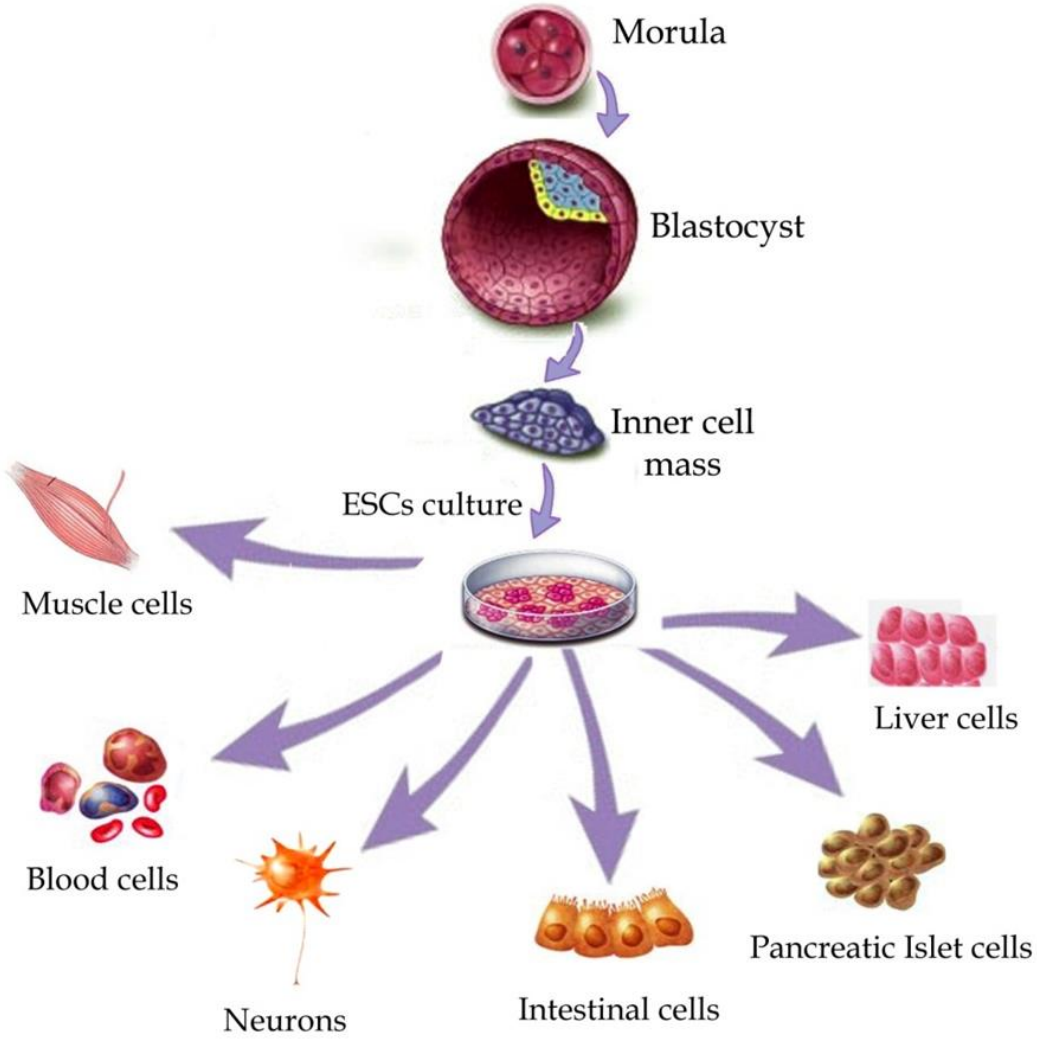
Epigenetics

Petr Müller



All cells of the body retain complete genetic information that remains unchanged throughout life.

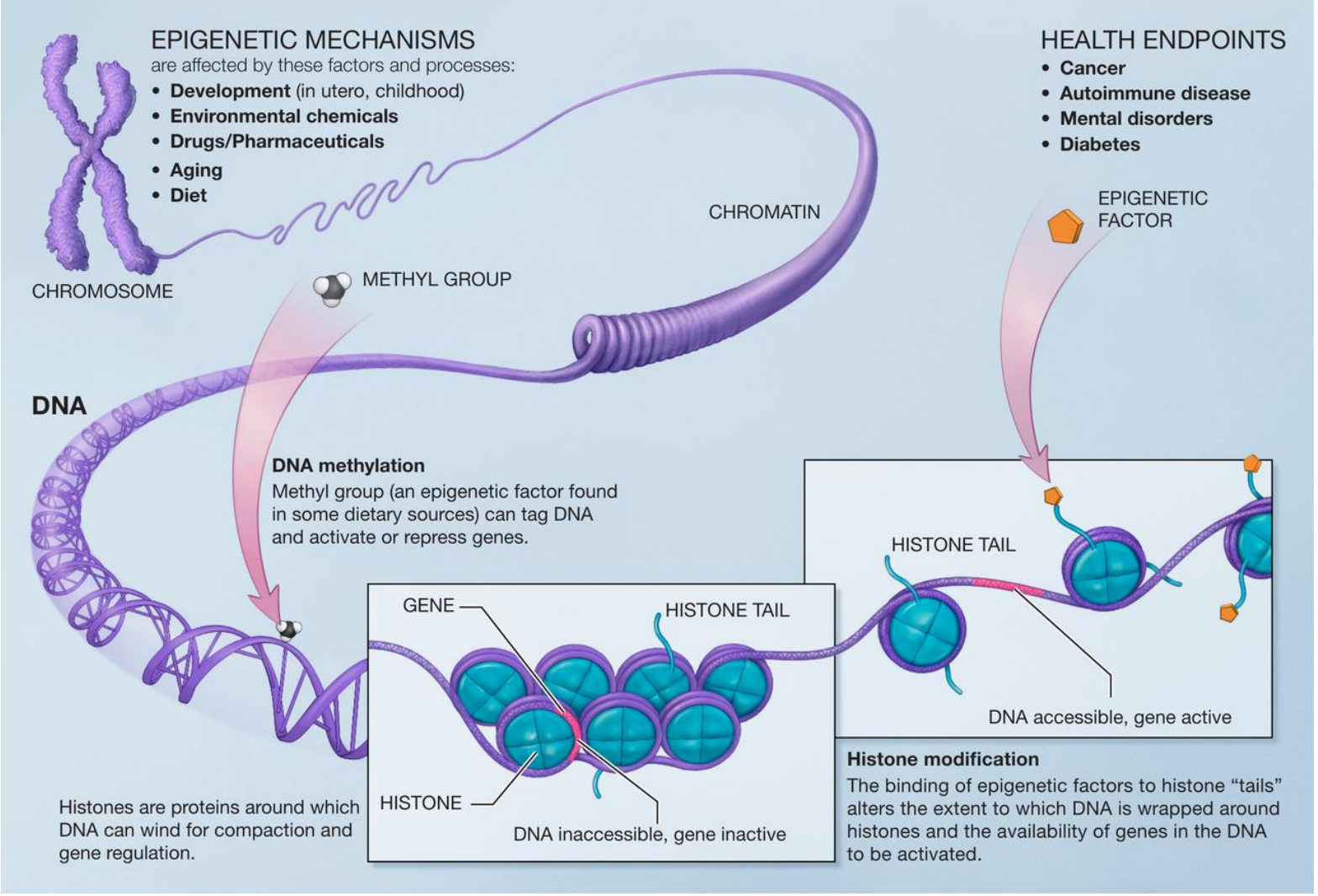
Human tissues are composed of differentiated cells
The daughter cells inherit the basic properties from parental cells



Epigenetics definitions and mechanisms

Epigenetics is the study of heritable phenotype changes that do not involve alterations in the DNA sequence.

Epigenetics most often involves changes that affect gene activity and expression, but the term can also be used to describe any heritable phenotypic change.

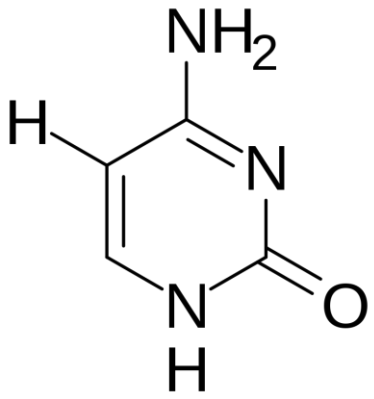


Mechanisms:

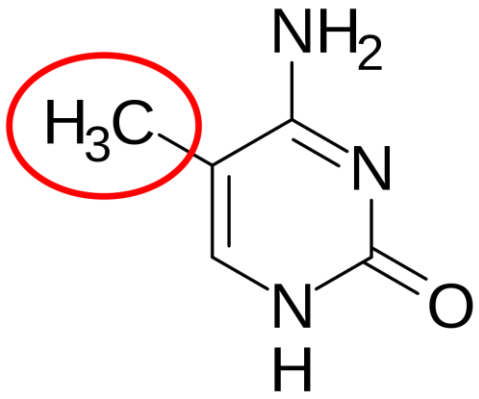
- Covalent modifications
- RNA transcripts
- MicroRNAs
- mRNA
- sRNAs
- Prions
- Structural inheritance
- Nucleosome positioning
- Histone variants
- Genomic architecture

DNA methylation

- process by which methyl groups are added to the DNA molecule.
- Methylation can change the activity of a DNA segment without changing the sequence

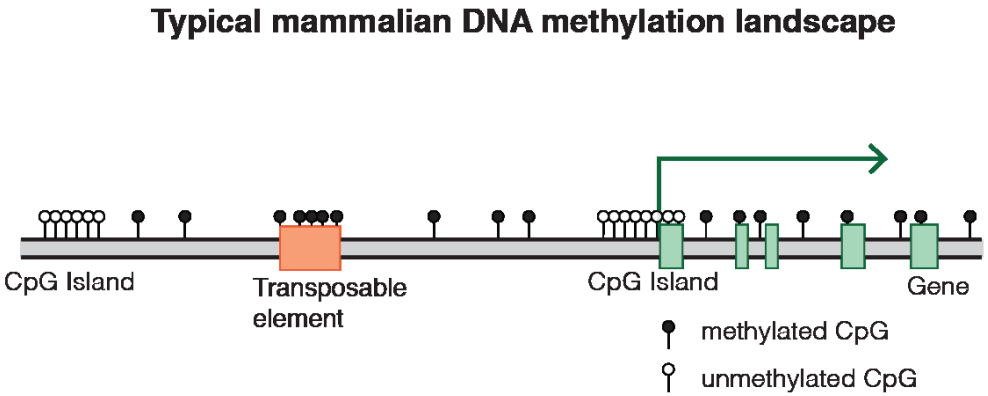


cytosine



methylated cytosine

In mammals however, DNA methylation is almost exclusively found in CpG dinucleotides, with the cytosines on both strands being usually methylated.



