Effect of aging on epigenetics of immune cells in dairy cattle

Helene Jammes[1], Aurélie Chaulot-Talmon[1], Charline Pontelevoy[1], Luc Jouneau[1], Christophe Richard[1], Valérie Gélin[1], Gilles Foucras[2]and Hélène Kiefer[1]

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[2]UMR1225 IHAP, Université de Toulouse, ENVT, INRA, 31076 Toulouse Cedex 3, France

SAPS Sciences Animales PARIS SACLAY

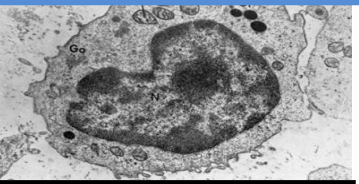
EAAP2019

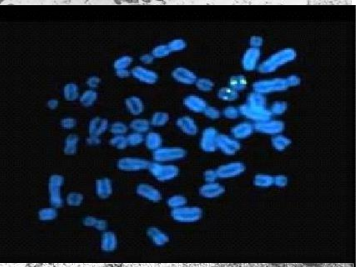
INRA SCIENCE & IMPACT

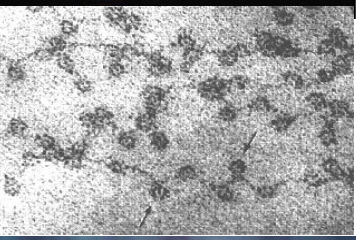
Part I – **Epigenetics** – Concepts – Generalities

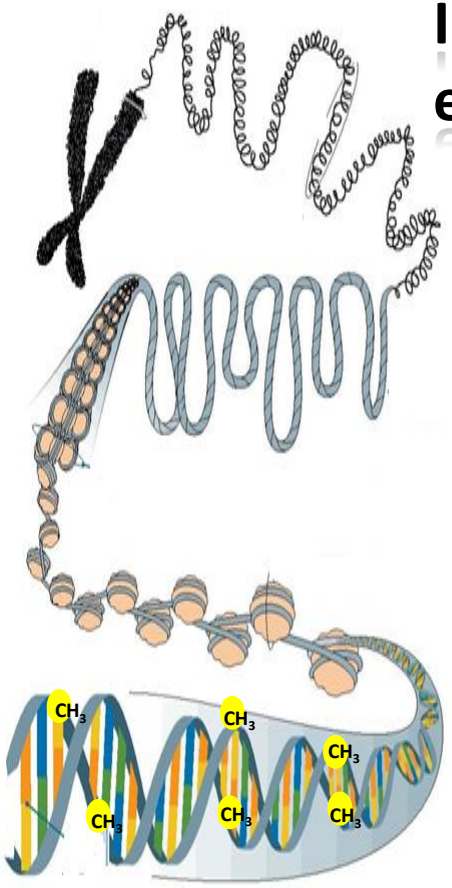
Part II – **Original data** from « Long Health program »

Effect of aging on epigenetics of immune cells in dairy cattle









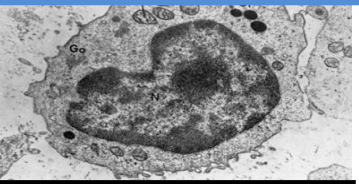
In Nucleus,
epigenetic marks are molecular processes

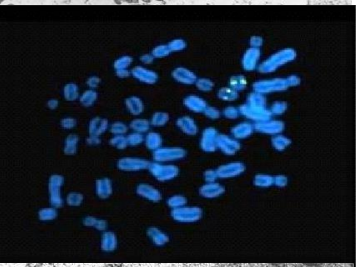
Post translational modifications of Histones

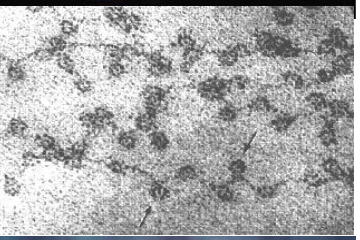
DNA methylation – 5meC

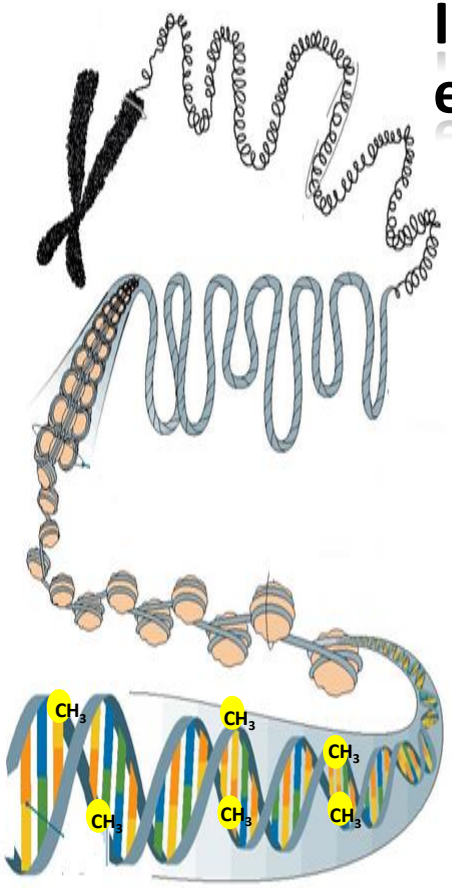
+ Small and long non-coding RNAs

Together, they regulate chromatin architecture and gene expression









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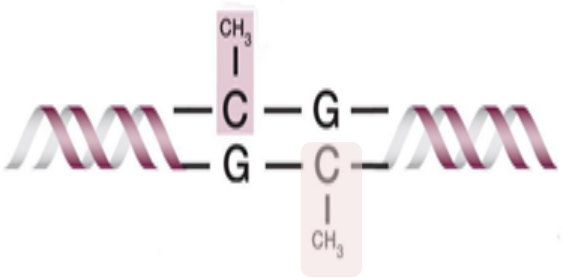
DNA Methylation is a covalent reaction



- Addition of methyl group
- Symetric manner
- Mainly in CpG context
- In mammals, 5-8% of CpG are methylated

Specific enzymatic activities

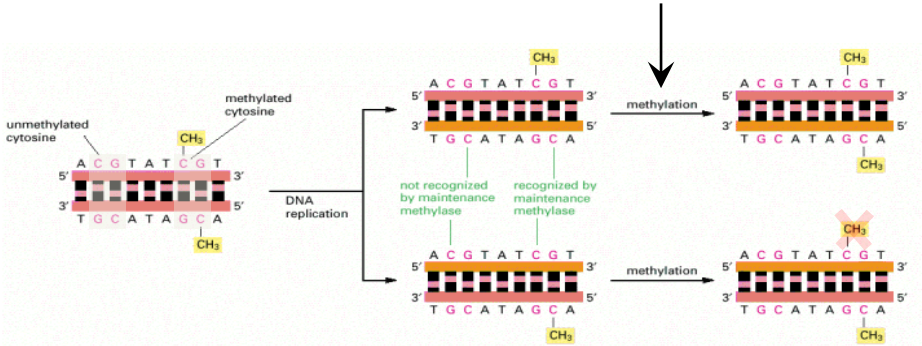
DNA methyltransferases

- **DNMT1**

higher affinity for hemimethylated DNA than unmethylated DNA

maintenance of DNA methylation, fidelity of replication of inherited epigenetic patterns

(Bestor, 1992; Lei et al., 1996; Li et al., 1992)



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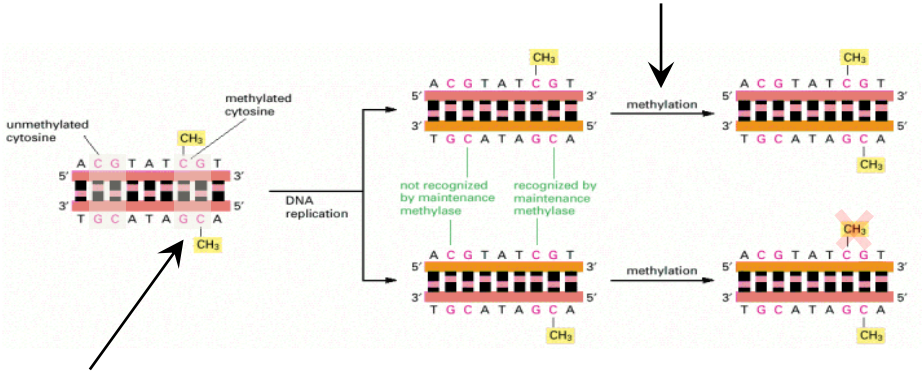


