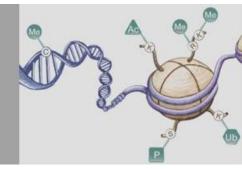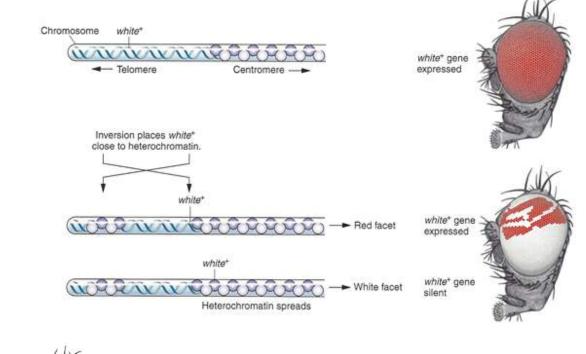# Epigenetics

Máté Varga

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Position-effect variegation: a matter of chromatin accessibility

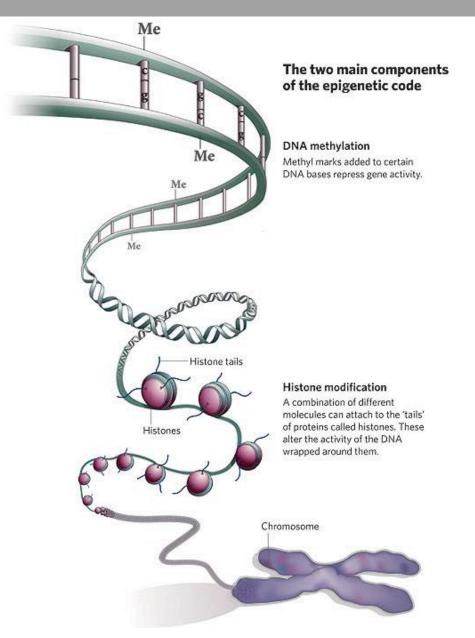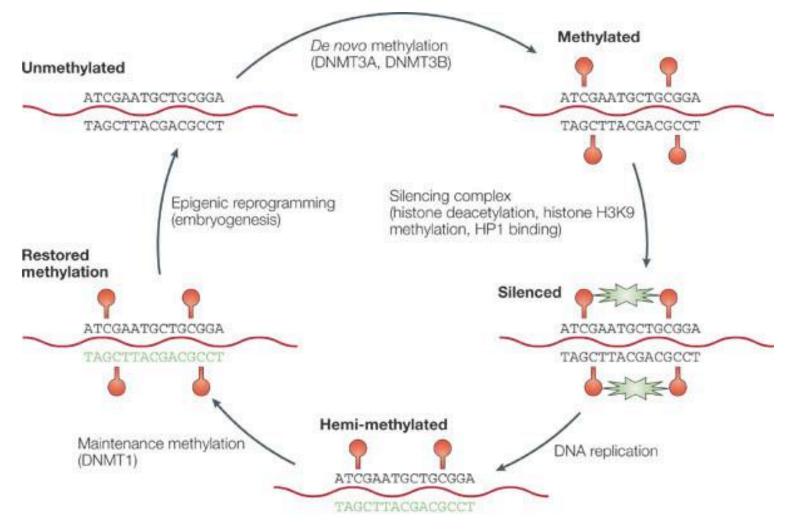*E(var)* Enhancers: e.g. Facilitator of Chromatin Transcription (FACT) proteins

Su(var) Supressors: e.g. Histone Deacetylases (HDACs)

The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

Histone tails

Histones

Chromosome
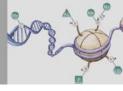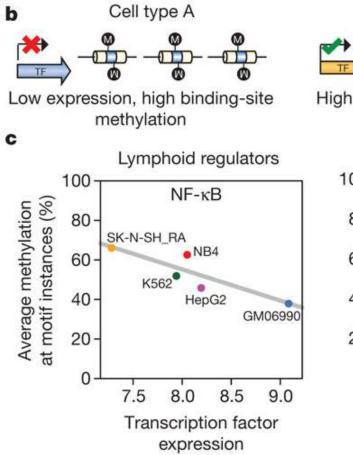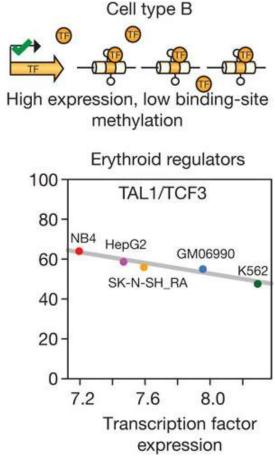
DNA methylation can be a passive process

- if DNA methylation would be an active process, methylation levels would be independent of TF concentration
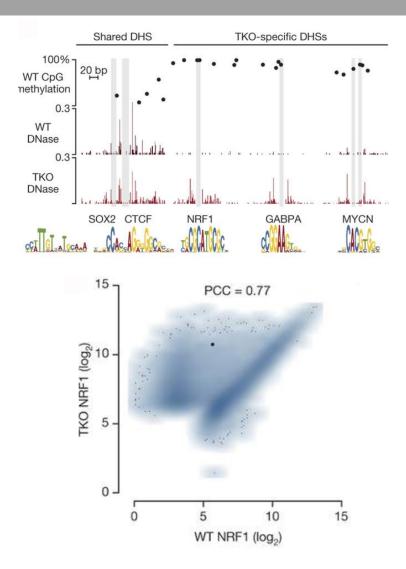
- what we observe, however, is that the higher the expression for a TF, the less methylation can be observed at its binding sequence

- this suggests that methylation is passive, and occurs wherever DNA is not bound by other factors
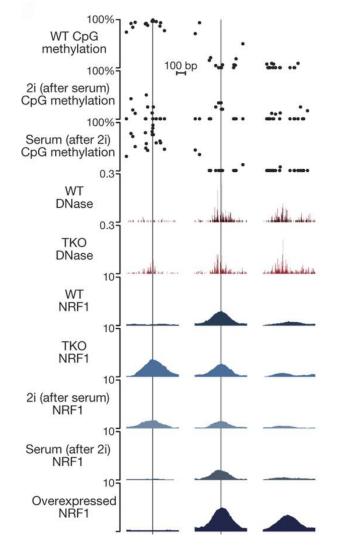
(Thurman et al. (2012) *Nature*)
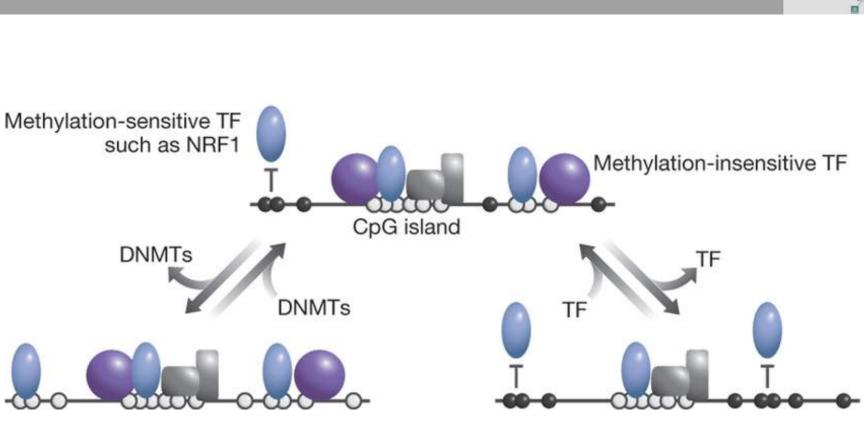
Some TFs are methylation sensitive