CpG islands are usually defined as regions with:

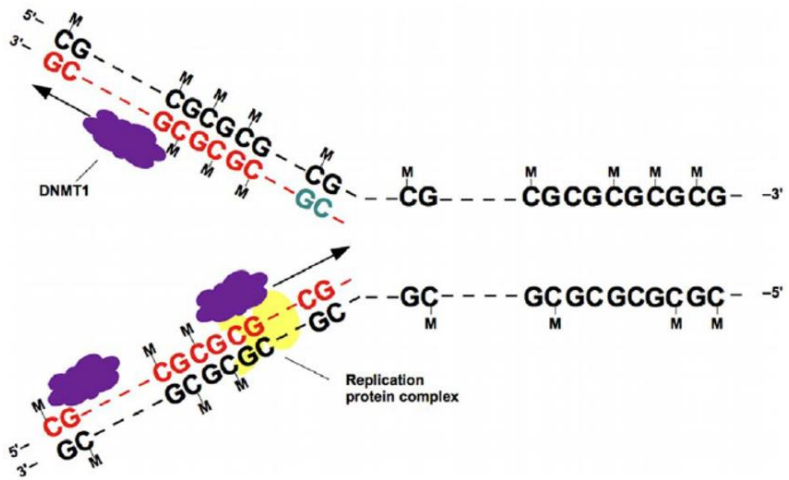
1) a length greater than 200bp,
2) a G+C content greater than 50%,
3) a ratio of observed to expected CpG greater than 0.6,

DNA methyltransferases (in mammals)

1. maintenance methylation (Maintenance methylation activity is necessary to preserve DNA methylation after every cellular DNA replication cycle).
2. *de novo* methylation

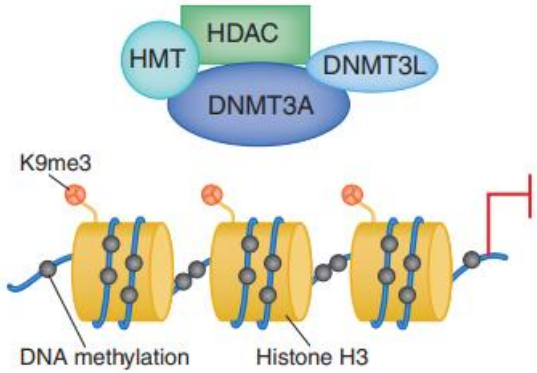
DNMT1

- maintanance



DNMT3a and DNMT3b

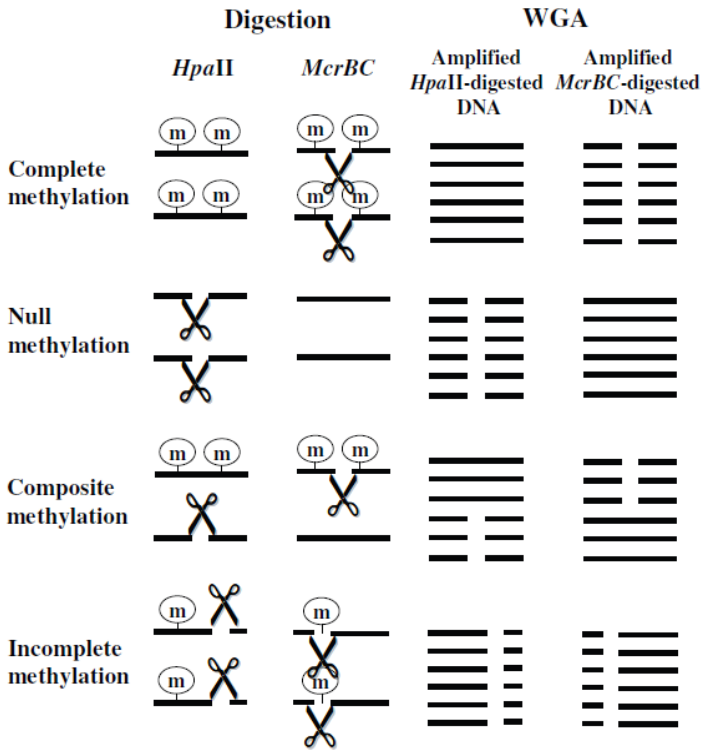
- the de novo methyltransferases that set up DNA methylation patterns



Model of DNMT3A activity. The DNMT3A protein complex is associated at promoters of silent genes in a complex with histone methyltransferase (HMT), histone deacetylase (HDAC) and DNA methyltransferase 3L (DNMT3L). These promoters are marked by DNA methylation, histone deacetylation and histone 3 lysine 9 methylation (K9me3).

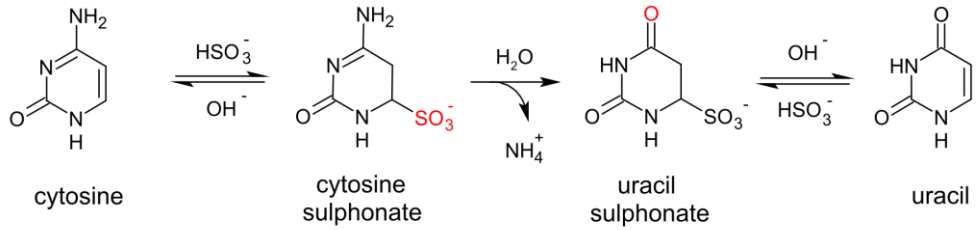
Detection of methylation

1) Using methylation sensitive restriction endonucleases

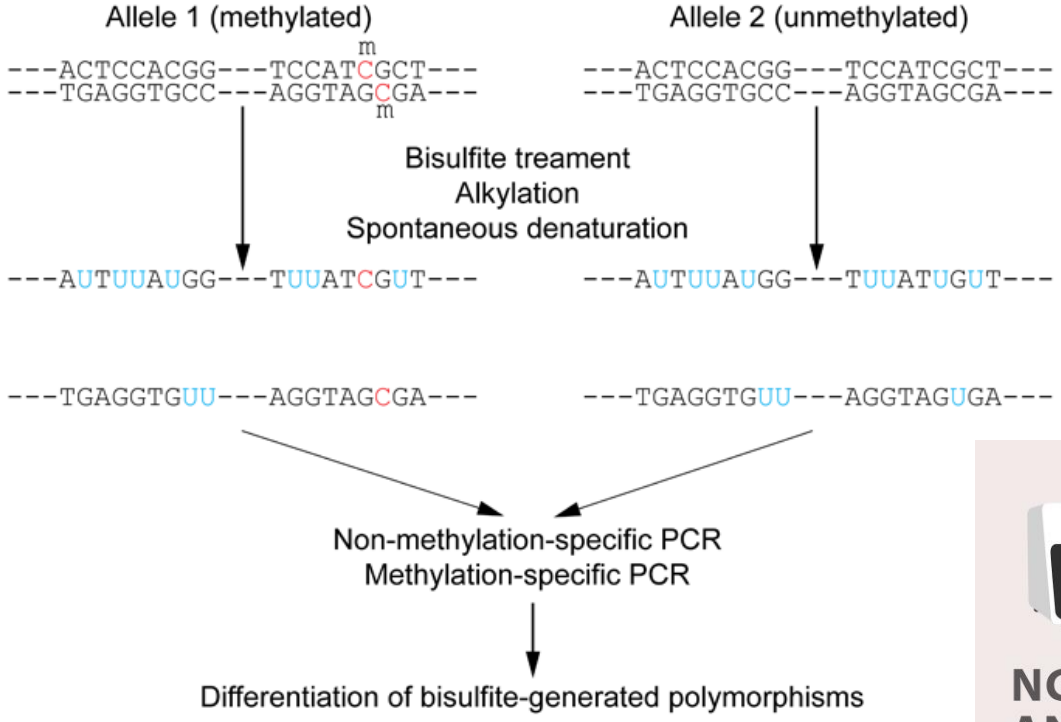


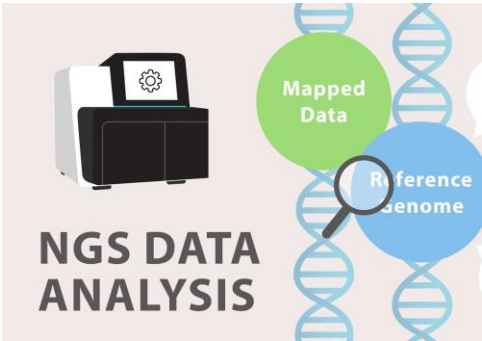
McrBC is an endonuclease which cleaves DNA containing methylcytosine* on one or both strands

2) Using bisulfite conversion



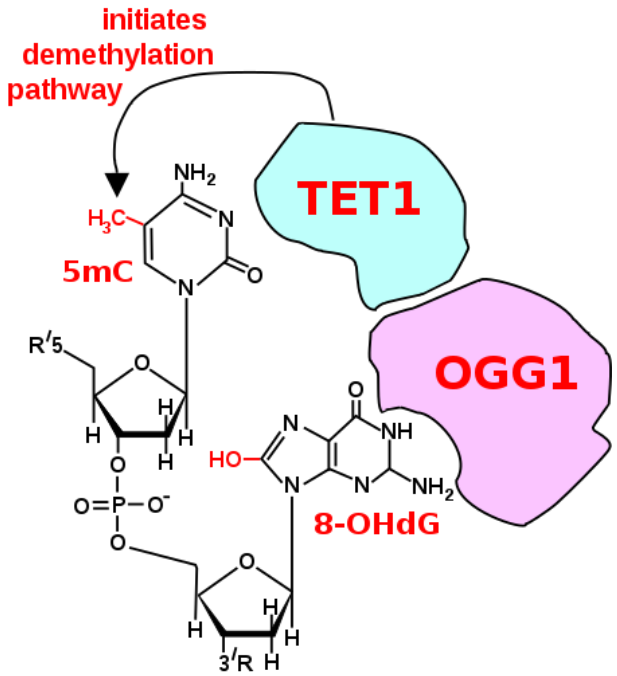
Outline of the chemical reaction that underlies the bisulfite-catalyzed conversion of cytosine to uracil.