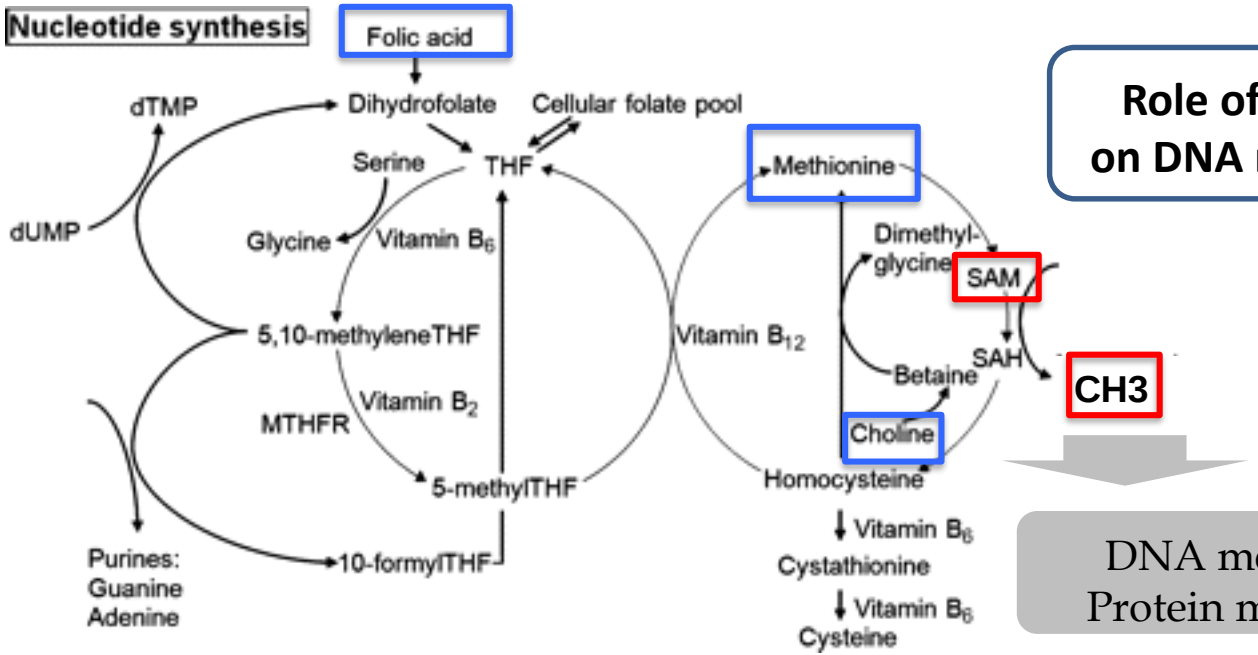
- **DNMT3A and DNMT3B**

act as *de novo* DNA methyltransferases responsible for establishment of DNA methylation Patterns

(Hata et al., 2002; Okano et al., 1999)

S adenosyl methionine, donnor of methyl group

- **SAM is one component of the one carbon metabolism**



Hcy: Homocysteine; Mat: methionine adenosyl transferase; **SAH**: S adenosylhomocysteine; **THF** tetrahydrofolate; **5, 10-MTHF**: 5, 10-methylenetetrahydrofolate; **5 Methyl THF**: 5 –methyl-tetrahydrofolate; **SHMT**: Serine hydroxymethyltransferase; **MTR**: Methyltetragydrofolate-homocysteine methyltransferase; **MTHFR**: Methylentetrahydrofolate

DNA methylation is also reversible

- **Passive manner by absence of DNMT1 in nucleus**

→ dilution throughout the cell division and DNA replication

→ loss of inheritability of DNA methylation

Kohli et Zhang, Nature, 2013

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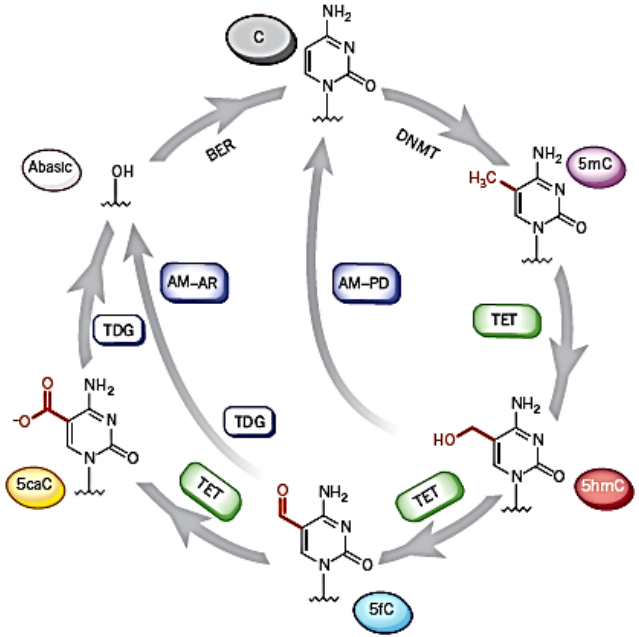
- **Active manner, enzymatically controlled**

- TET enzyme family (Ten eleven translocation proteins)
- Deaminase (AID Activation induced cytidine deamination)
- Reparation enzyme (APOBEC Apolipoprotein B mRNA editing)
- Glycosylase (TDG Thymine DNA glycosylase)

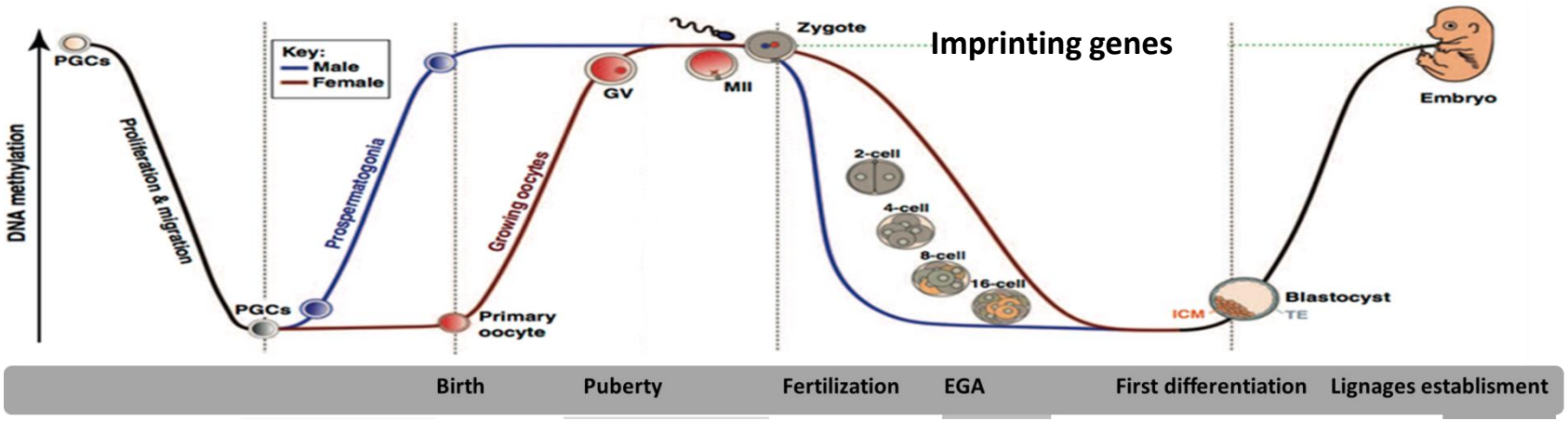


Kohli et Zhang, Nature, 2013

- **5 hydroxymethylation,**

- a new epigenetic marks with specific regulatory roles
- Mainly present in neurones