- in the absence of DNMT function, TFs can bind previously methylated positions

- after new methylation, these NRF1 sites disappear
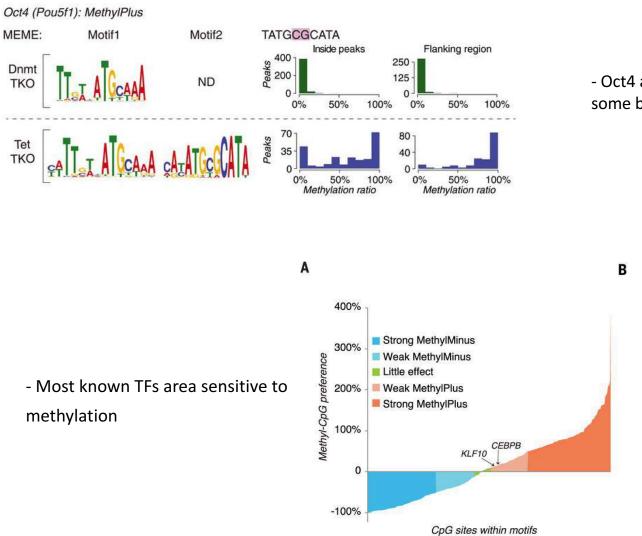
(Domcke et al. 2015 *Nature*)
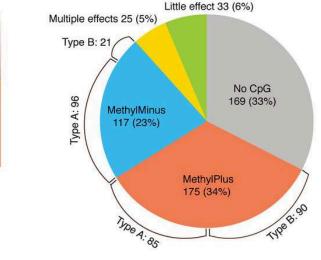
Some TFs are methylation sensitive

- Binding of methylation-sensitive TFs can happen after DNMT-function impairment, or as the result of the binding of methylation-insensitive TFs (these induce local demethylation)
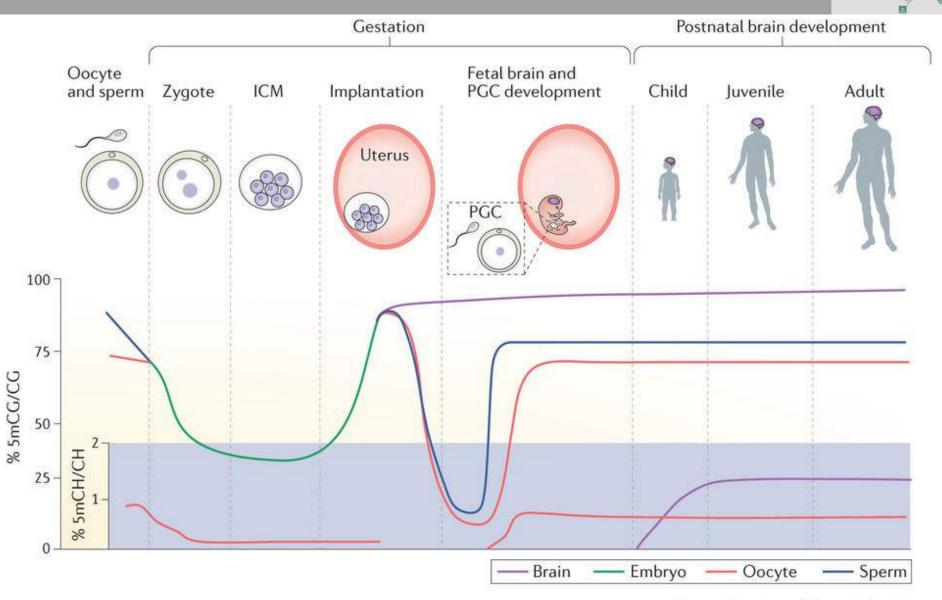
(Domcke et al. 2015 *Nature*)

Oct4 (Pou5f1): MethylPlus

- Oct4 actually *prefers* methylation at some binding sites

- Most known TFs area sensitive to methylation

(Yin et al. 2017 *Science*)
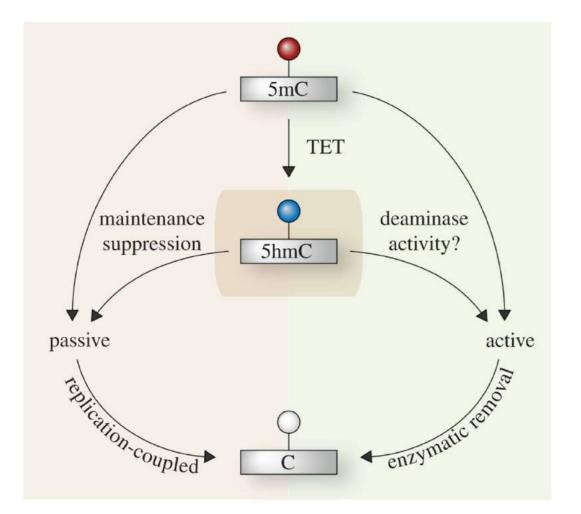
Changes in methylation during development

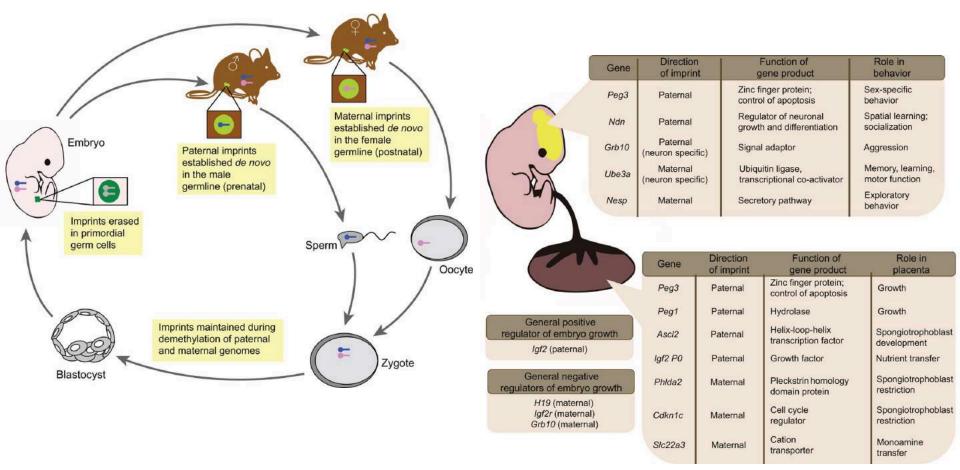(Ciernia and LaSalle 2016 *Nat Rev Neurosci*)

- The 5mC-5hmC conversion could be the first step in the passive demethylation of the paternal genome at the beginning of development
- Active (Tet-dependent) demethylation occurs in PGCs

(Hackett and Surani 2012 *Phil Trans Roy Soc B*)

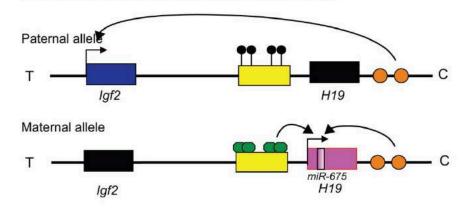# The role of parental imprinting in development
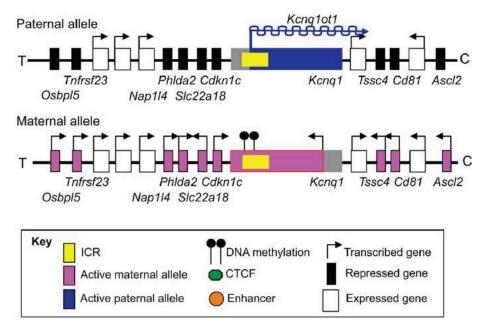

(Plasschaert and Bartolomei 2014 *Development*)

A Insulator model of imprinting: the *H19*/*Igf2* locus
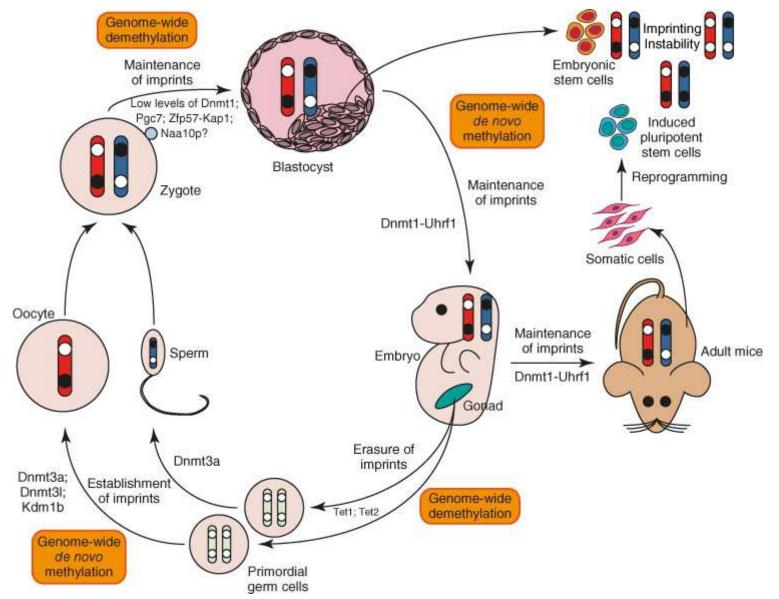
B ncRNA model of imprinting: the *Kcnq1* locus