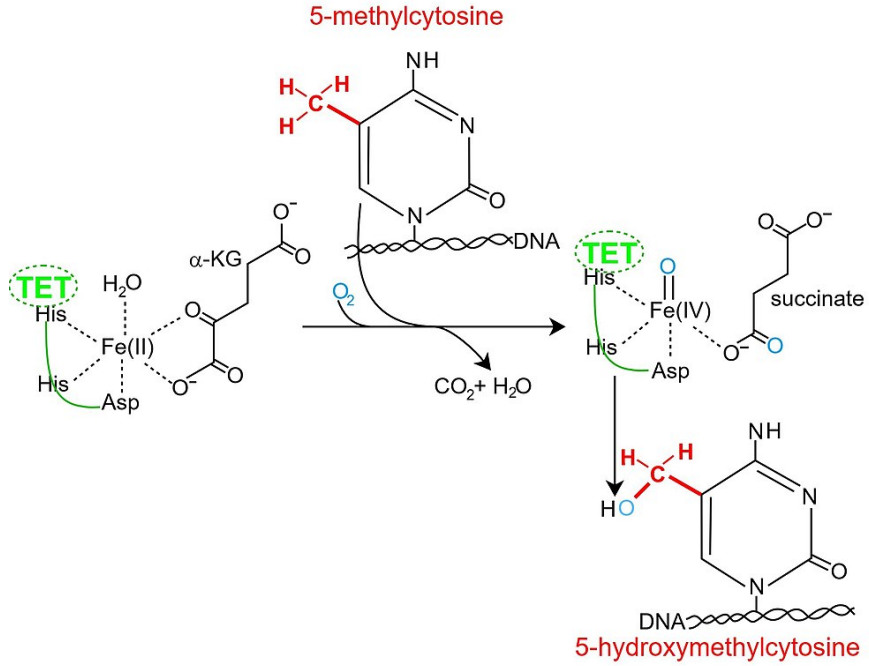


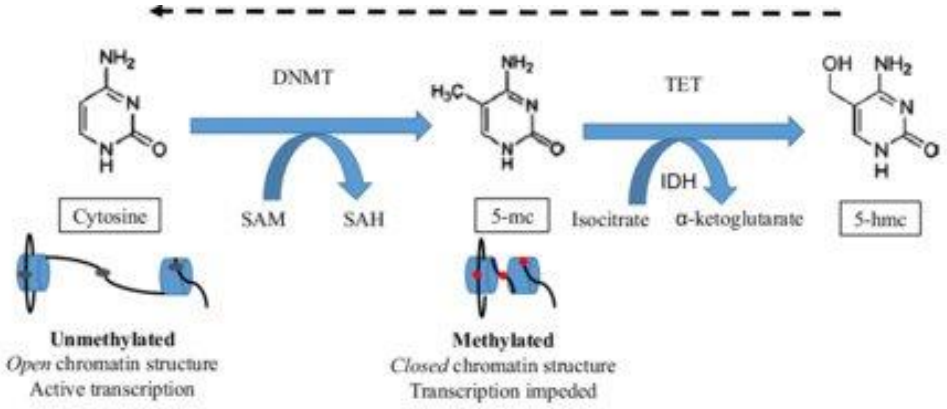
DNA demethylation

- TET enzymes are a family of ten-eleven translocation (TET) methylcytosine dioxygenases.
- They are instrumental in DNA demethylation.

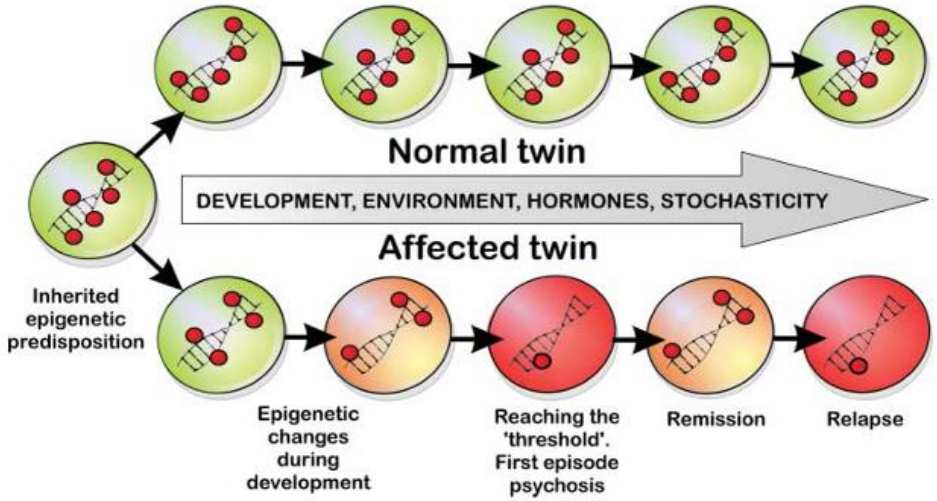


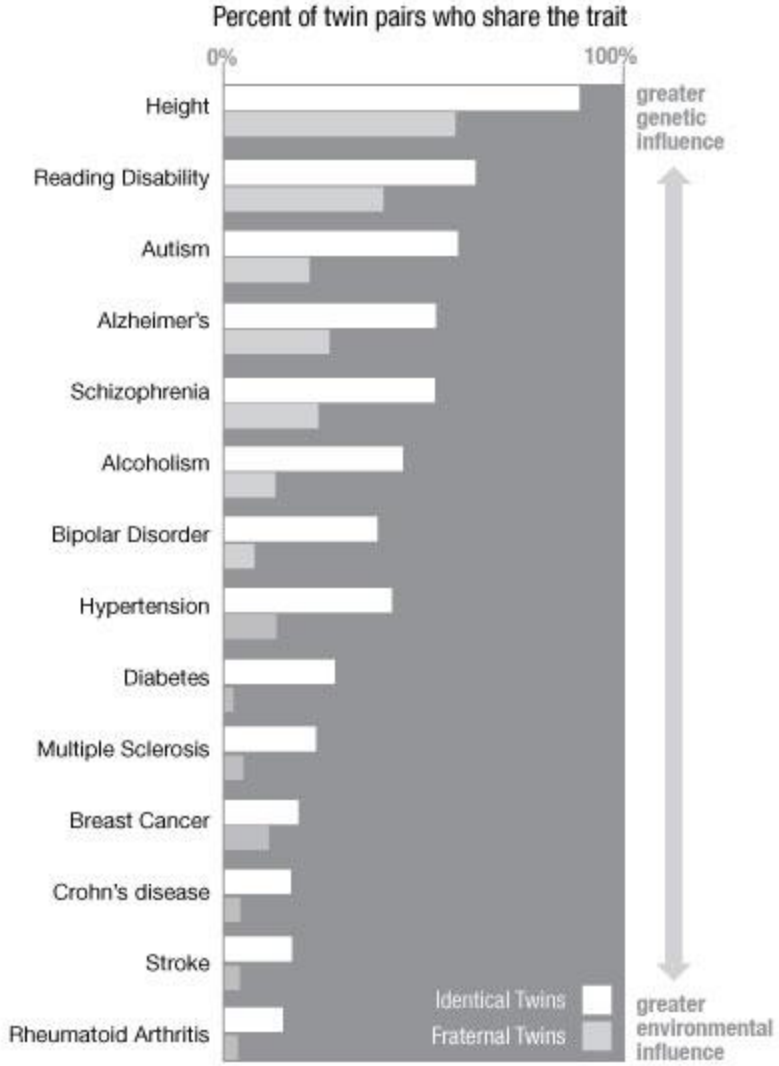
Oxoguanine glycosylase (OGG1) recruits TET enzyme





The effect of epigenetic regulation can be observed in identical twins.