Apposition and erasure of DNA methylation contribute to a normal program driving gene expression profile and cell differentiation



➢ **Opening of sensitive windows to environmental effects**

- Influence of maternal environment (pre conceptionnal period; gestation) → Fœtal programming
- Paternal effect (pre conceptionnal period) →epigenetic maturity (acquisition and maintenance)

Epigenetic reprogramming controls the future of individual

Nutrition•

Climatic influence •

Toxic exposure •

Managment •

Stress •

Epigenetics

cell identity and function

Acts as cell memory of external events

Birth Puberty Adult life

Expression of selected genetic merit

Performances
Milk Yield and composition
Meat
Female Fertility
Male fertility & semen quality

Biotechnologies of reproduction

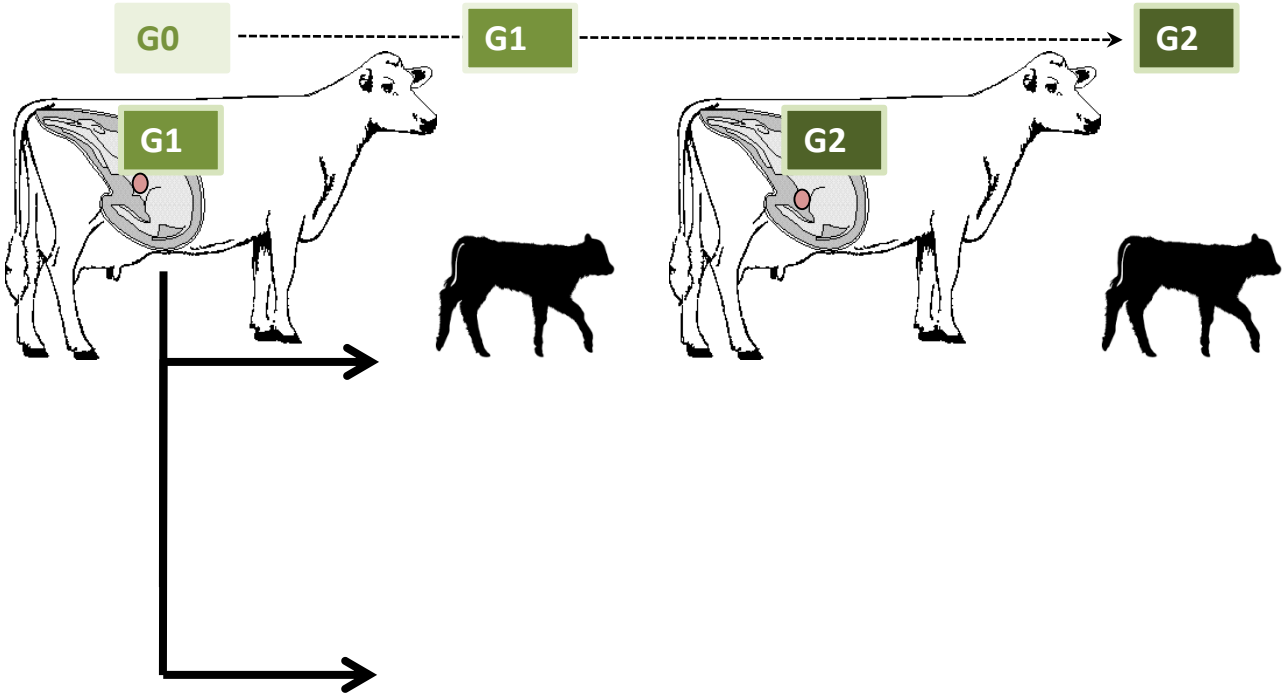
- **Semen Cryopreservation**
- **Oocytes maturation**
- ***In vitro* fertilization**
- **Embryo culture & transfer**

Maternal environment Nutrition
Uterus sensitivity and foeto-placental development

Calf environment Nutrition
Growth, health, puberty

Cross generations transmission of epigenetic information

Environment effect



1 - Effect on G0

- Alterations of cell specific marks
- Modifications of functionnality of genome

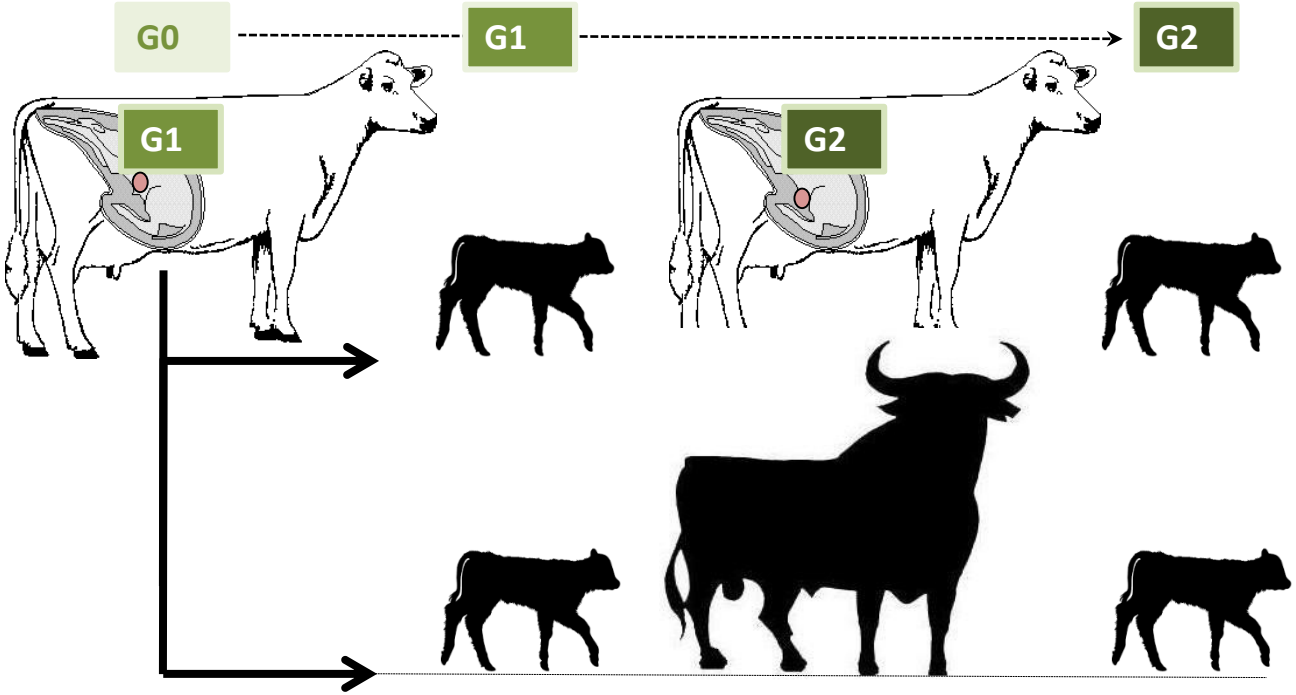
2 - Fœtal programming for G1

- Alterations of fœtal epigenetic programming
  - Somatic cells consequences at long terms
  - Germinal cells consequences on fertility

3 – Transmission inter-generationnal (G2)

Cross generations transmission of epigenetic information

Environment effect



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3 – Transmission inter-generationnal (G2)

1 – Effect on G0

2 – Fœtal programming
- Alteration of DNA methylation apposition
- Alteration of spermaogenesis
- Alteration of fertility

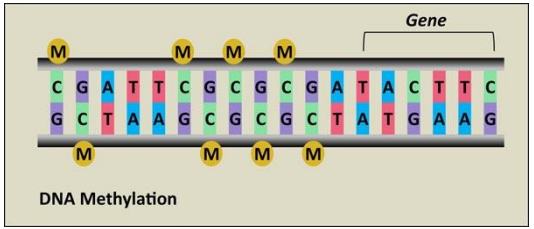
3 – Transmission trans-generationnal (G2)