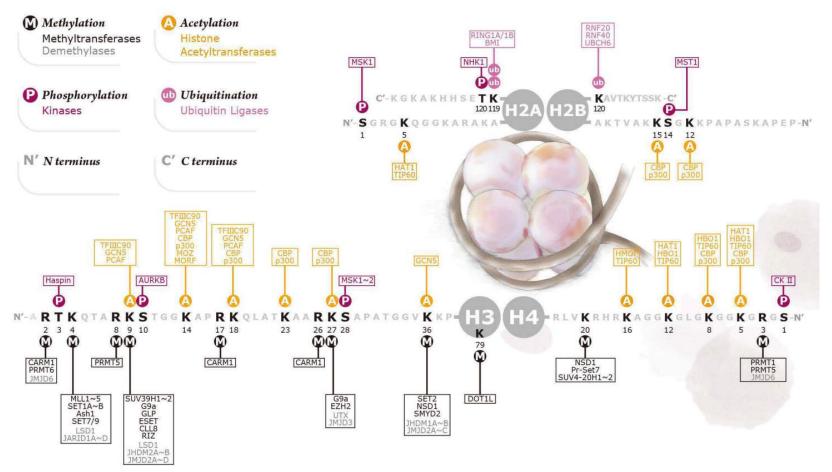# How parental imprinting can be conserved during epigenetic reprogramming?

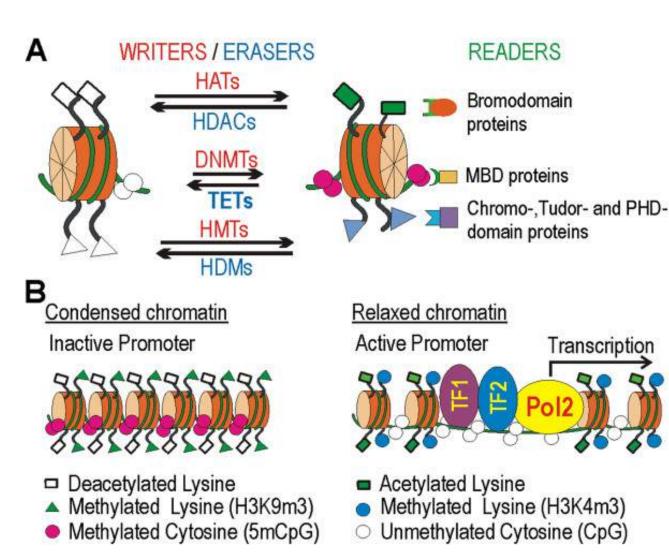(Brandao et al. 2018 *eLS*)

Epigenetic histone modifications

Inactive state: acetylation (Ac) - lysine (K) 12 (histone 4 - H4) and methylation (Me) K9, K27 (H3)

Active state: acetylation - K9, K14 (H3), K5 (H4), methylation - K4 (H3), arginine (R) 3 (H4)

Epigenetic histone modifications

HATs = histone acetyl transferases
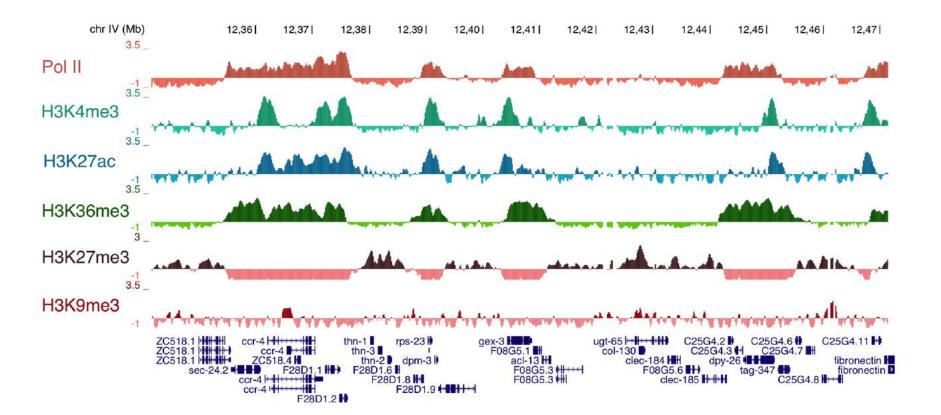
HDACs = histone deacetylases

DNMTs = DNA methyltransferases

TETs = ten-eleven translocation enzymes
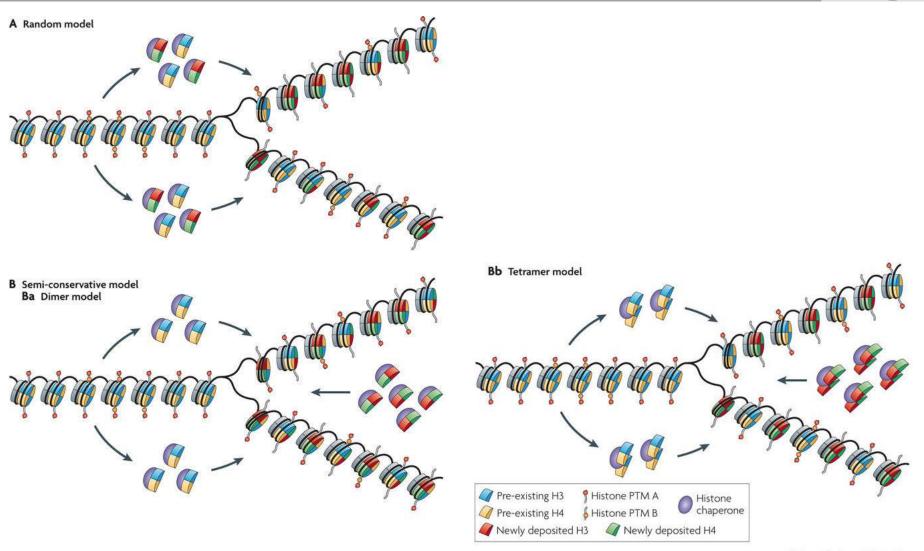
HMTs = histone methyl transferases

HDMs = histone demethylases

(Glant et al. 2014 *BMC Medicine*)

Epigenetic histone modifications and gene expression

(Wormbook)

(Margueron and Reinberg 2010 *Nat Rev Gen*)

(Laprell et al. 2017 *Science*)

Inheritance of epigenetic histone modifications: the role of PcG proteins
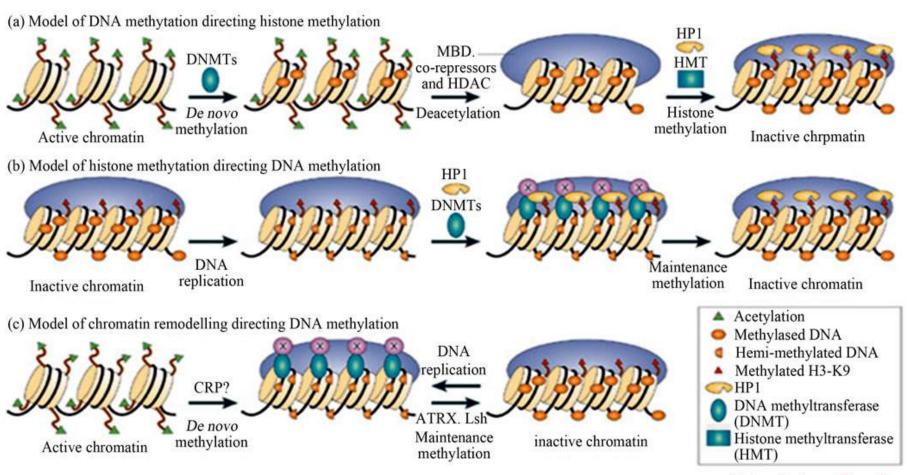

