

Epigenetics and phenotypical variability in domestic species:
can epigenetic marks be used as new co-variables in animal breeding?

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INRA, 78352, Jouy en Josas France



Definition(s)

Molecular mechanisms

Examples of epigenetic modifications affecting the phenotype

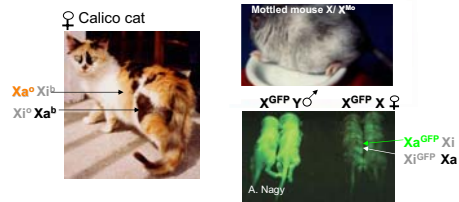
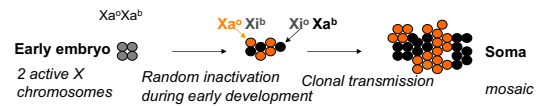
Transgenerational transmission of epigenetic modifications

Genome wide studies of epigenetics

EPIGENETICS

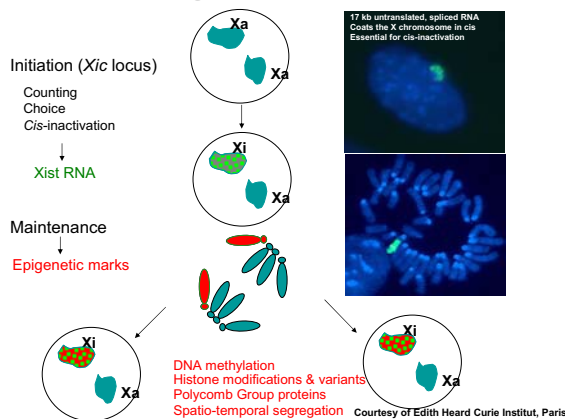
- 1- Most cells contain the same set of genes, but their phenotype can vary according to which genes are expressed and repressed.
- 2- Alterations in gene expression patterns that are heritable (through mitosis or meiosis), without changes in DNA sequence, are known as « **epigenetic mechanisms** ».
- 3- Epigenetic states are potentially **reversible** - during development, or in the germ line, or in somatic cells (eg stem cell differentiation), or in disease (eg epimutations in cancer)
- 4- Epigenetics contribute to the « intangible » phenotypical variations

X chromosome inactivation: a classical example of epigenetics



X-linked coat colour mutations produce variegated phenotypes only in females and not in males
Mary Lyon, 1961, Nature 199:372 "Gene action in the X chromosome of the mouse (*Mus musculus*)"

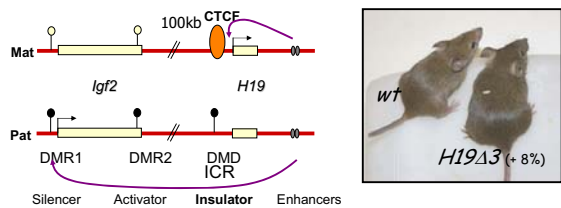
Regulation of X Inactivation



Parental imprinting

Parent-of-origin specific gene expression whereby a single allele is differentially expressed depending on the sex of the parent transmitting allele

The paradigmatic case of the IGF2- H19 locus



Tilghman, 1995 ; Dandolo, 1997

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Different types of epigenetic phenomena

X inactivation

Parental imprinting

Position-effect variegation

Paramutation

Transposon activity « changes in phase »

Definition(s)

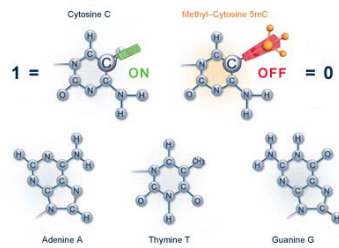
Molecular mechanisms

Examples of epigenetic modifications affecting the phenotype

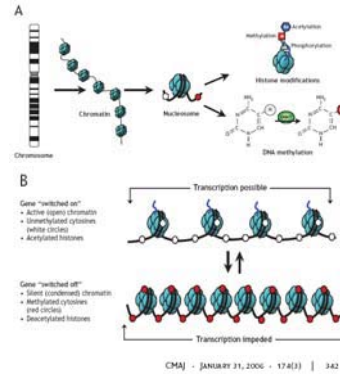
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DNA methylation is the most studied epigenetic modification