Why DNA methylation can be used as biomarker

- Acquisition of methylation profiles during development
- Stable and inheritable throughout the mitosis → cell identity
- Reversible
- Sensitive to environment

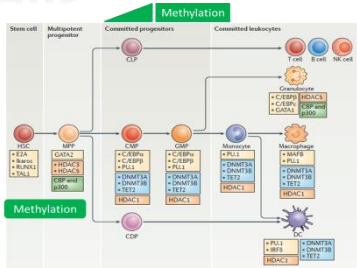
- Individual identity
  - Dependency to genomic sequence
  - SNP → Loss or acquisition of cpG position
  - Accumulation of modifications under environment effects
  - Accumulation of stochastic errors during the life

→ cell memory



DNA methylation can be used as biomarker using blood cells

- Hematopoietic cell differentiation Is dependent of methylation profiles



- Alterations of DNA methylation in blood cells reflect immunity diseases

Review

Cell

Special Issue: Human Genetics

DNA methylation: a promising landscape for immune system-related diseases

Beatriz Suarez-Alvarez[1], Ramon M. Rodriguez[2], Mario F. Fraga[2] and Carlos López-Larrea[1,3]

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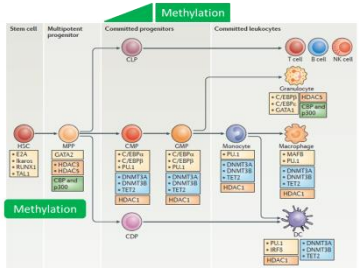

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- Alterations of DNA methylation reflect also no immune diseases

(Cancers, Obesity, Cardiovascular diseases, Autism….)

cancers
Review
Whole-blood DNA Methylation Markers for Risk Stratification in Colorectal Cancer Screening: A Systematic Review

GENOMICS & INFORMATICS
DNA Methylation Profiles of Blood Cells Are Distinct between Early-Onset Obese and Control Individuals

Review Article
The Management of Cardiovascular Risk through Epigenetic Biomarkers
Laurent Metzinger,[1] Stefano de Franciscis,[2,3] and Raffaele Serra[2,3]

Frontiers in Cellular Neuroscience
Epigenetics and Autism Spectrum Disorder: Is There a Correlation?

ELSEVIER
Alzheimer's & Dementia
Review Article
Blood DNA methylation as a potential biomarker of dementia: A systematic review
Peter D. Fransquet[a,b], Paul Lacaze[a], Richard Saffery[b], John McNeil[a], Robyn Woods[a], Joanne Ryan[a,b,c,*]

- Alterations of DNA methylation reveal environmental effects

Taylor & Francis
RESEARCH PAPER
OPEN ACCESS
Tobacco exposure-related alterations in DNA methylation and gene expression in human monocytes: the Multi-Ethnic Study of Atherosclerosis (MESA)
Lindsay M. Reynolds[a], Kurt Lohman[a], Gary S. Pittman[b], R. Graham Barr[c], Gloria C. Chi[d], Joel Kaufman[d], Ma Wan[b], Douglas A. Bell[b], Michael J. Blaha[a], Carlos J. Rodriguez[a] and Yongmei Liu[a]

Tobacco exposure

EPIGENOMICS, VOL. 10, NO. 11 | RESEARCH ARTICLE
Physical activity and epigenetic biomarkers in maternal blood during pregnancy
Sylvia E Badon[1], Alyson J Littman, Kwun Chuen Gary Chan, Mahlet G Tadesse, Patricia L Stapleton, Theo K Bammler, Tanya K Sorensen, Michelle A Williams & Daniel A Enquobahrie
Published Online: 16 Oct 2018 | https://doi.org/10.2217/epi-2017-0169

Physical activity

SCIENTIFIC REPORTS
OPEN
Persistent DNA methylation changes associated with prenatal mercury exposure and cognitive performance during childhood
Andres Cardenas[1], Sheryl L. Rifas-Shiman[2], Golareh Agha[3], Marie-France Hivert[4], Augusto A. Litonjua[5], Dawn L. DeMeo[5], Xihong Lin[6], Chitra J. Amarasiriwardena[3], Emily Oken[2], Matthew W. Gillman[2,7] & Andrea A. Baccarelli[3]

Toxic exposure

PLOS ONE
DNA Methylation Signatures Triggered by Prenatal Maternal Stress Exposure to a Natural Disaster: Project Ice Storm
Lei Cao-Lei[1], Renaud Massart[1], Matthew J. Suderman[2], Ziv Machnes[2], Guillaume Elgbeili[4], David P. Laplante[4], Moshe Szyf[2]*, Suzanne King[1]

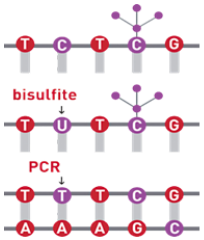
Maternal stress

How analyze the DNA methylation ?
A large panel of methodologies, at different scales

A large panel of methodologies to analyse the DNA methylation

DNA methylation

- Native genomic DNA
  - Global analysis
    - **Descriptive analysis:** Antibody –based detection in cells and tissue-sections
    - **Quantitative analysis:**
      - **All CpGs:** Mass Spectrometry
      - **Selected CpGs methyl sensible enzymatic cleavage - quantification:** LUMA
  - Sequence specific analysis
    - **Selected CpGs:** Methyl sensible Enzymatic cleavage, electrophoressis, PCR
  - Genomic region Enrichment
    - **MeDip-seq, MeDIP-Chip**
    - **Methyl-CpG binding Domain sequencing (MBD-seq)**
- Bisulfite converted DNA
  - Quantification at CpG-level resolution
    - Genome wide analysis
      - **Whole-Genome Bisulfite sequencing (WGBS)**
      - **Reduced Representation Bisulfite Sequencing (RRBS)**
      - **Infinium array hybridization** (exclusively human and rodent; Illumina)
    - Locus specific
      - **Locus specific:**
        - Single CpG resolution - PCR and pyrosequencing
        - Methyl-specific PCR
        - Methyl-Specific PCR (QM-MSP) assay
        - Methyl-Sensitive High Resolution Melting (MS-HRM)
      - **Highly multiplexed targeted regions**
        - Microdroplet PCR for Highly Multiplexed targeted bisulfite sequencing
        - Targeted Bisulfite Sequencing using SeqCapEpi enrichment system
        - Multiplexed MSRE-qPCR



Unmethylated C converted in T
Methyl protects again desamination
5meC / C analyzable as a single SNP (C/T)

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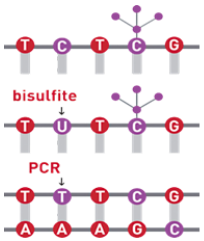

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Part II – « LongHealth »

INRA's Metaprogram leading by Pierre GERMON

2017-2020

Integrated management of ruminant health for a sustainable dairy production

LongHealth

Objectives

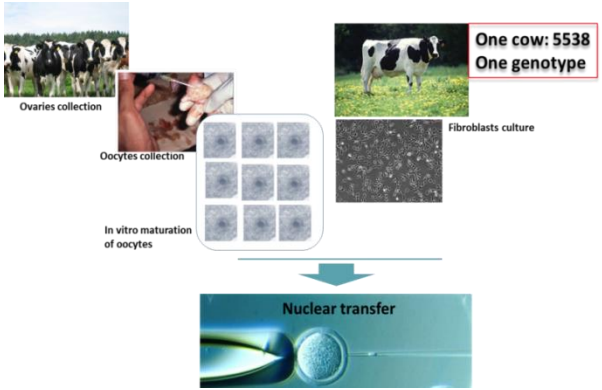
- to investigate the **trade-offs** between **health traits, growth** and **milk production** in **biological** and **economical** terms (WP1)
- to investigate the **trade-offs between health, production and welfare** using monitoring tools newly available among **precision livestock farming** solution (WP2)
- to decipher how **interactions** between **genetics** and **epigenetics** when either environmental changes (nutrition) or physiological changes (age) modulate the **response to infection** (WP3)