- Repressive histone-modifications are lost during cell divisions

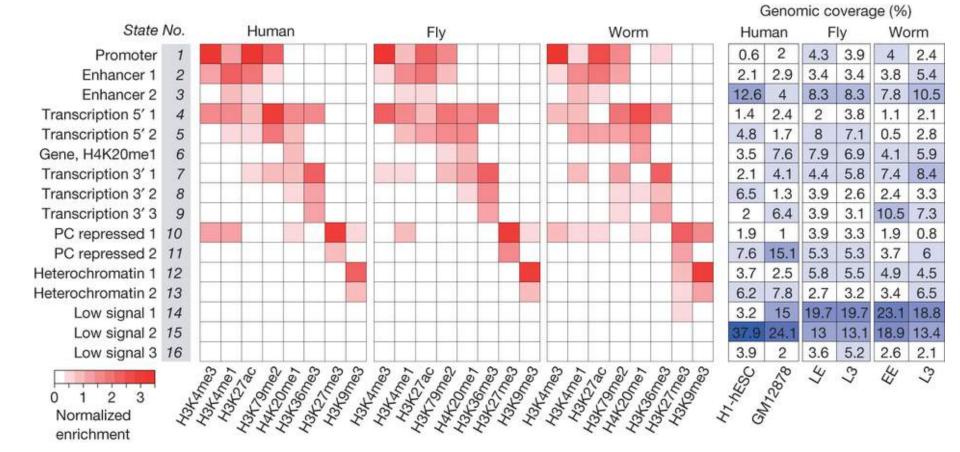(Coleman and Struhl 2017 *Science*, Laprell et al. 2017 *Science*)

(a) Model of DNA methytation directing histone methylation

(b) Model of histone methytation directing DNA methylation

(c) Model of chromatin remodelling directing DNA methylation

Nature Reviews I Genetics

(Li (2002) *Nat Rev Gen*)

Promoter modifications are similar across Metazoa
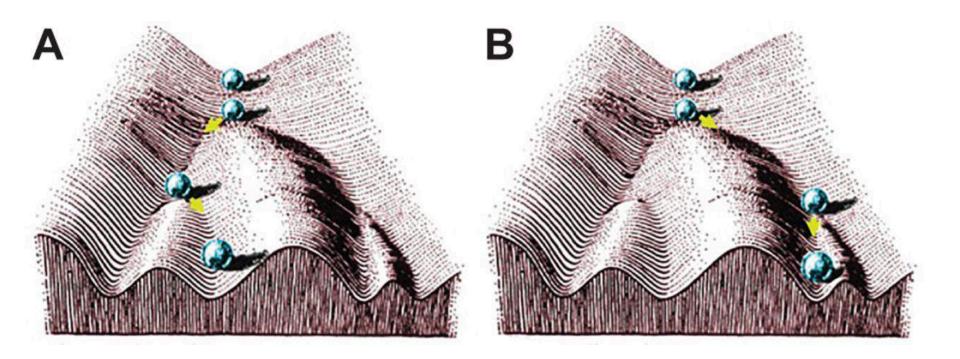
(Ho et al., 2014 *Nature*)

- Cells in different tissues express different sets of genes, but they all have the same genome
- Epigenetics has a major role in deciding which genes are transcribed at a given timepoint in a cell
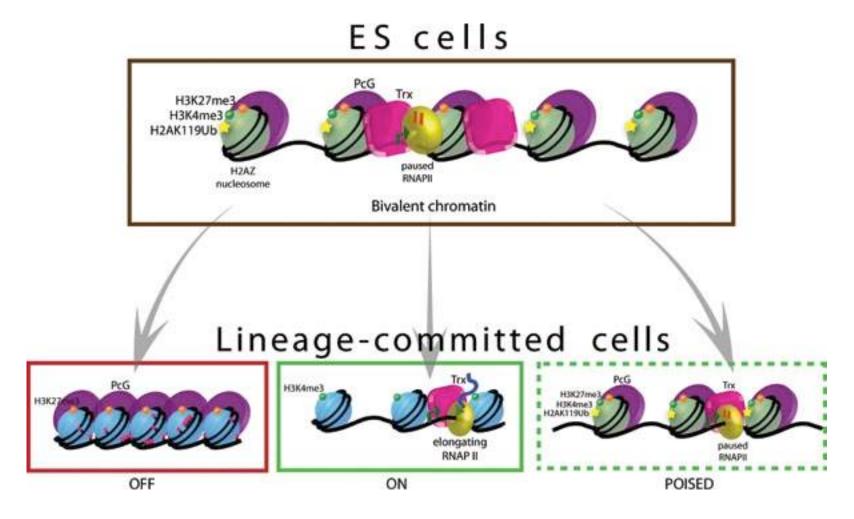
Bivalent epigenetic modifications
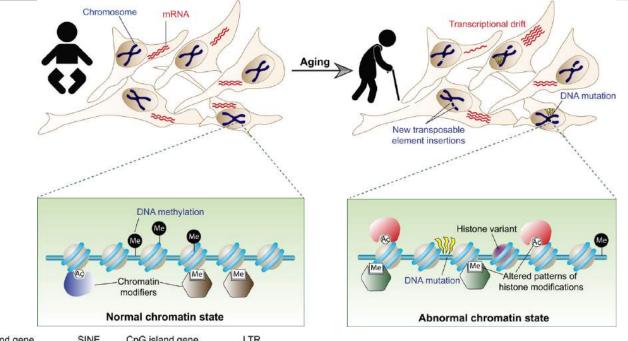
- most often occur at developmental genes
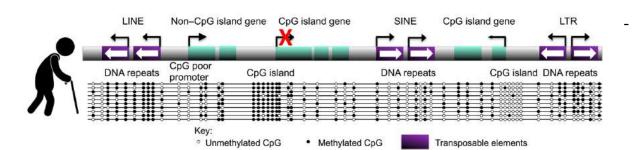- the bivalent modifications get resolved over time
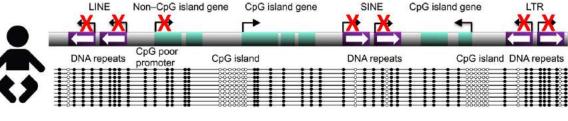
The epigenetics of aging

- in *general* heterochromatin loosens up during aging, and there are less repressive epigenetic modification
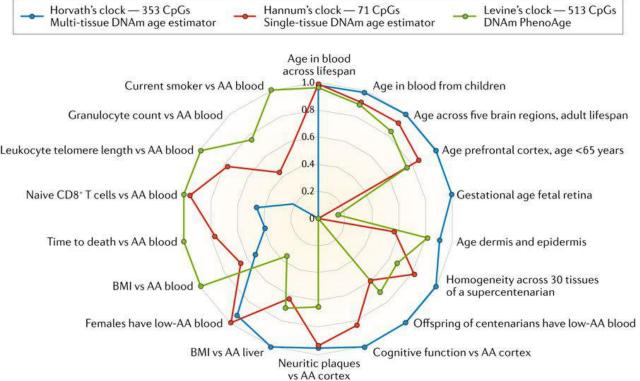
- While most sites become hypomethylated, there are some that will be hypermethylated (and consequently some genes are silenced)
- Transposons become overactive, with deleterious consequences
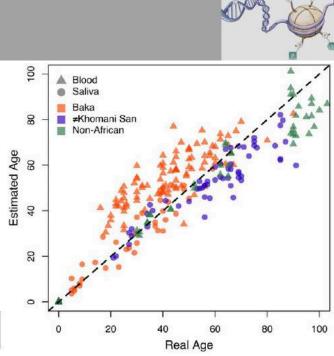
(Pal and Tyler, 2016 *Science Adv*)