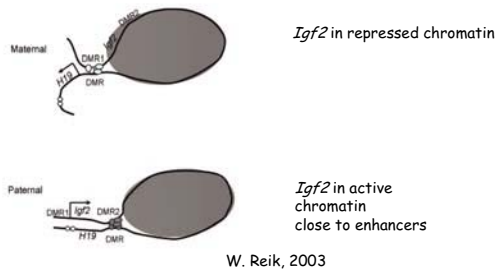


Histone modifications are linked to DNA methylation



The *H19* locus has a cis effect on *Igf2*

***Igf2* expression is affected by a chromatin mediated DNA looping**



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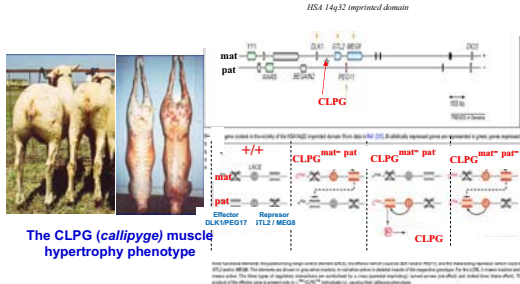
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The emblematic case of the callipyge locus in livestock

A single base mutation resulting in muscle hypertrophy but only for heterozygous animals inheriting the paternal mutated allele



The CLPG (*callipyge*) muscle hypertrophy phenotype

Charlier et al. 2001 Nat Genet
 Takeda et al. 2006 PNAS
 Michel George's group

Other examples in domestic mammals

Pig

An intronic (G-A) mutation in the porcine IGF2 gene which abrogates the binding of a trans acting factor to a muscle-specific silencer element

Results in an increase in IGF2 levels with resulting muscle hyper trophy

Van laere et al. 2003, Nature

Sheep

Defective interaction of the myostatin MSTN transcripts with skeletal Muscle-specific miRNAs (miR-1 and miR-206miRNAs)

A mutation in the MSTN 3'untranslated region (3'UTR) of Texel sheep created an illegitimate target site for miRNAs leading o Myostatin down regulation and hence muscular hyper trophy

Clop et al, Nature Genetics 2006

Definition(s)

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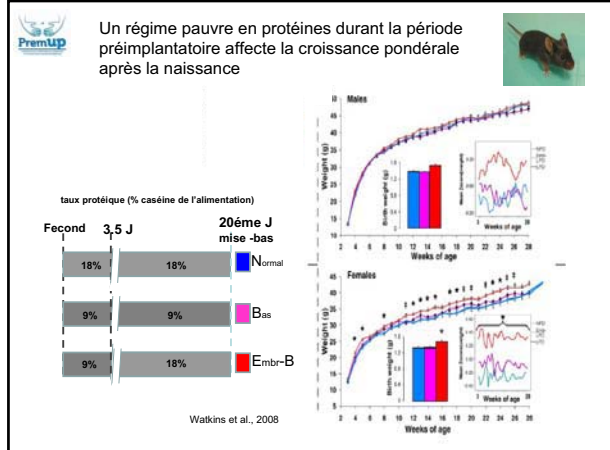
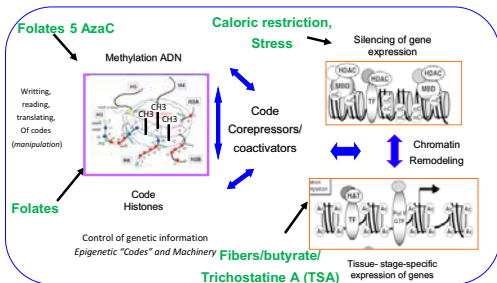
Transgenerational inheritance of epigenetic modifications

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Epigenetic Transgenerational Actions of Endocrine Disruptors and Male Fertility

Transient exposure of a gestating female rat during the period of gonadal sex determination to the endocrine disruptors vinclozolin (an or methoxychlor induced an adult phenotype in the F1 generation of decreased spermatogenic capacity (cell number and viability) and increased incidence of male infertility. These effects were transferred through the male germ line to nearly all males of all subsequent generations examined (F1 to F4).