➢ **To explore the aging effect on monocyte methylome**

An original model to explore the aging effect on monocyte methylome

- **Cloned animals from the same cell line**

- Each somatic nucleus is transfered in a single oocyte
- Epigenetic reprogramming drived by oocyte competences allows the embryonic development



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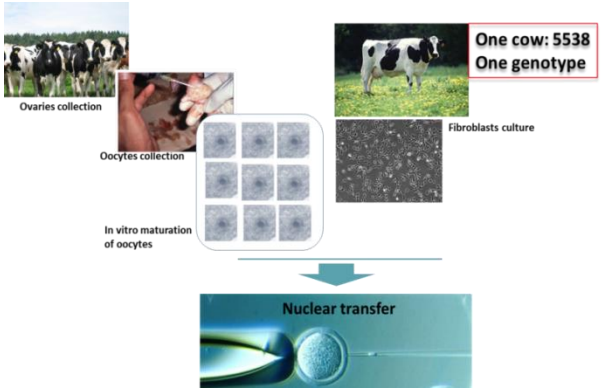


- **Two groups of cloned animals with different ages, managed in the same farm under the same conditions**

Holstein females generated
by **somatic cell nuclear transfer** (SCNT)

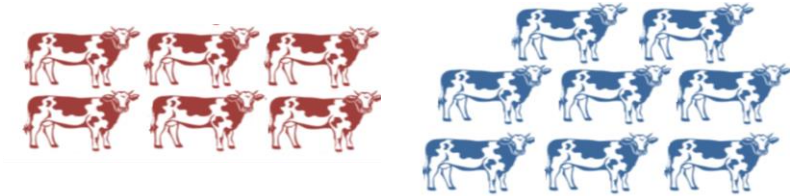
n= 6 n= 8

Experimental design

ONT

YNT

Constant genotype



Hormonal synchronisation

Ovulation control by ultrasound → Efficiency 100%

Blood sampling at D15 (at 8 am, before feeding)

Experimental design

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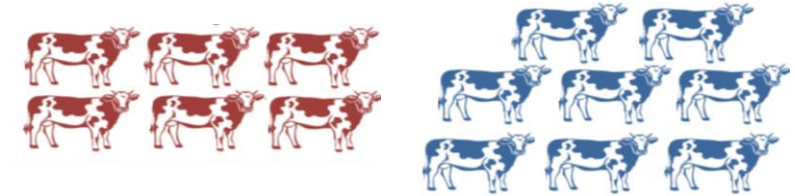

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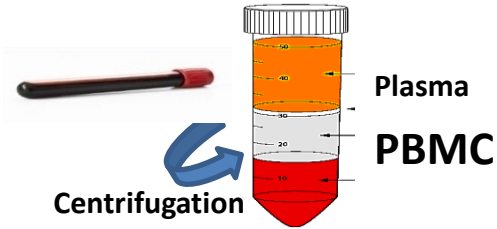


Ficoll gradient

Monocytes selection
Magnetic beads coated with anti CD14+ antibody

DNA extraction

RRBS library construction

Pan genomic DNA methylation analysis, construction of RRBS library

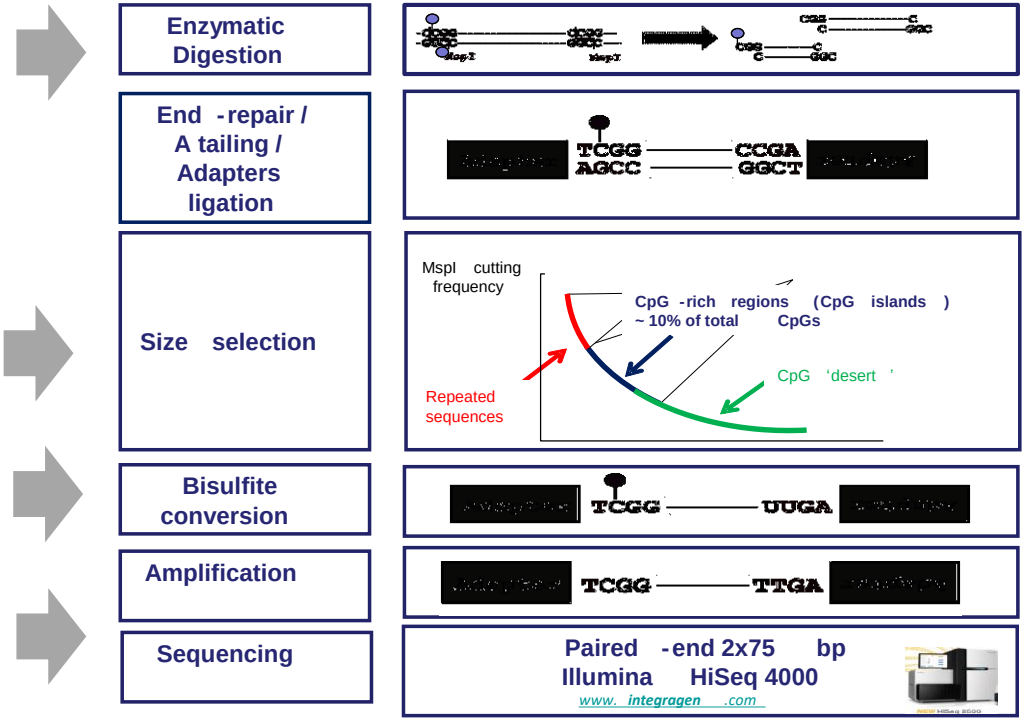
- Targeting of CCGG sites

- Selection of 40-290 base fragments
- Only a small part of genome (3%)
- Representative of CpG rich regions

- Transformation of epigenetic mark as SNP

- Limited amplification
- Sequenced using HiSeq4000

(Integragen, Evry, France



(Gu et al., 2011)

Overview of the bioinformatic pipeline, including quality controls

Perrier et al., 2018

1

Trimming
Trim Galore

- Suppression of adapters and nucleotides incorporated during the end-repair step
- Suppression of bad quality nucleotides (phred 33)
- Suppression of short reads (<20 bp)

- **Only sequences with high quality are selected**

2

Read Alignment
Bismark

Krueger F. et al., 2011

- **ARS-UCD1.2** as bovine genome reference

- **The alignement Is performed with Bismark software**
- **Only the uniquely mapped reads are considered**

3

Methylation extraction
Bismark

- Selection of CpGs with appropriate sequencing depth (10 to 500 x)
- Counting of C/T polymorphisms

4

Identification of DMCs
MethylKit

Akalin *et al.*, 2012

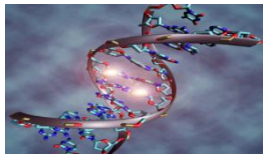
- qvalue<0.001
- Minimum of 25% methylation difference between two conditions
- DMCs can be aggregated into DMRs (home made script)

5

Annotation of DMCs and DMRs
Homemade script integrating

http://homer.salk.edu/homer/ngs/annotation.html

- Genes, CpG islands, and repeats associated with DMCs/DMRs



« Longhealth » RRBS libraries construction

Perrier et al, 2018

Adaptation from method previously described

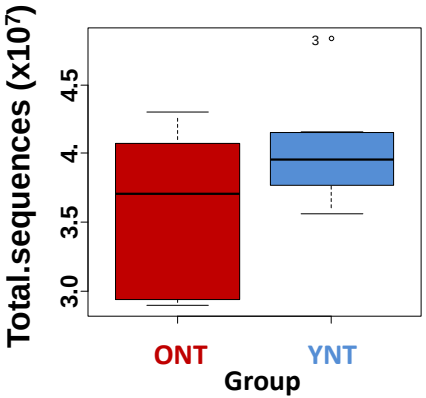
Semi-automatized method / Size selection of fragments using magnetic beads