- the right combination of hypo- and hypermethylated sites can predict the age of the sample tissue with high accuracy

(Gopalan et al., 2017 *Genetics*)
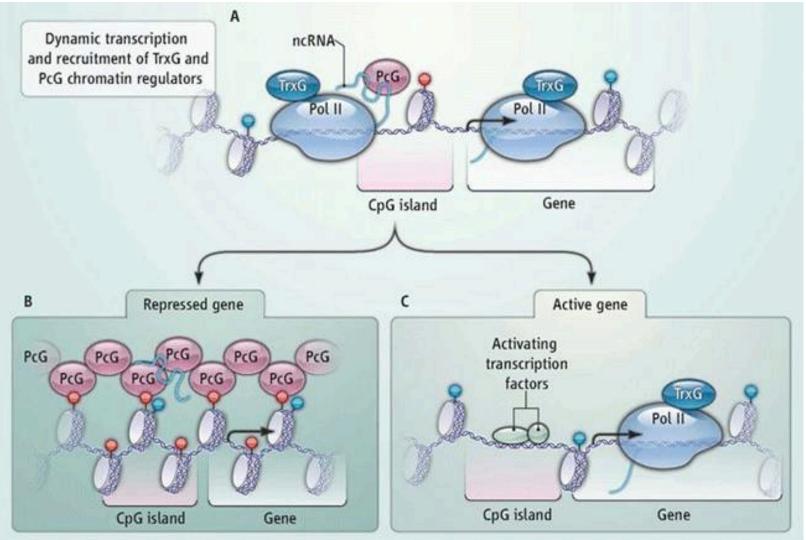
(Horvath and Raj, 2018 *Nat Rev Gen*)
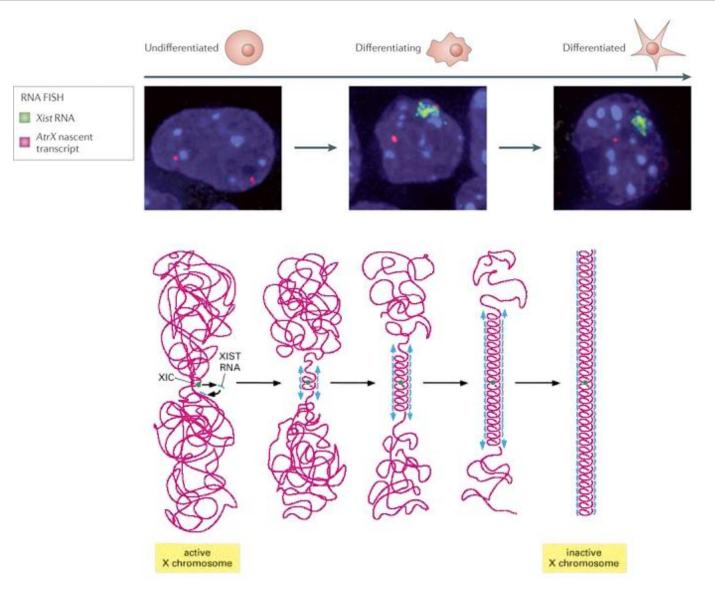
(PcG = Polycomb Group Protein; TrxG = Trithorax Group Protein)

(Guenther and Young (2010) *Science*)
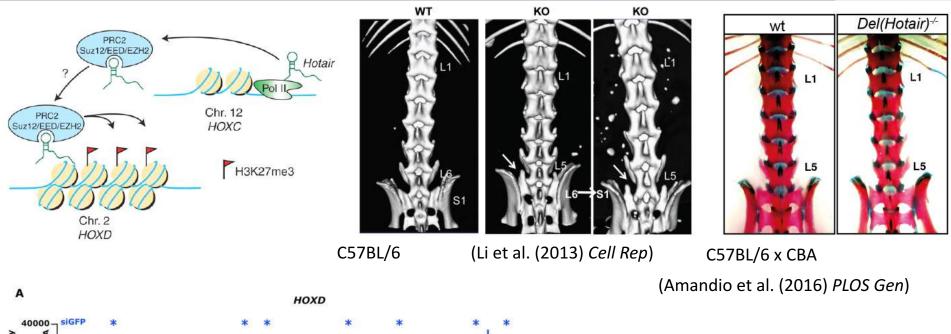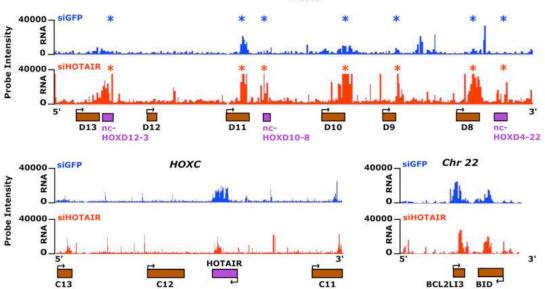
(Augui et al. (2011) *Nat Rev Gen*))

C57BL/6   (Li et al. (2013) *Cell Rep*)   C57BL/6 x CBA

(Amandio et al. (2016) *PLOS Gen*)
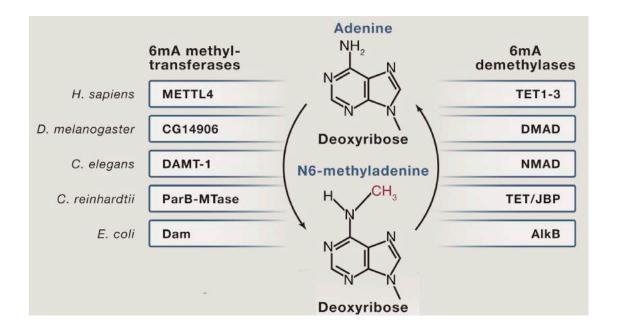
BUT: Denis Duboule's group has knock out the whole HoxC cluster before (including *HOTAIR*) with no phenotype. How is this possible?

(Rinn et al. (2007) *Cell*)
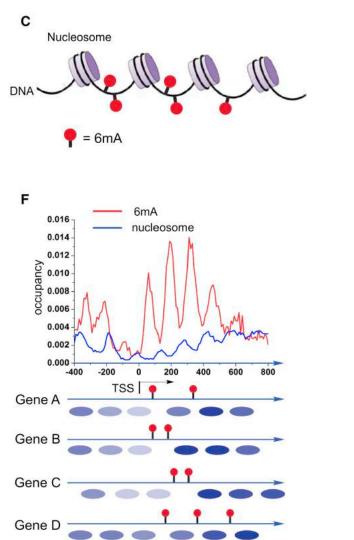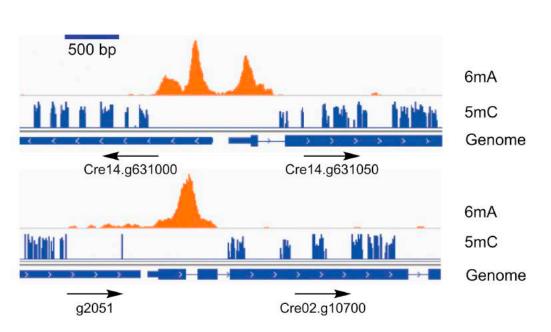
In prokaryotes m6A methylation is essential (m5C is not observed), but recently has been detected in eukaryotes as well.

m6A DNA-methylation in the regulation of *Chlamydomonas* gene expression
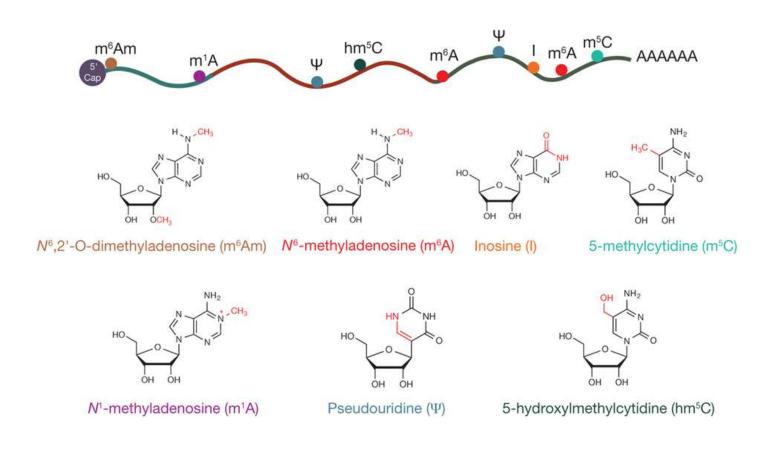
- m6A is characteristic for the linker regions of *Chlamydomonas,* in the proximity of the TSS (m5C is seen in the inside of the genes)