PNAS

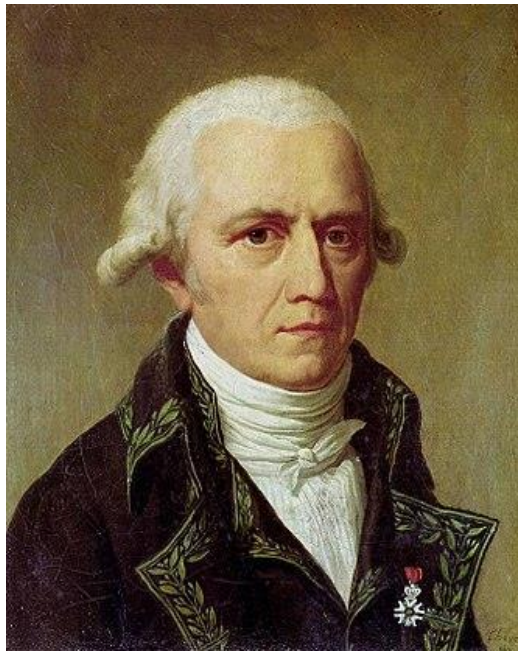
Epigenetic differences arise during the lifetime of monozygotic twins

Mario F. Fraga*, Esteban Ballestar*, Maria F. Paz*, Santiago Ropero*, Fernando Setien*, Maria L. Ballestar†, Damia Heine-Suñer‡, Juan C. Cigudosa§, Miguel Urioste¶, Javier Benitez¶, Manuel Boix-Chornet†, Abel Sanchez-Aguilera†, Charlotte Ling‖, Emma Carlsson‖, Pernille Poulsen**, Allan Vaag**, Zarko Stephan††, Tim D. Spector††, Yue-Zhong Wu‡‡, Christoph Plass‡‡, and Manel Esteller*§§

*Epigenetics, §Cytogenetics, and ¶Genetic Laboratories, Spanish National Cancer Centre (CNIO), Melchor Fernandez Almagro 3, 28029 Madrid, Spain; †Department of Behavioral Science, University of Valencia, 46010 Valencia, Spain; ‡Molecular Genetics Laboratory, Genetics Department, Son Dureta Hospital, 07014 Palma de Mallorca, Spain; ‖Department of Clinical Sciences, University Hospital Malmö, Lund University, S-205 02 Malmö, Sweden; **Steno

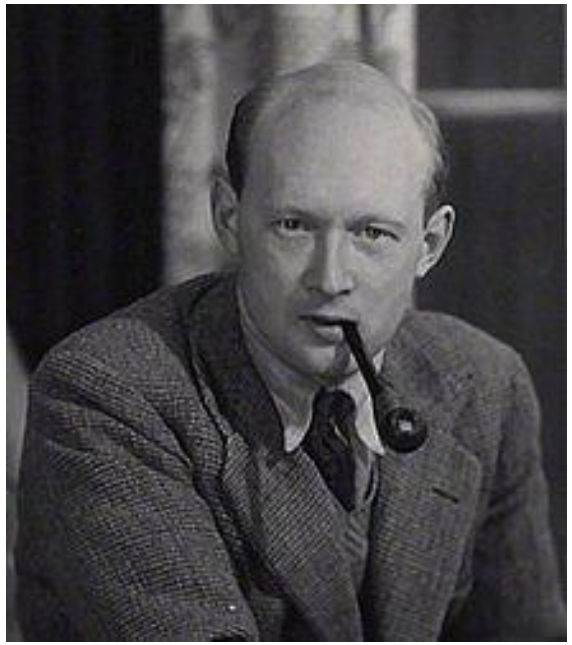
Epigenetics and inheritance

Jean-Baptiste Lamarck



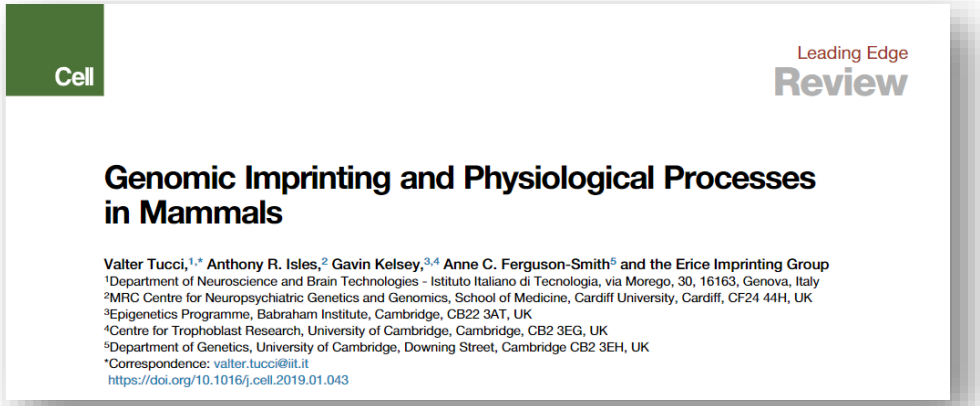
- 1744 – 1829
- French biologist, first author of evolutionary theory
- Theory of inheritance of acquired characteristics, called Lamarckism

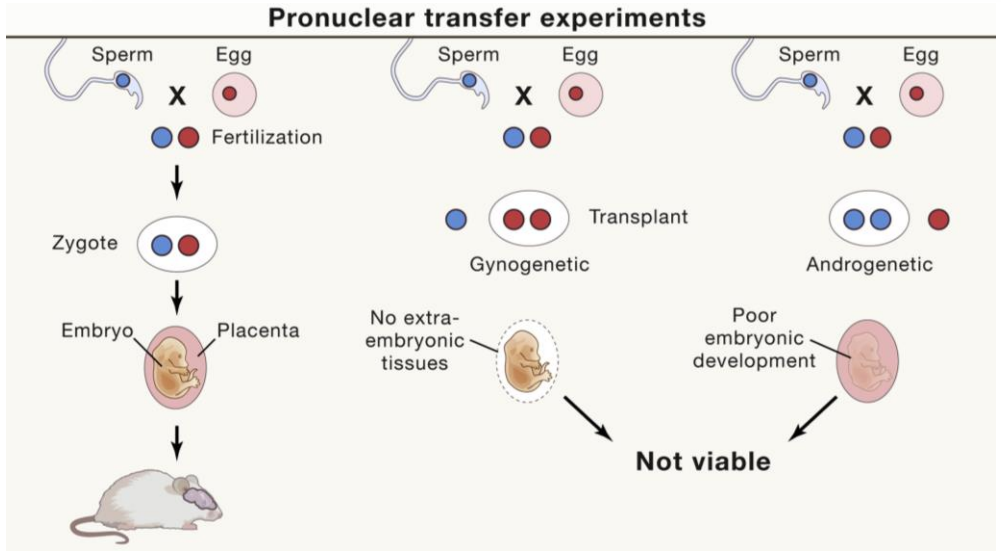
C. H. Waddington

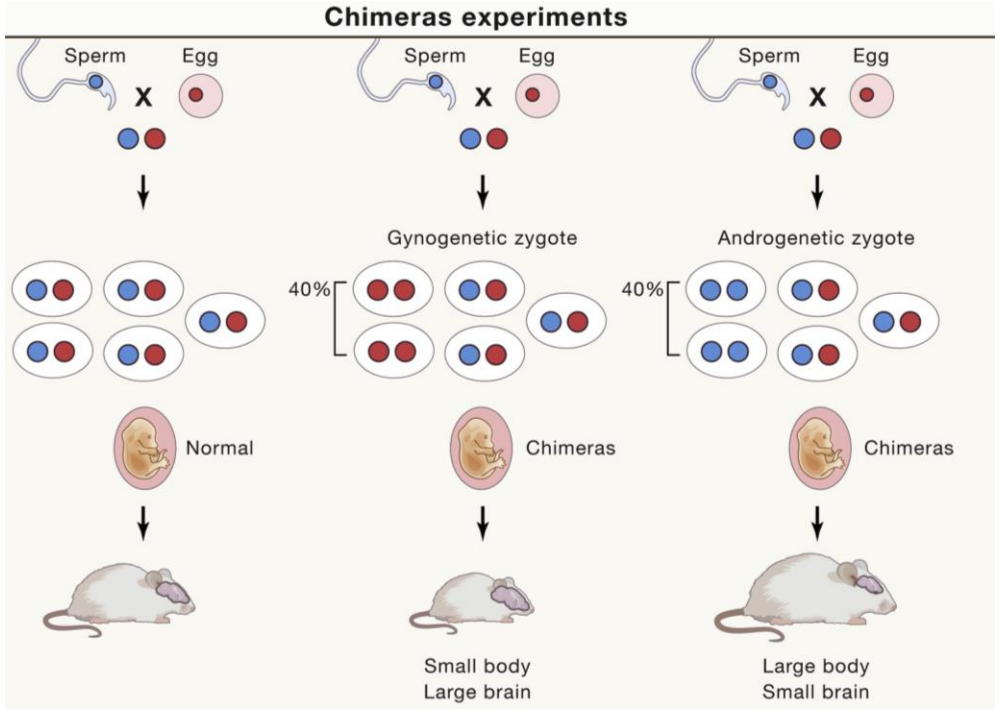


- 1905 – 1975
- British developmental biologist
- proposed an evolutionary process, "genetic assimilation", as a Darwinian mechanism that allows certain acquired characteristic to become heritable
- Proposed imprinting and epigenetic landscape

Can epigenetic information be passed on to the next generation ?