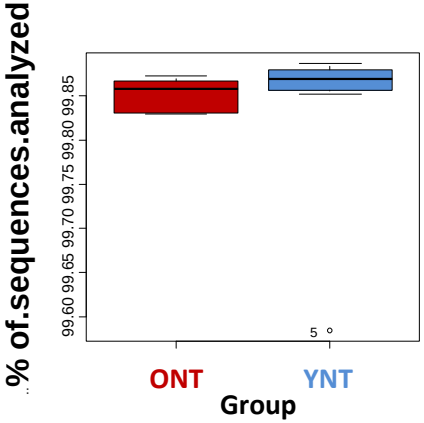
Sequencing HiSeq 4000

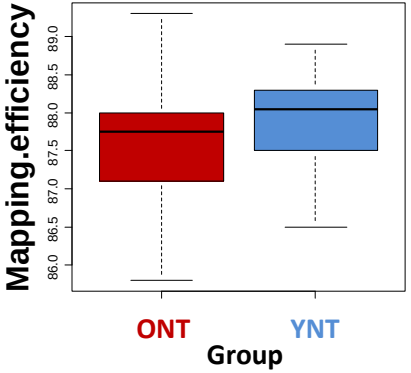
Service compagny , Integragen, Evry France

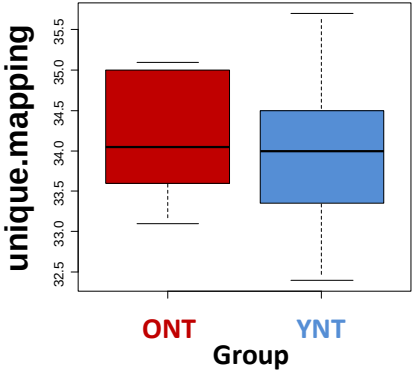
Overview of basic RRBS statistics (1)

- 39 millions of sequences/library
- 99.8% of concerved sequences after triming
- 88% of mapping efficiency
- 34% of unique mapping





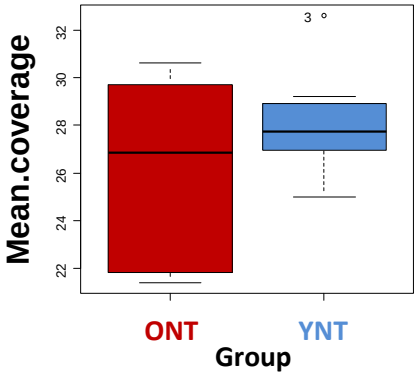




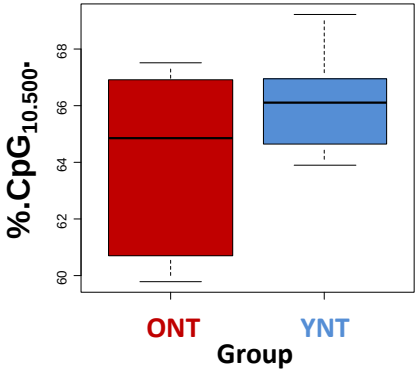
- High number of sequences with a high quality index
- High mapping efficiency
- Low unique mapping due to surabondance of repeat sequences in bovine genome
- No difference between groups

Overview of basic RRBS statistics (2)

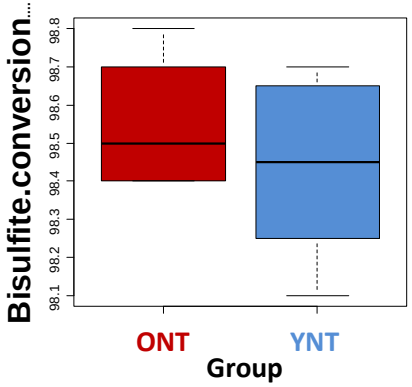
- Mean coverage of 27.7



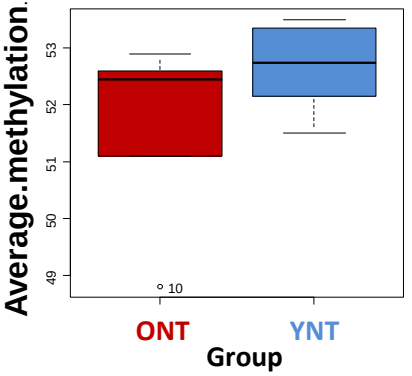
- 66 % of selected CpG with a coverage [10-500]



- 98.5% effeciency of bisulfite conversion



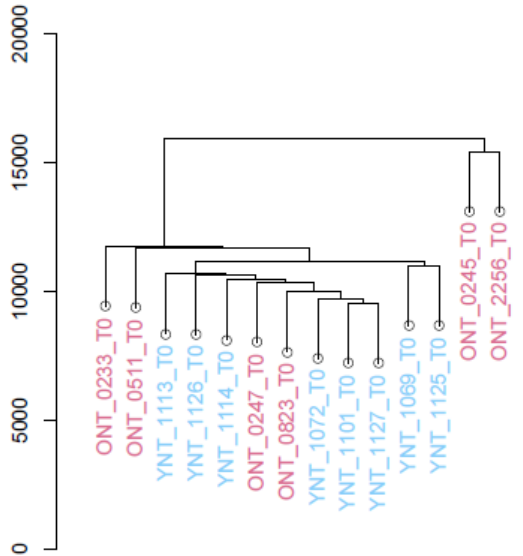
- 52.5 % of global methylation No significant difference



- Mean coverage of 27.7
- Average of 1.8 million of $CpG_{10\text{-}500}$ selected
- Good bisulfite conversion efficiency
- No difference of average of methylation on $CpG_{10\text{-}500}$ selected between groups

Descriptive analysis

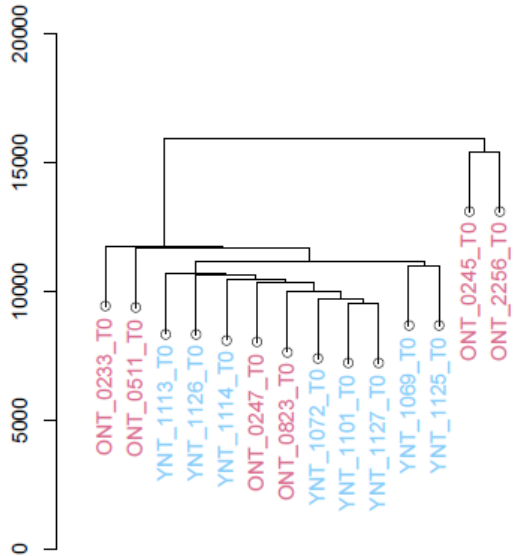
Analysing 1.8 millions of $CpG_{10\text{-}500}$



- **No clear separation between old and young groups in accordance with the global methylation %**

Descriptive analysis

Analysing 1.8 millions of $CpG_{10\text{-}500}$

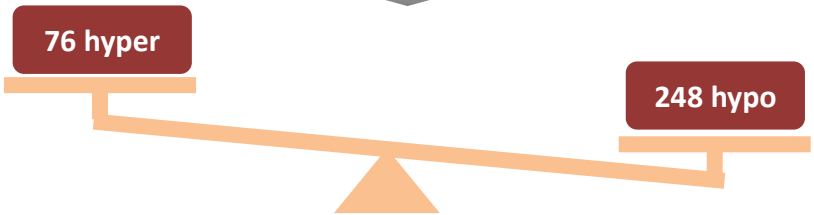


- No clear separation between old and young groups in accordance with the global methylation %

Differential analysis

Methylkit analysis
25% of methylation difference

324 DMCs

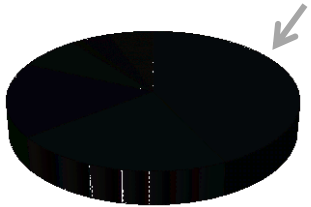


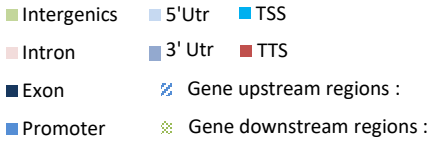
- A limited number of DMCs
- Mainly hypomethylated in ONT group

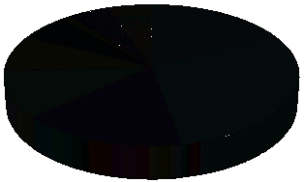
324 DMCs exhibit a specific genomic distribution