(Fu et al. (2015) *Cell*)

Epitranscriptome: posttranscriptional modification of the RNA

N^6,2'-O-dimethyladenosine (m⁶Am) N^6-methyladenosine (m⁶A)   Inosine (I)   5-methylcytidine (m⁵C)

N^1-methyladenosine (m¹A)   Pseudouridine (Ψ)   5-hydroxylmethylcytidine (hm⁵C)
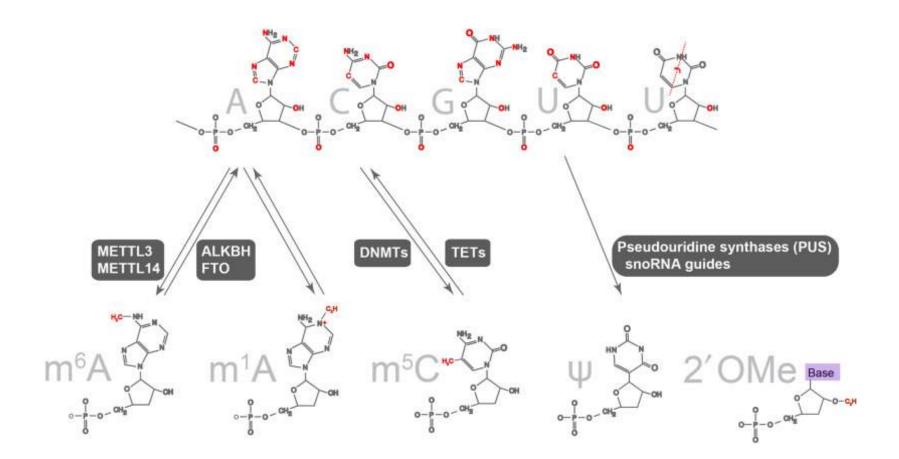
- Posttranscriptional modification can affect RNA stability
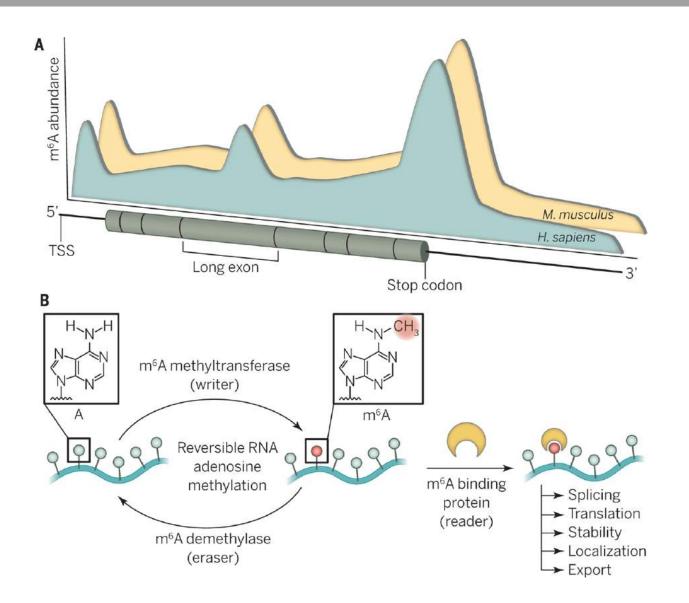
- We can distinguish "writer", "eraser" and "reader" proteins

m6A regulates the stability of maternal transcripts during zebrafish development
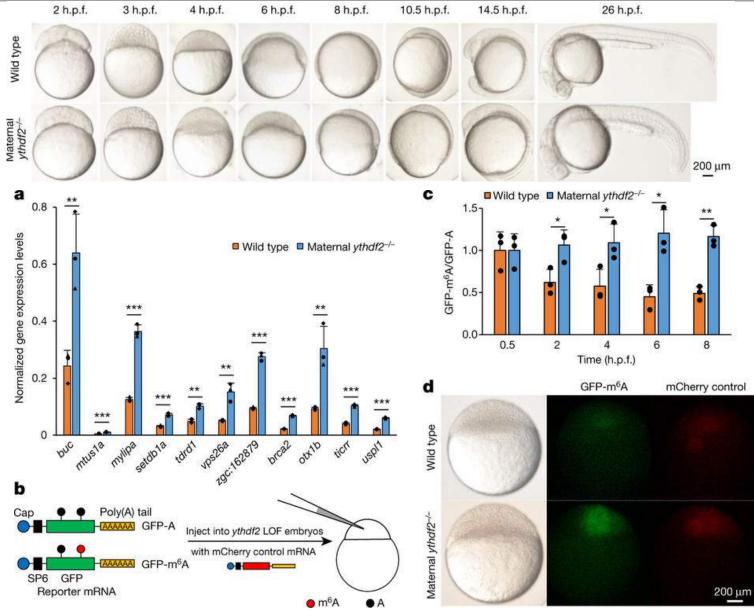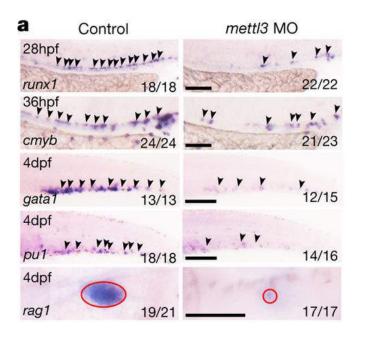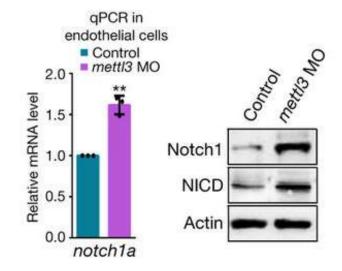
(Zhao et al., 2017 *Nature*)

- overactive Notch1a inhibits the formation of HSCs from endothelial tissue
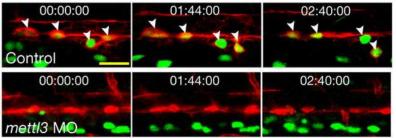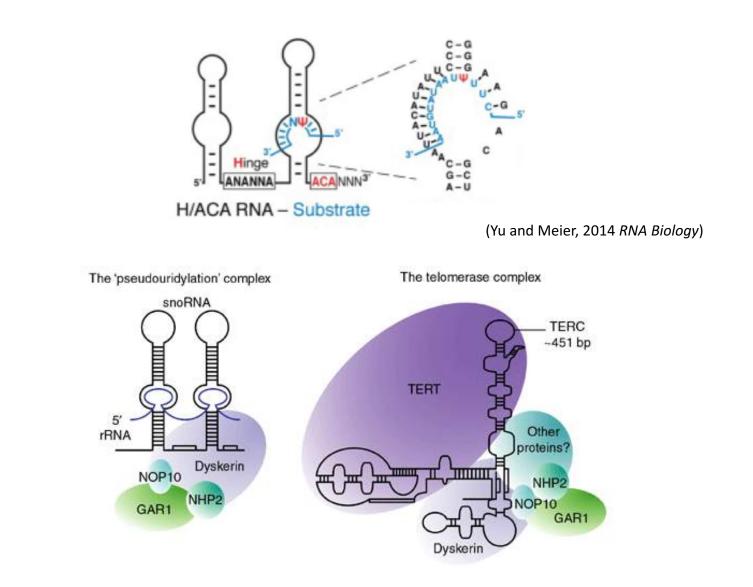
- In the absence of Mettl3 (an m6A wirter) neither HSCs, nor differentiated blood lineages can develop

(Zhang et al., 2017 *Nature*)