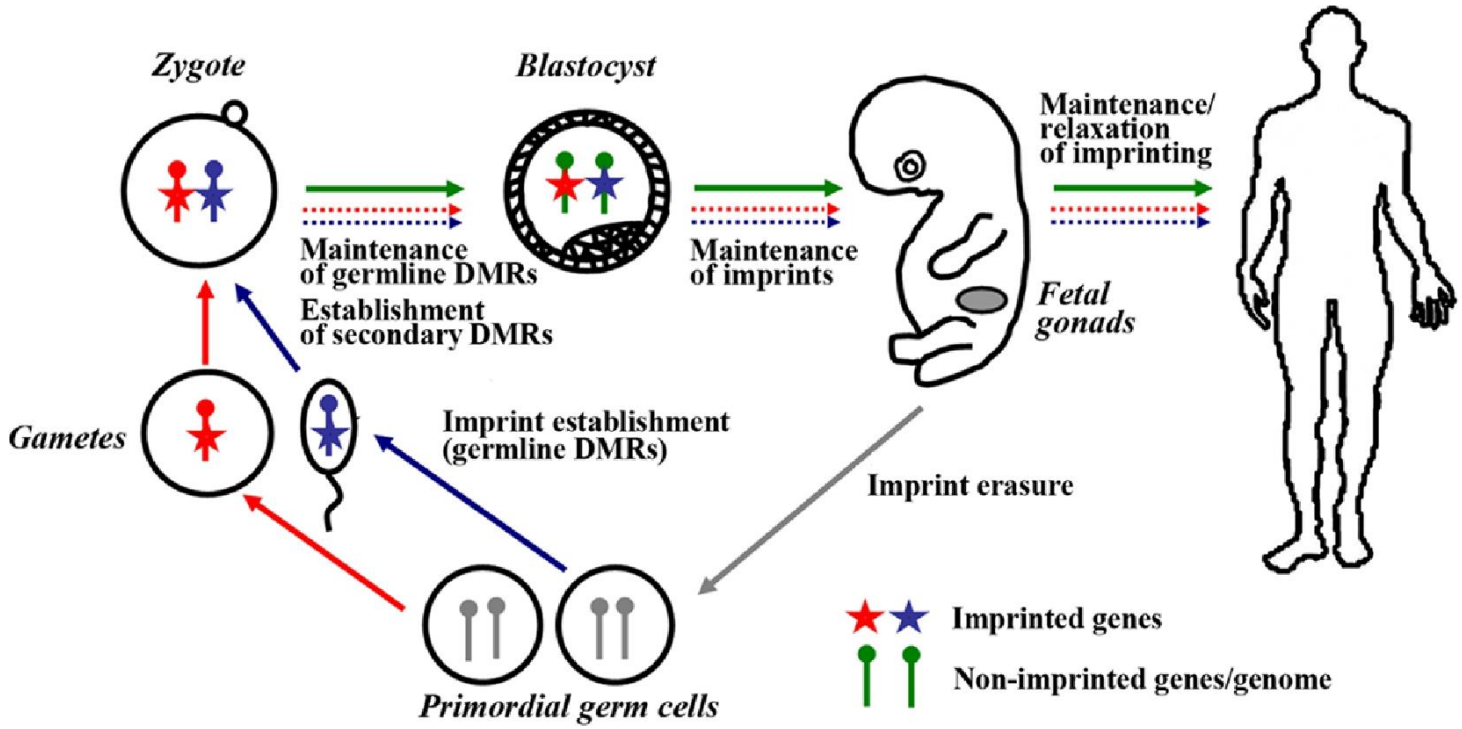






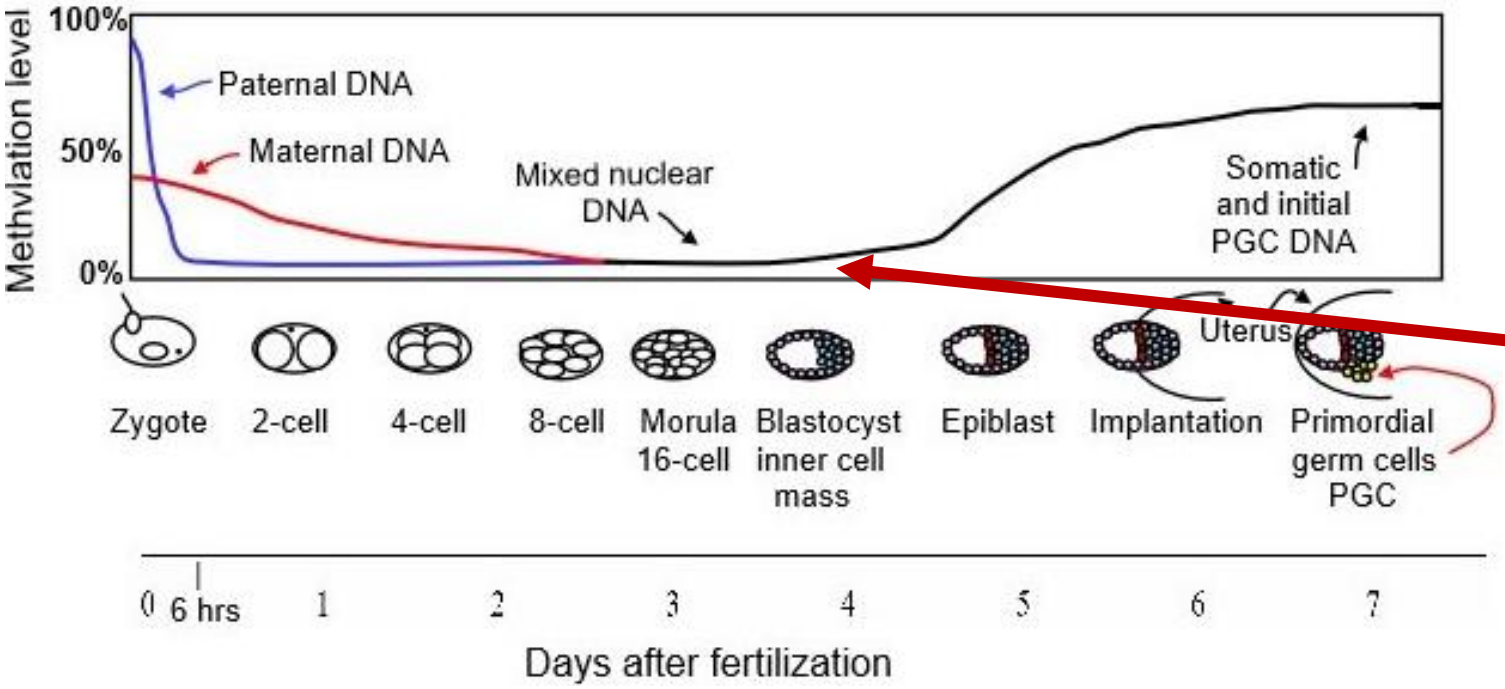
The term ‘‘imprinting’’ was first used by the cytogeneticist HelenCrouse in the 1960s to describe the elimination of paternally derived X chromosomes in flies

Genomic imprinting in mammals: its life cycle, molecular mechanisms and reprogramming



The epigenetic imprints regarding the parental origin are established during male and female gametogenesis, passed to the zygote through fertilization, maintained throughout development and adult life, and erased in primordial germ cells before the new imprints are set.

Can we detect imprinting by detection of methylation in sperm or oocyte?

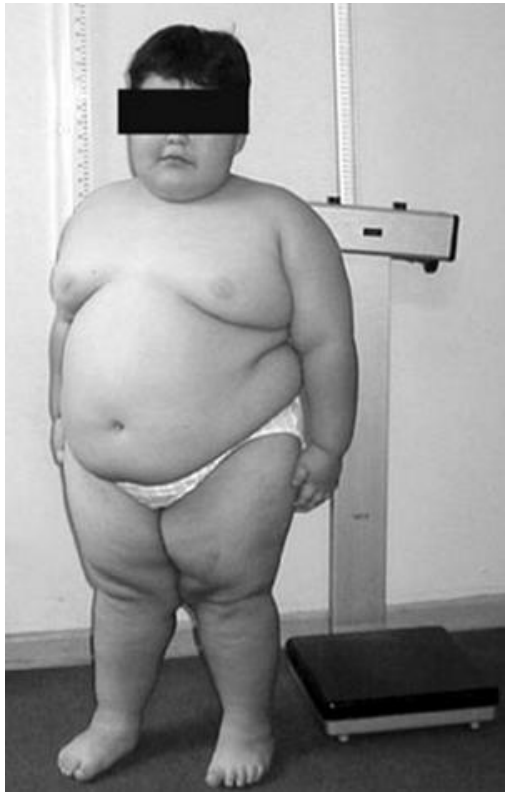


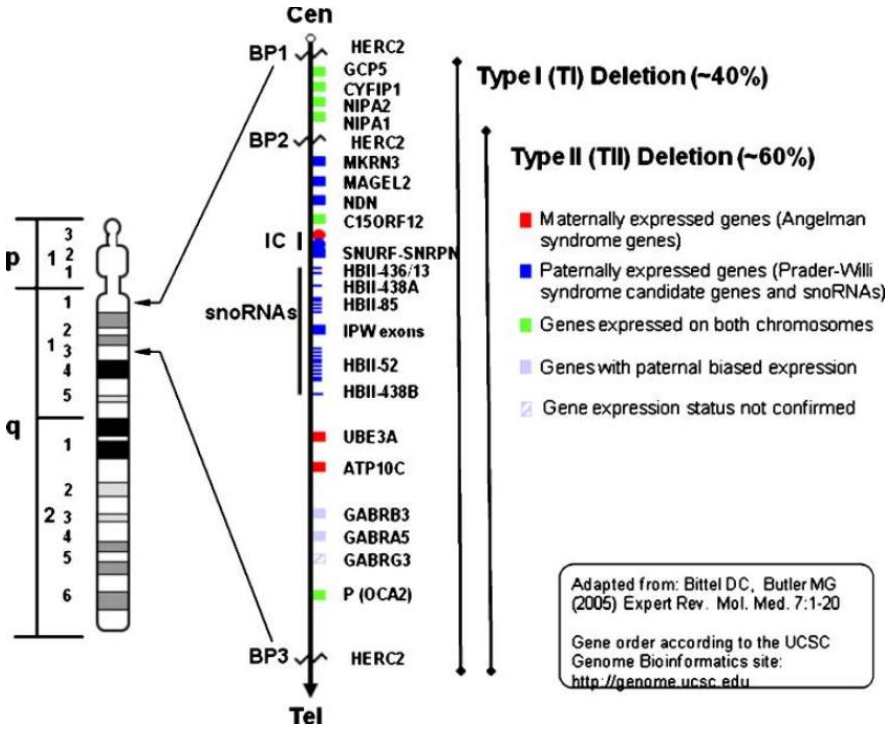
In 2019, 260 imprinted genes have been reported in mice and 228 in humans.

- post-fertilization epigenetic reprogramming
- DNA methylation is reset before implantation exept imprinted genes
- DNA methylation marks at the DMRs of imprinted genes are stable through embryogenesis and early development, until they are reprogrammed in primordial germ cells.

Diseases associated with impaired genomic imprinting in human

- **Prader-Willi syndrome** (PWS) is a complex neurodevelopmental genetic condition due to **paternal loss of imprinted genes on chromosome 15**
- characterized by a range of mental and physical
- 350,000–400,000 people worldwide.