- Reference: - 1.8 million of $CpG_{10\text{-}500}$

Gene feature

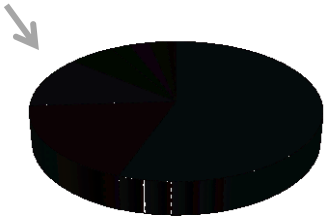


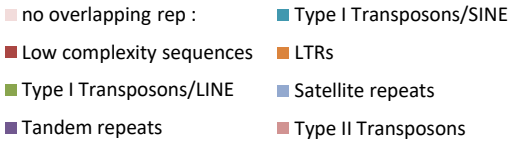


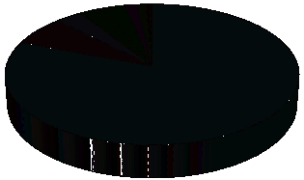


- More associated with intergenic regions (42.9% vs 17.4%) in detriment of genic regions
- Mainly hypomethylated in ONT group (94%)

Repeat sequences







- More associated with repeat sequences (44.4% vs 19.3%)
- Mainly hypomethylated in ONT group (99%)

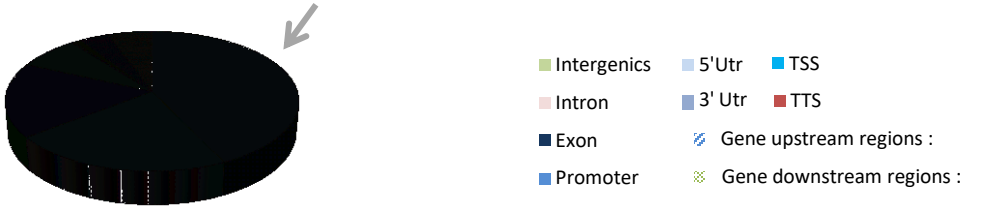
- Age effect is a global hypomethylation events are enriched for repetitive sequences and for intergenic regions
- Thought to be responsible for the reactivation of retrotransposon elements and genome instability
- According to previous data published in human.

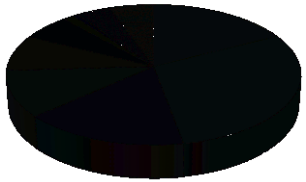
Jung and Pfeifer *BMC Biology* (2015) 13:7
DOI 10.1186/s12915-015-0118-4

Rare DMCs are associated with gene features

- Reference: - 1.8 million of $CpG_{10\text{-}500}$

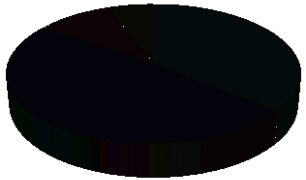
Gene feature





- More associated with intergenic regions (42.9% vs 17.4%) in detriment of genic regions

CpG islands

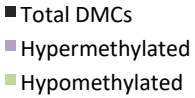


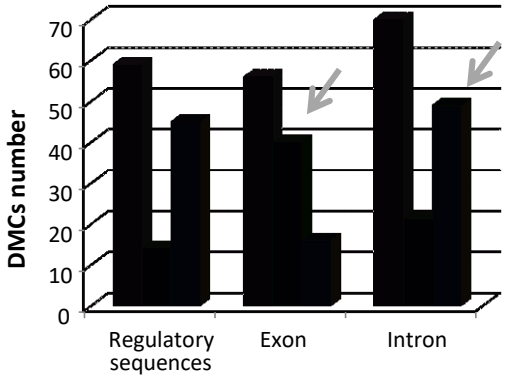
- No difference

185 DMCs targeted 112 unique genes

in ONT group

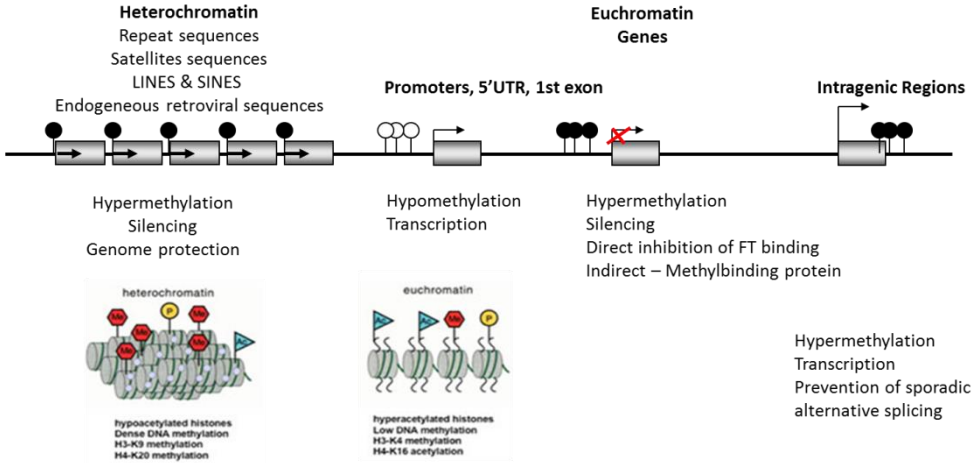
- More associated with intronic regions and mainly hypomethylated
- DMCs associated with exonic regions and mainly hypermethylated





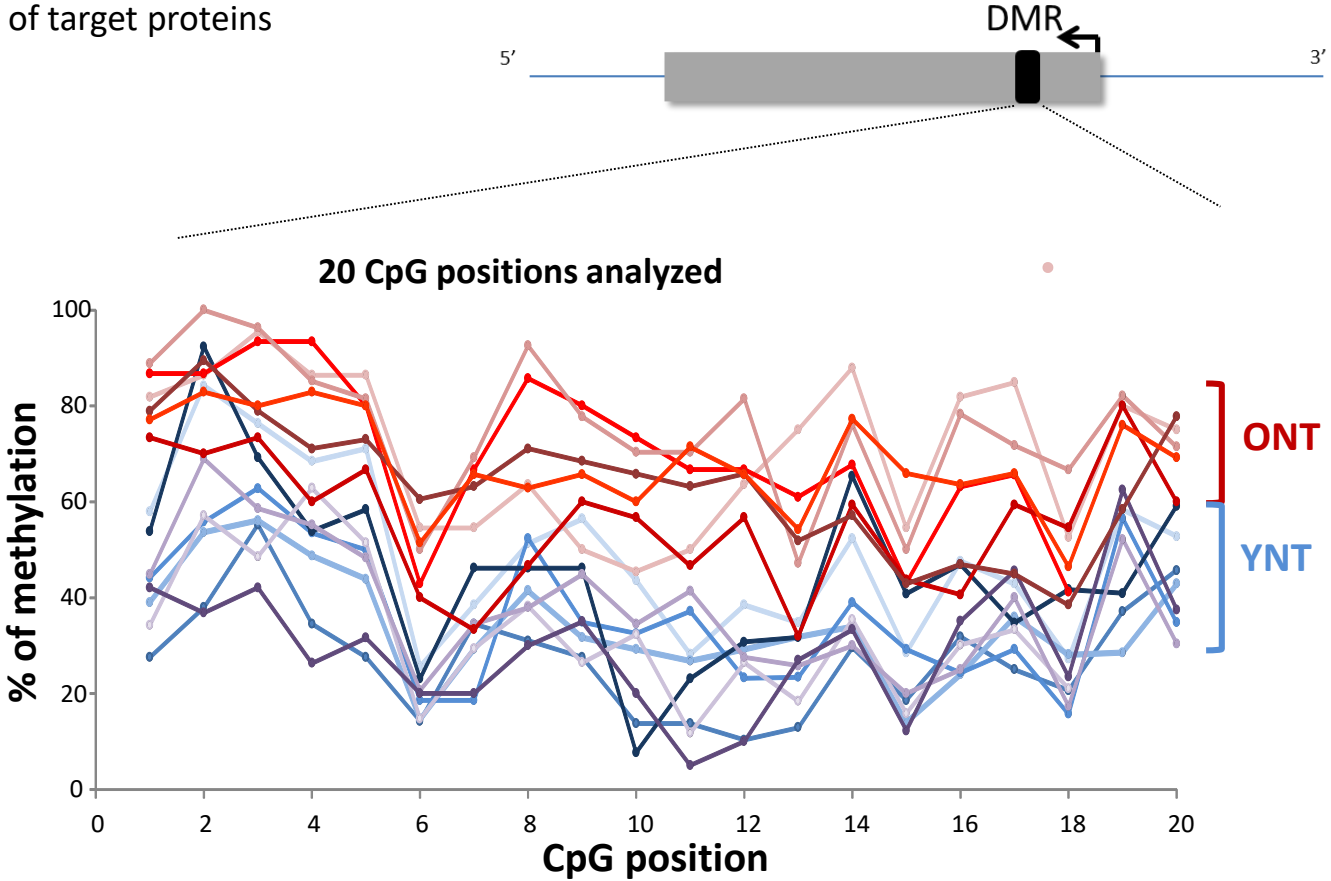
DMCs could be aggregated in DMRs
Only 12 genes are targeted by a DMR