H/ACA RNA – Substrate

(Yu and Meier, 2014 *RNA Biology*)
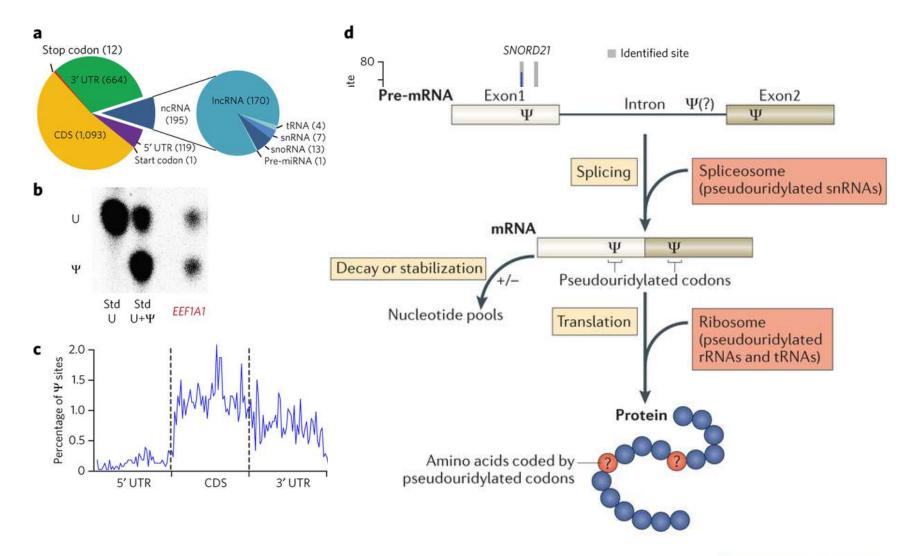
The 'pseudouridylation' complex

The telomerase complex

Adenosine (A) – inosine (I) RNA editing and its srole in neuronal function

- A-to-I RNA editing is catalyzed by ADAR (Adenosine deaminase acting on RNA) enzymes

- In the case of GluR2 the Ca^{2+} permeability of the AMPA-receptor is altered after editing and this can change the dynamics of the membrane potential
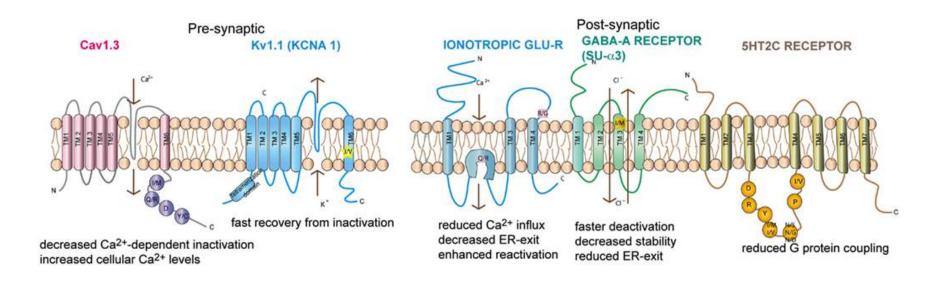
(Behm et al., 2017 *J Cell Sci*, Liu and Zukin, 2007 *Trend Neurosci*)
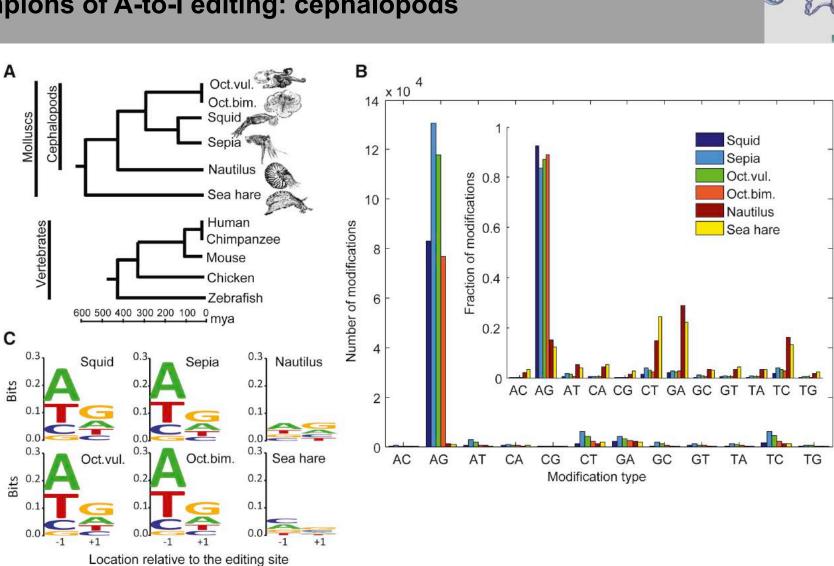
- The properties of many other ion-channels can be changed after RNA-editing
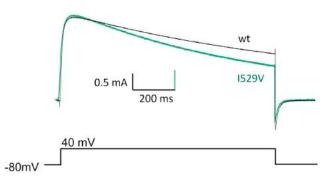
- In the case of Coleoid cephalopods A-to-I editing is the rule
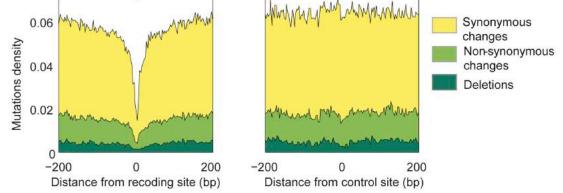
Sepia K$_v$2.1 specific editing site
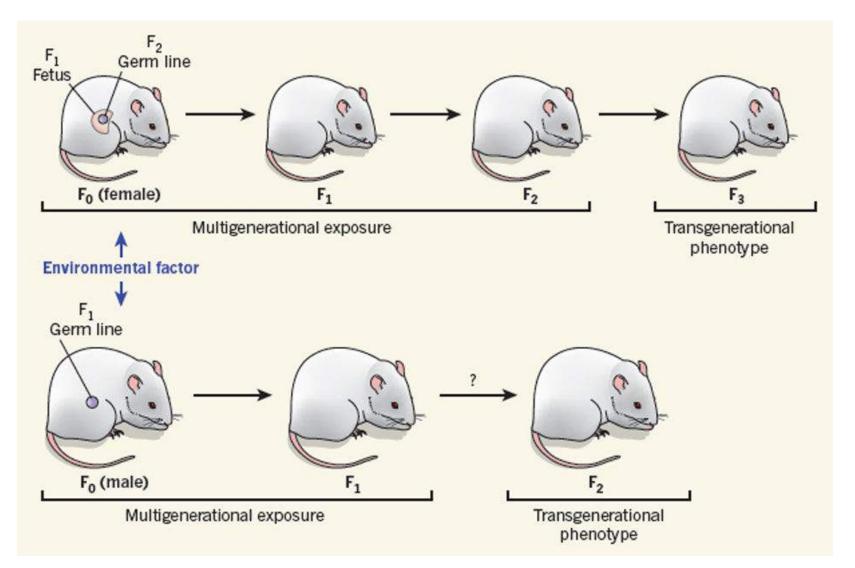
- the K$_v$2 potassium channel closes faster as the result of the editing

- A large number of editing-sites are under positive selection, genome evolution is slower in Cephalopods

(Liscovitch-Brauer, 2017 *Cell*)