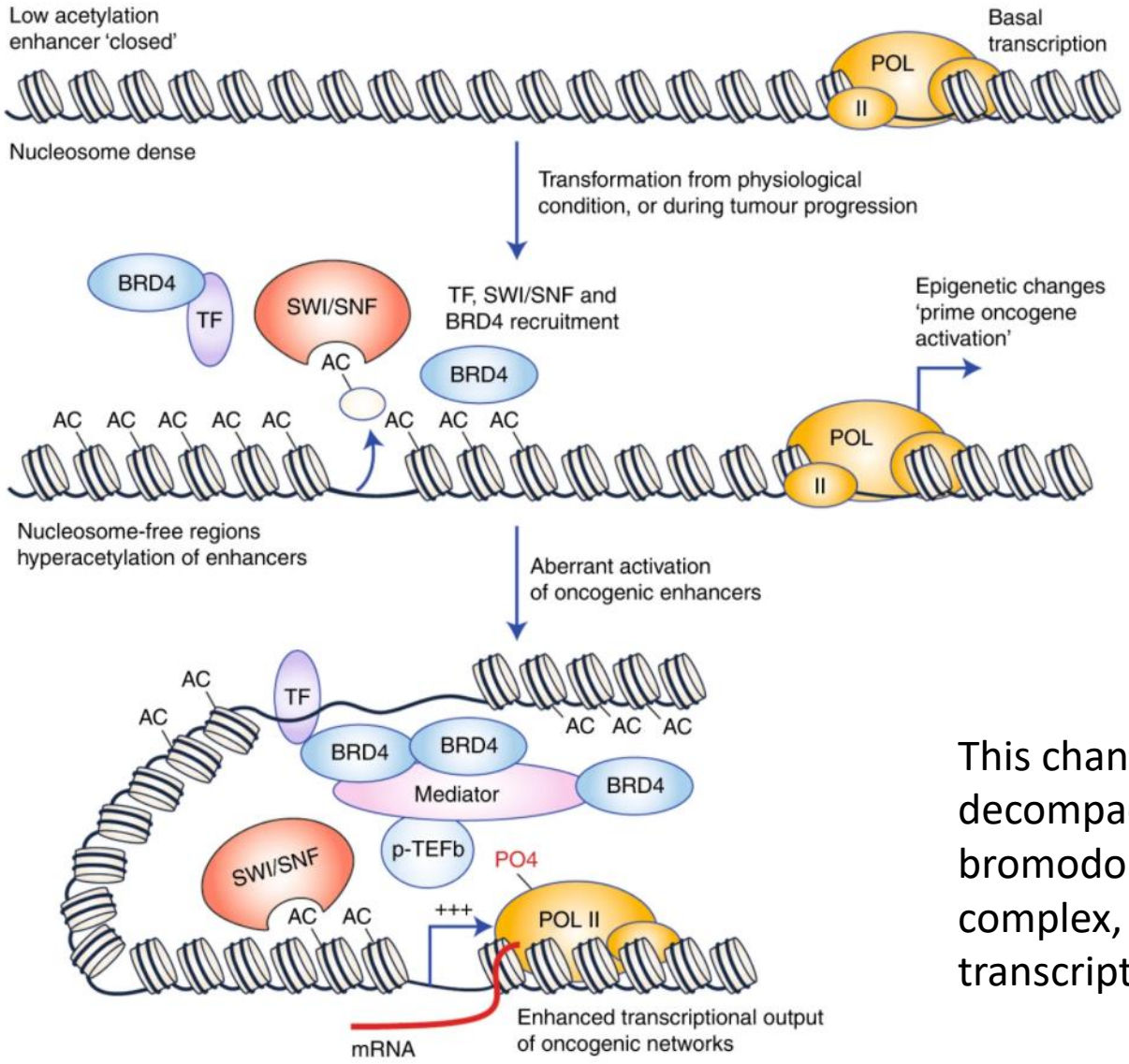




- **Angelman syndromes**
- **Silver-Russell syndrome**
- **Beckwith-Weidemann syndrome**
- **Albright hereditary osteodystrophy**
- **uniparental disomy 14**

Assisted Reproductive Technology (ART) related genomic imprinting

Epigenetics and cancer

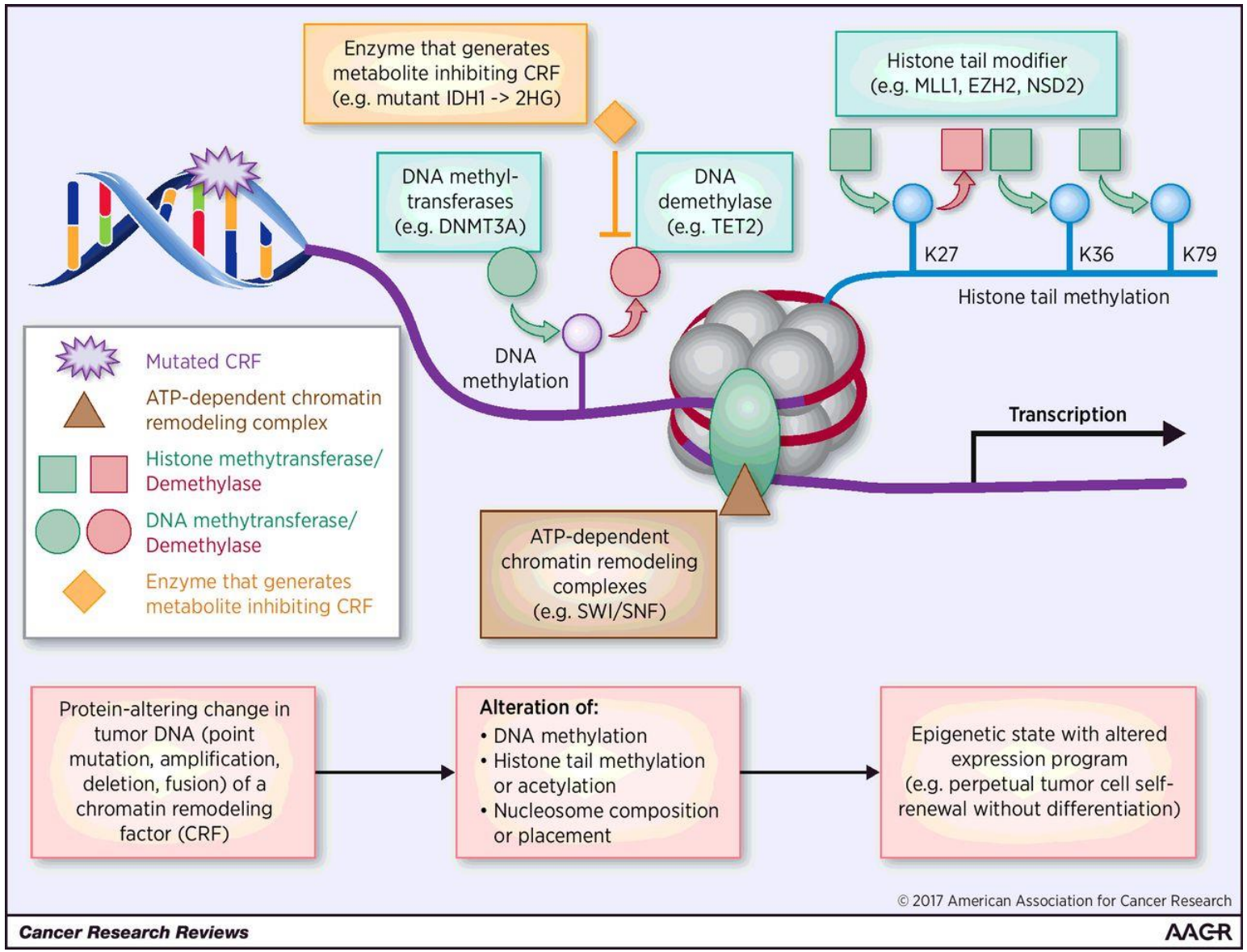


Under physiological conditions, proliferation and survival genes are transcribed at a basal rate to maintain homoeostasis.

During the transformation chromatin surrounding proto-oncogenes becomes enriched for histone acetylation, especially at enhancer regions.

This change in chromatin programming allows nucleosome decompaction, which facilitates the recruitment of bromodomain chromatin remodellers, such as the SWI/SNF complex, that further open chromatin to allow the binding of transcription factors (TF).

Mutated Chromatin Regulatory Factors as Tumor Drivers in Cancer



IDH1, IDH2

- Isocitrate dehydrogenase
- Production of 2-HG -> DNA hypermethylation
- Mutated in glioblastoma and hematologic malignancies

SWI/SNF complexes

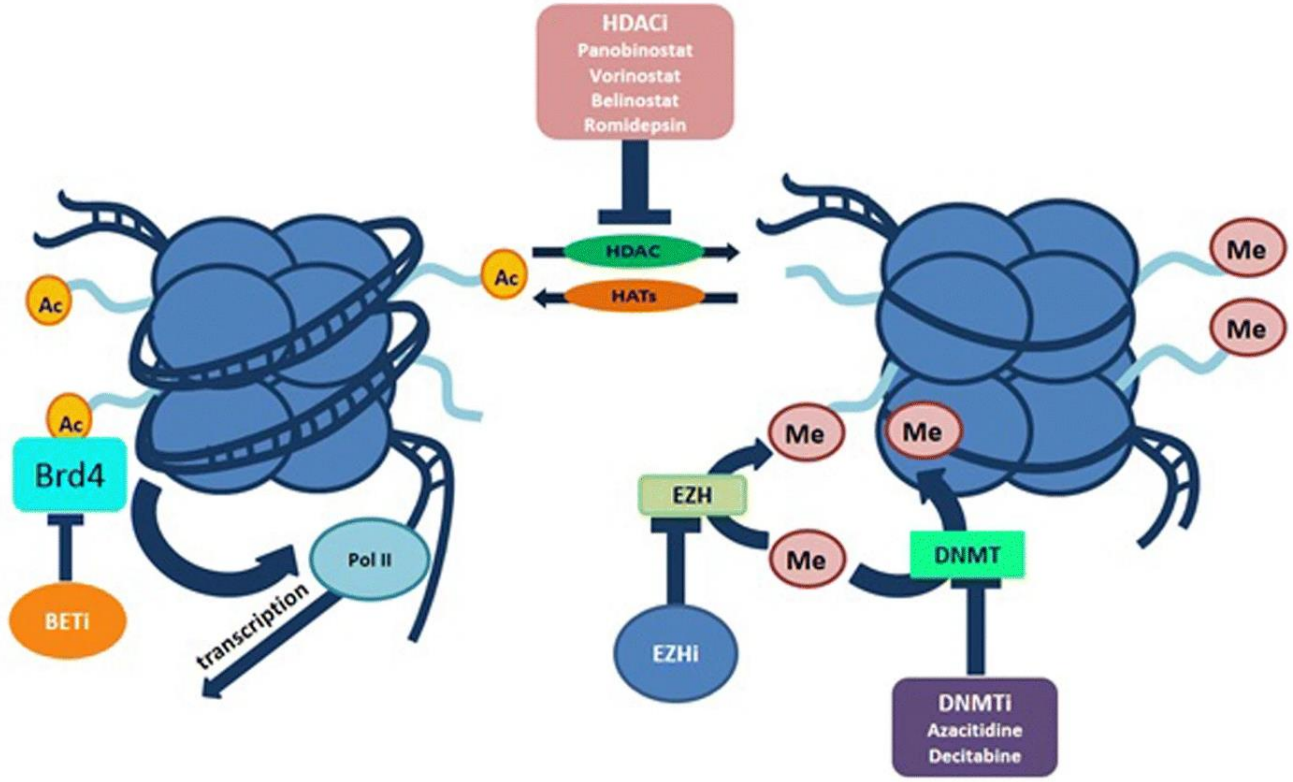
- ATP dependent chromatin remodeling
- SMARCA4- the most frequently mutated chromatin remodeling ATPase in cancer
- SMARCB1
- ARID1A