Anway et al (2005). Science 308,1466-1469



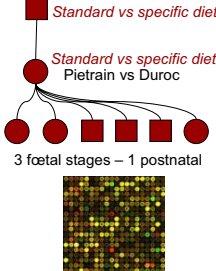
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Nutritional effects on epigenetic modifications:
Studies within WP2 of the SABRE program

- Standard versus methyl-supplemented diet :
 - Breed effects [task 2.2.1.](#)
 - Developmental stages [task 2.3.1](#)
 - Transgenerational inheritance of epigenetic modifications [task 2.3.3.](#)
- Genome-wide and local analysis [task 2.2.2.](#)
 - All genome expression profile analysis
 - Methylation status analysis of differentially expressed genes and of candidate genes (IGF2, IGF2r; Ins...)



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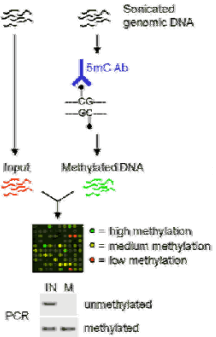
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Genome-wide methylation analysis
Methylated DNA ImmunoPrecipitation (MeDIP)

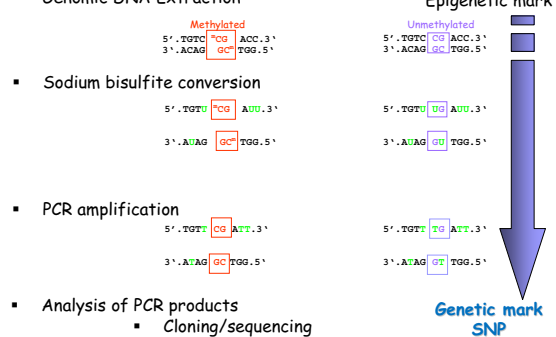
Principle: Total genomic DNA is sonicated and methylated DNA is immunoprecipitated with an antibody directed against 5-methylCystidine (5mC). Input DNA (IN) and methylated DNA (M) can be differentially labeled with Cy5 (red) and Cy3 (green) and co-hybridized as a twocolor experiment on micro-arrays (CpG island arrays, Promoter-arrays...), or used for single-gene analysis by PCR



• Weber M, Davies JJ, Wittig D, Oakeley EJ, Haase M, Lam WL, Schubeler D: Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nat Genet* 2005, 37:853-862.
 • Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, Rebhan M, Schubeler D: Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat Genet* 2007, 39:457-466.
 • Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S: Genome-wide analysis of Arabidopsis thaliana DNA methylation uncovers an interdependence between methylation and transcription. *Nat Genet* 2007, 39:151-60.

Genome-wide methylation analysis
Bisulfite treatment of genomic DNA

- Genomic DNA Extraction
- Sodium bisulfite conversion
- PCR amplification
- Analysis of PCR products
 - Cloning/sequencing
 - Pyrosequencing



Massive sequencing

- Roche / 454
- 2006 : 20 Mb per run (100 bases per reading)
- 2007 : 100 Mb per run (250 bases per reading)
- 2008 : 1 Gb per run (500 bases per reading)
- 1000 euros / Mbase
- error rates > 1% in homopolymeric regions
- quality assembling ~20 genome equivalent; no supercontigs required

- Solexa/Illumina 1G
- 2007 : 1 Gb per run (32 bases per reading)
- 2008 : 3 Gb per run (50 bases per pair base)
- <10 euros / Mbase
- error rates < 99.9%, no assembling ~

P. Wincker, 2007; source Genoscope, Paris