Toadflax floral symmetry: the oldest example of transgenerational epigenetic inheritence
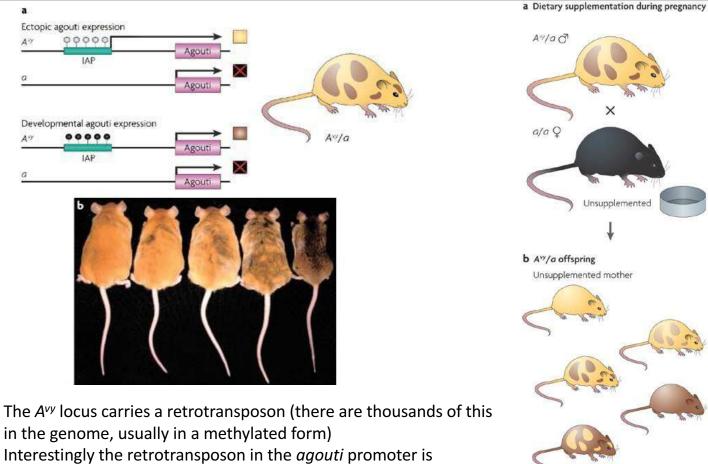
- Toadflax (*Linaria vulgaris*) plants normally have flowers with bilateral symmetry, but some plants have flowers with radial symmetry
- Plants with radial flowers sometimes have offspring with bilateral flowers, but the inheritance pattern is nowhere close to the mendelian ratio
- Floral symmetry is linked to the function of the *CYCLOIDEA-like* (*Lcyc*) gene, but this genes seems to be intact in plants with radial flowers!
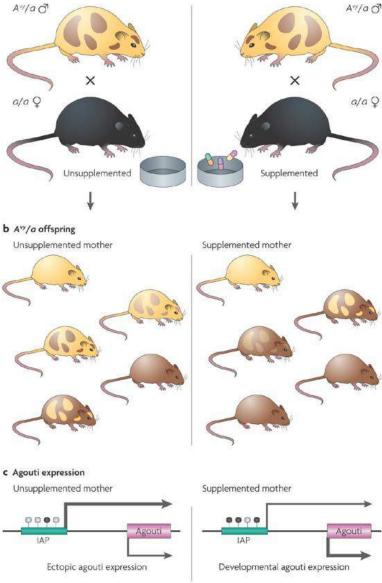
- Expansion in the methylation of a nearby TE can spread into the *Lcyc* locus

The agouti color: a classic example for non-mendelian, epigenetic inheritance
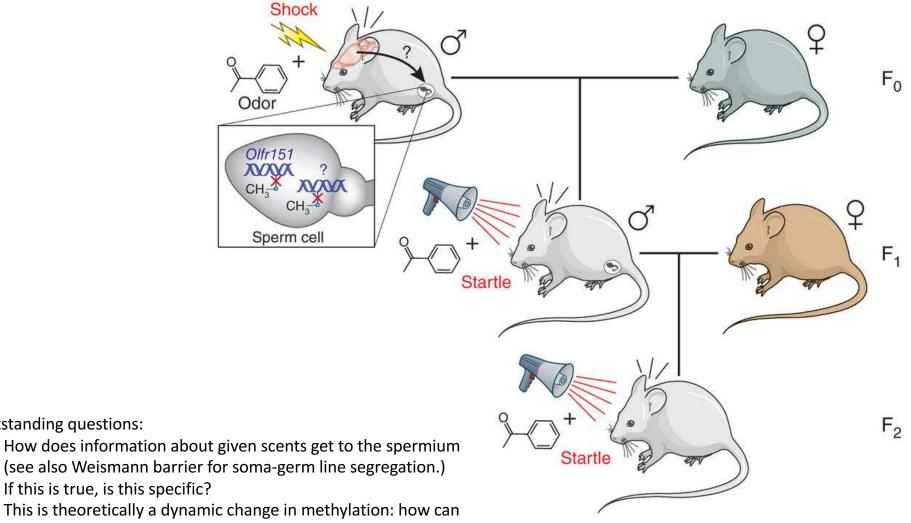
- The *A^vy* locus carries a retrotransposon (there are thousands of this in the genome, usually in a methylated form)
- Interestingly the retrotransposon in the *agouti* promoter is metastable - if it becomes hypomethylated acts as a cryptic constitutive promoter.
- The five mice on the picture have exactly the same allelic combination, their differences are epigenetic
- Agouti mice are not only lighter, but also heavier (pleiotropic effects)

(Jirtle and Skinner, 2007 *Nat Rev Gen*)

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Outstanding questions:
- How does information about given scents get to the spermium (see also Weismann barrier for soma-germ line segregation.)
- If this is true, is this specific?
- This is theoretically a dynamic change in methylation: how can be this protected during the demethylation wave that occurs early in development.

(Szyf, 2014 *Nat Neurosci*)

- A heat-shock inducible, multi-copy transgene remains turned on for 14 (!!) generations post heat-shock.

- SET-25: histone methyltransferase, H3K9me3 specific
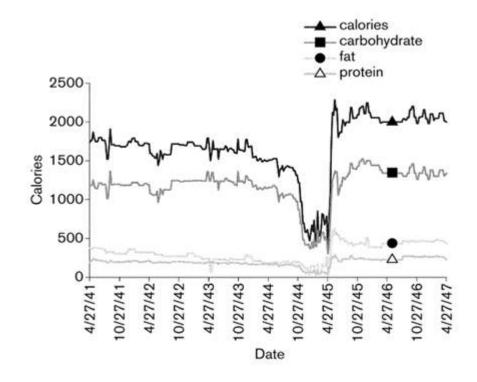
- As a result of the heat-shock a general SET-25 repression occurs (therefore it takes several generations at other loci as well to revert to the original)

(Klosin et al., 2017 *Science*)
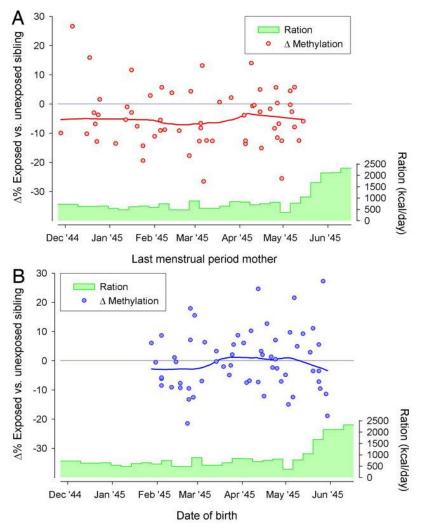
- az ez idő alatt embrionális fejlődésben levő gyerekeben felnőtként nagyobb a cukorbetegség, elhízás, kardiovaszkuláris betegségek, bizonyos pszichiátriai problémák gyakorisága

BUT: no significant effect in F2

Table 2. Prevalence of F2 self-reported disease* according to F1 gender

	F1 exposed	F1 unexposed	All
F2 of F1 men			
n	52	99	151
Cardiovascular%	5.9	2.0	3.4
Pulmonary%	9.8	8.2	8.7
Hay fever%	19.6	30.6	26.8
Eczema%	25.5	24.5	24.8
Cholesterol%	0.0	2.0	1.3
Diabetes%	0.0	2.0	1.3
Hypertension%	3.9	2.0	2.7
F2 of F1 women			
n	106	103	209
Cardiovascular%	1.9	4.0	3.0
Pulmonary%	4.9	5.0	4.9
Hay fever%	20.6	18.0	19.3
Eczema%	19.4	24.0	21.7
Cholesterol%	0.0	0.0	0.0
Diabetes%	1.0	0.0	0.5
Hypertension%	3.9	5.0	4.4

*Defined as answering in the affirmative to questions phrased as 'has a doctor ever diagnosed' or 'has a doctor ever prescribed medication for' the different conditions.

- Fasting during pregnancy results in the hypomethylation of the DMR region of *IGF2,* what may persist over decades

(Veenendaal et al., 2013 *BJOG*)

(Heijmans et al , 2008 *PNAS*)

Human examples: China, after Mao's Big Leap (1959-60)

- As the result of the "Big Leap" 30-55 million people may have starved to death
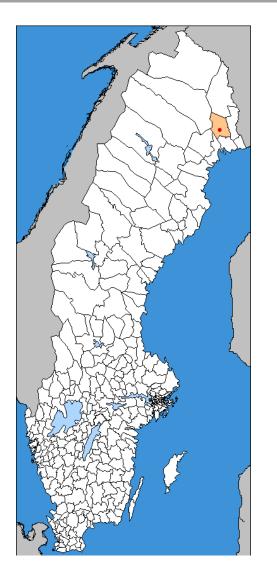
- Today only for schizophrenia can be some association observed.

(Li and Lumney, 2017 *Int J Epid*)

- This is correlation between two generation – we have no idea, what is causing it, epigenetics is just one possibility (but the results also could be a statistical fluke due to covariate mining)

(Pembrey et al , 2006 *Eur J Hum Gen*)