DNA and histone methyl transferase/demethylase

Pharmacological intervention of chromatine remodeling (in oncology)

Histon deacetylase inhibitors

IDH inhibitors

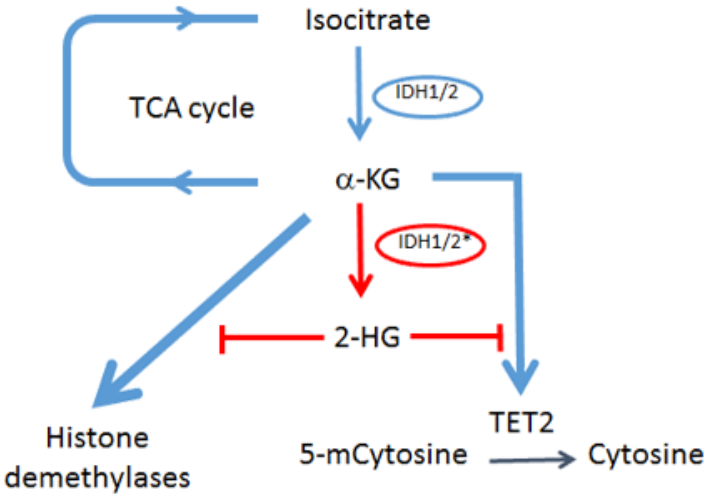


BET inhibitors

Histon methyl-transferace inhibitors inhibitors

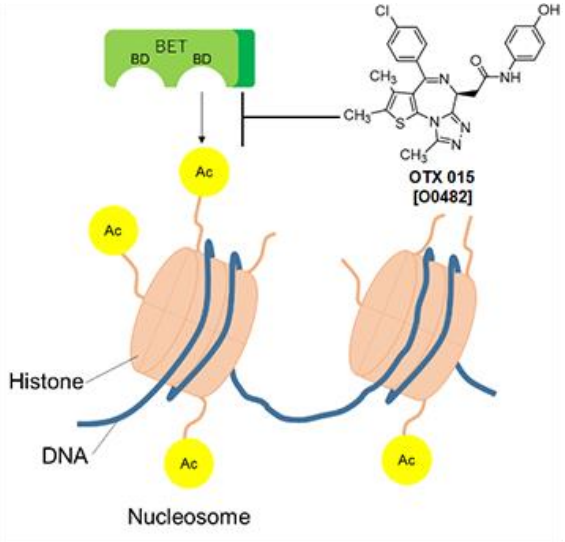
Pharmacological intervention of chromatine remodeling (in oncology)

IDH inhibitors



IDH1 and IDH2 are both nicotinamide adenine dinucleotide phosphate (NADP)-dependent enzymes that catalyze the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (α-KG), while producing NADPH.

BET inhibitors



bromodomain is an approximately 110 amino acid protein domain that recognizes acetylated lysine residues

Histon deacetylase inhibitors

Histon methyl-transferace inhibitors inhibitors

FDA has so far approved four HDAC inhibitors for the treatment of cancer (romidepsin, belinostat panobinostat, vorinostat)

Methylation and aging

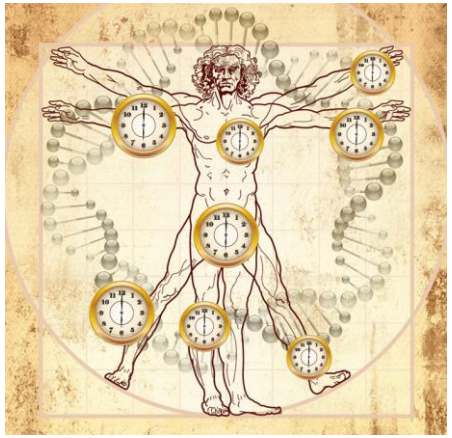
Horvath *Genome Biology* , **14**:R115
http://genomebiology.com//14/10/R115

Genome Biology

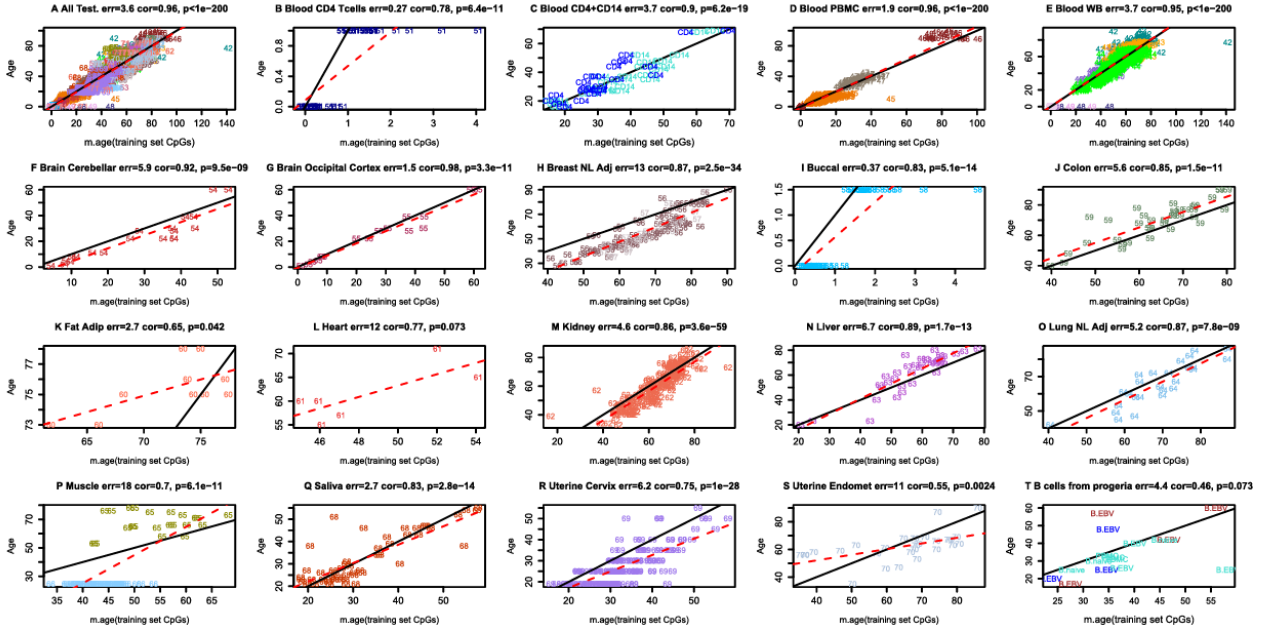
RESEARCH Open Access

DNA methylation age of human tissues and cell types

Steve Horvath[1,2,3]



In humans and other mammals, DNA methylation levels can be used to accurately estimate the age of tissues and cell types, forming an accurate epigenetic clock



Chronological age (y-axis) versus DNAm age (x-axis) across different cells and tissues