Chromosome	Methylation in ONT	Gene ID	Gene feature	Gene name	Gene description	CpG island
5	Hypomethylated	ENSBTAG00000000507	exon	NR4A1	Bos taurus nuclear receptor subfamily 4 group A member 1 (NR4A1), mRNA.	
5	Hypomethylated	ENSBTAG00000008036	intron	CELSR1	cadherin EGF LAG seven-pass G-type receptor 1	shore
6	Hypomethylated	ENSBTAG00000013980	exon	SOD3	superoxide dismutase 3	island
12	Hypomethylated	ENSBTAG00000008656	promoter	KBTBD6	kelch repeat and BTB domain containing 6	island
3	Hypermethylated	ENSBTAG00000014132	exon	SNED1	sushi, nidogen and EGF like domains 1	island
7	Hypermethylated	ENSBTAG00000017349	intron	PCDHGA8	Bos taurus protocadherin gamma subfamily B, 4 (PCDHGB4), mRNA.	island
10	Hypermethylated	ENSBTAG00000025329	exon	IRF2BPL	interferon regulatory factor 2 binding protein like	island
12	Hypermethylated	ENSBTAG00000022991	intron	NBEA	neurobeachin	island
12	Hypermethylated	ENSBTAG00000034069	promoter	MAB21L1	Bos taurus mab-21 like 1 (MAB21L1), mRNA.	island
12	Hypermethylated	ENSBTAG00000012019	exon	IRS2	insulin receptor substrate 2	island
13	Hypermethylated	ENSBTAG00000044047		SKIDA1	SKI/DACH domain containing 1	island
16	Hypermethylated	ENSBTAG00000006515	intron	ESPN	espin	island



IRF2BPL

encodes E3 ubiquitin protein ligase involved in the proteasome-mediated ubiquitin-dependent degradation of target proteins



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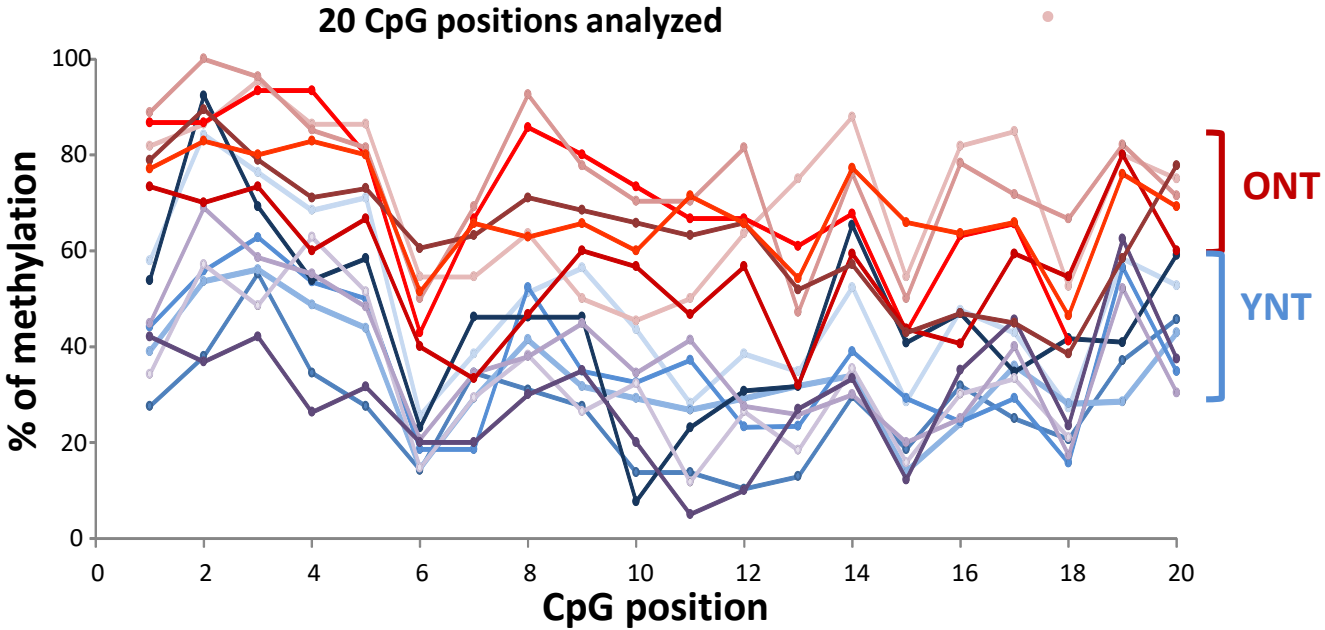

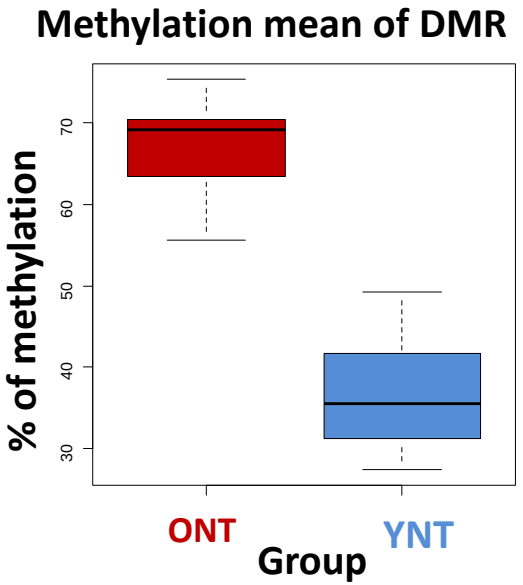

Approximative Kruskal-Wallis Test
p-value = 0.000645

Conclusions

Using original model of cloned animals,

Aging affects

- **a limited set of CpG positions**
- **associated with intergenic regions and repeat regions**
- **mainly hypomethylated**

Reactivation of retrotransposon elements
Induction the genome instability

Aging targets

- **a limited set of genes**
- **DMRs associated with intronic region than exonic or regulatory regions**

Function of some genes makes sense with diseases development
Epigenetic drift → de differentiation of cells

More investigations

- **Correlation between alteration of methylation of DMRs and gene expression**
- **Other epigenetic marks associated with these DMRs (Histone modifications, Chip-seq PCR)**

Conclusions

To continue to identify DMCs as biomarkers in various conditions

- **in response to inflammatory challenge**
- **at different physiological stages**
- **after diet changes…**
- **in association with different traits (fertility, milk production…)**

In female and in male

To develop new tools to rountinely analyze this individual variability of epigenome

- **usefull to better determine the health status of animals**
- **used as new phenotypic parameters to improve the GWAS study**

The team

- **Hélène Kiefer**
- **Aurélie Chaulot-Talmon**
- **Charline Pontlevoy**
- **Anne Gabory**
- **Mélanie Jouin**
- **Luc Jouneau & Anne Aubert** - Bioinformatics and statistics

INRA experimental farm

- **Christophe Richard**
- **Valérie Gélin**

Collaboration

- **Gilles Foucras**

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Epigenetics integrates a part of environment

Phenotype = Genotype + Epigenotype + Environment $+I_{GEnv} +I_{GEp} + I_{GEnvEp}$