Article

Nature | Vol 588 | 3 December 2020

Reprogramming to recover youthful epigenetic information and restore vision

https://doi.org/10.1038/s41586-020-2975-4

Received: 31 July 2019

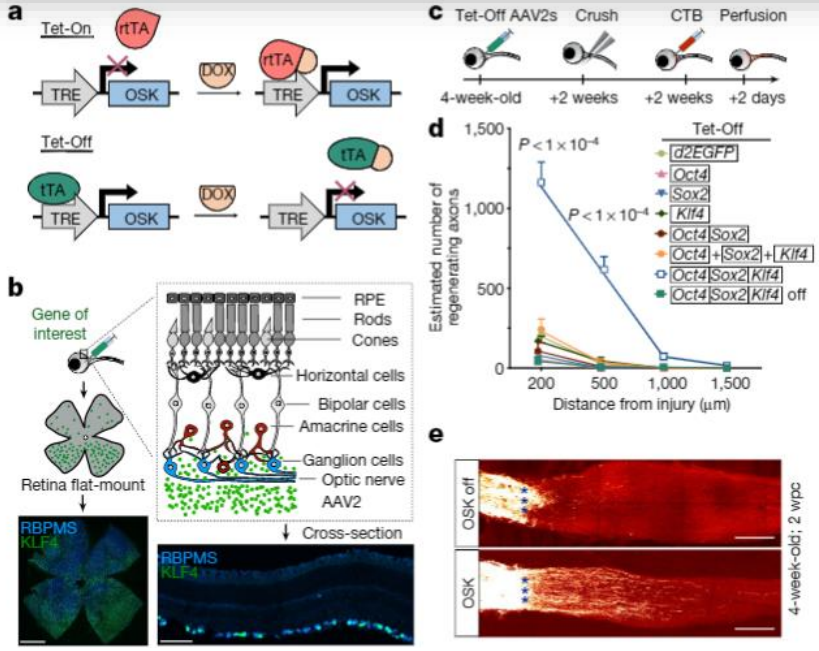
Accepted: 22 October 2020

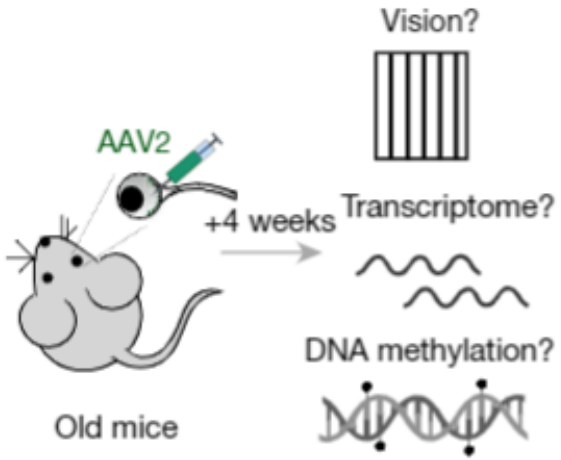
Published online: 2 December 2020

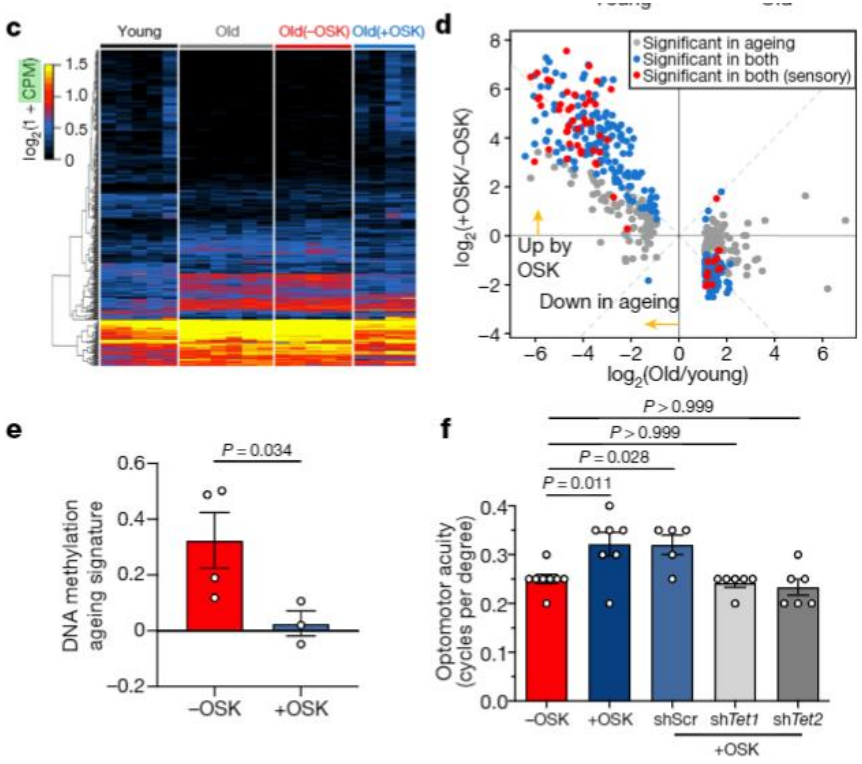
Check for updates

Yuancheng Lu[1], Benedikt Brommer[2,3,11], Xiao Tian[1,11], Anitha Krishnan[3,4,11], Margarita Meer[5,6,11], Chen Wang[2,3], Daniel L. Vera[1], Qiurui Zeng[1], Doudou Yu[1], Michael S. Bonkowski[1], Jae-Hyun Yang[1], Songlin Zhou[2,3], Emma M. Hoffmann[3,4], Margarete M. Karg[3,4], Michael B. Schultz[1], Alice E. Kane[1], Noah Davidsohn[7], Ekaterina Korobkina[3,4], Karolina Chwalek[1], Luis A. Rajman[1], George M. Church[7], Konrad Hochedlinger[8], Vadim N. Gladyshev[5], Steve Horvath[9], Morgan E. Levine[6], Meredith S. Gregory-Ksander[3,4,12], Bruce R. Ksander[3,4,12], Zhigang He[2,3,12] & David A. Sinclair[1,10,12]

Changes to DNA methylation patterns over time form the basis of ageing clocks, but whether older individuals retain the information needed to restore these patterns—and, if so, whether this could improve tissue function—is not known.







- Ectopic expression of Oct4 (also known as Pou5f1), Sox2 and Klf4 genes (OSK) in mouse retinal ganglion cells restores youthful DNA methylation patterns and transcriptomes, promotes axon regeneration after injury, and reverses vision loss in a mouse model of glaucoma and in aged mice.
- The beneficial effects of OSK-induced reprogramming in axon regeneration and vision require the DNA demethylases TET1 and TET2.

Chromatine remodelation to DNA methylation

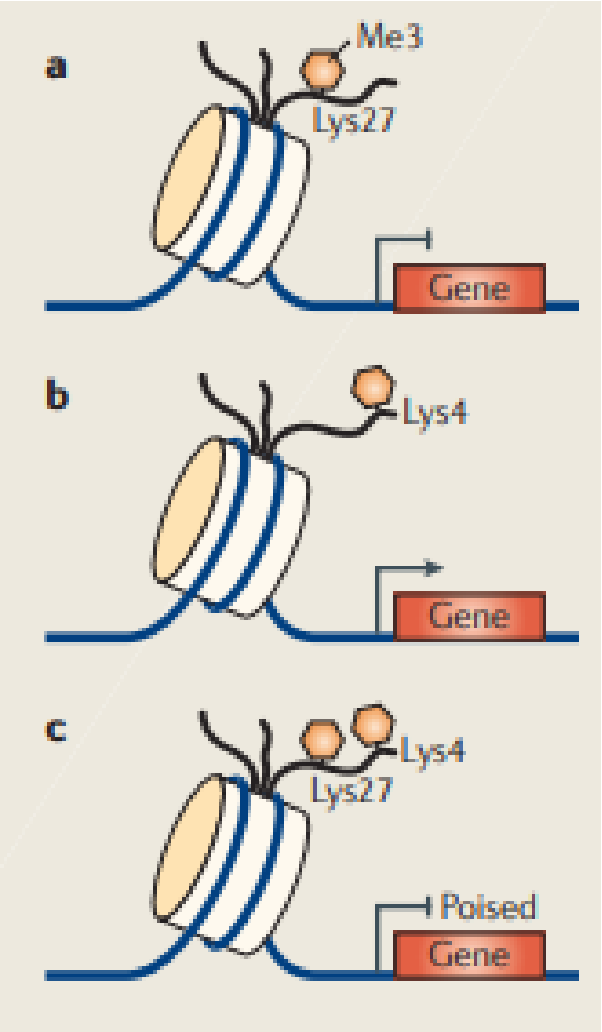


Table 1 | **Genes used to induce dedifferentiation, transdifferentiation or reprogramming**

Gene symbol*	Class	Role *in vivo*	Mouse knockout phenotype
Arf (*Cdkn2a*)	Protein kinase inhibitor	Negative regulator of proliferation	Increased tumorigenesis
Ascl1	Transcription factor	Neural lineage specification	Impaired development of various brain centres; neonatal lethality
Baf60c (*Smarcd3*)	Chromatin modulator	Neuron differentiation	Defective cardiogenesis and somitogenesis
Bcl11b	Transcription factor	Fetal thymocyte development and survival	Prenatal and perinatal lethality; haematopoietic defects
Brn2 (*Pou3f2*)	Transcription factor	Neuroectoderm specification	Perinatal lethality
Cebpa	Transcription factor	Broad target range	Neonatal lethality; multi-organ defects
Cebpb	Transcription factor	Immune and inflammatory response; brown fat specification	High neonatal hypoglycaemia and mortality
Fgf1	Growth factor	Angiogenic	Normal
Gata4	Transcription factor	Heart tube and foregut formation	Lethal; ventral defects
Klf4	Transcription factor	Differentiation of epithalial cells	Perinatal death owing to skin defects
Lin28	Transcription factor	Suppressor of microRNA biogenesis	Unknown
Mafa	Transcription factor	Activates insulin gene expression	Diabetes and pancreatic islet abnormalities
Mef2c	Transcription factor	Controls cardiac morphogenesis and myogenesis	Prenatal death and cardiovascular abnormalities
Myc	Transcription factor	Broad action on cell cycle and growth	Prenatal lethality and growth defects
Myt1l	Transcription factor	Pan-neural transcription factor with roles in neuronal differentiation	Unknown
Nanog	Transcription factor	Imposes pluripotency on embryonic stem cells and prevents their differentiation	Early embryonic death
Ngn3	Transcription factor	Neurogenesis and pancreatic endocrine cells specification	Deficiency of endocrine cells and insulin-producing cells; postnatal diabetes
p38 mapk (*Mapk14*)	Protein kinase	Inflammation and response to stress	Embryonic to perinatal lethal with multi-system defects
Pdx1	Transcription factor	Specifies early pancreatic epithelium	Postnatal lethality and abnormal pancreatic and liver development
Oct4	Transcription factor	Crucial for early embryogenesis and for embryonic stem cell pluripotency	Peri-implantation lethality; failure to develop the inner cell mass
Pu.1 (*Spi1*)	Transcription factor	Lymphoid-specific enhancer	Postnatal lethality and haematopoietic defects
Rb1	Transcription factor and chromatin modulator	Key regulator of entry into cell division	Prenatal lethality and neuronal and haematopoietic defects
Tbx5	Transcription factor	Mesoderm differentiation	Prenatal lethality and cardiovascular defects