

# Cancer Epigenetics



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## I. Epigenetics: Essential Notions



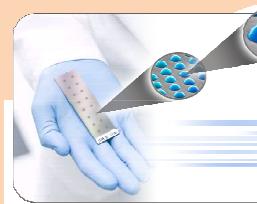
## II. Epigenetics & Cancers



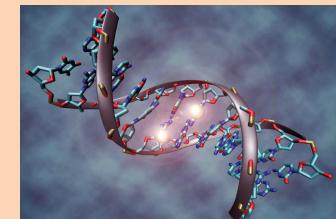
## III. Epigenomics is coming of age **(technologies)**



## IV. Applications/ Translational Cancer Epigenomics



## V. New Epigenetics field



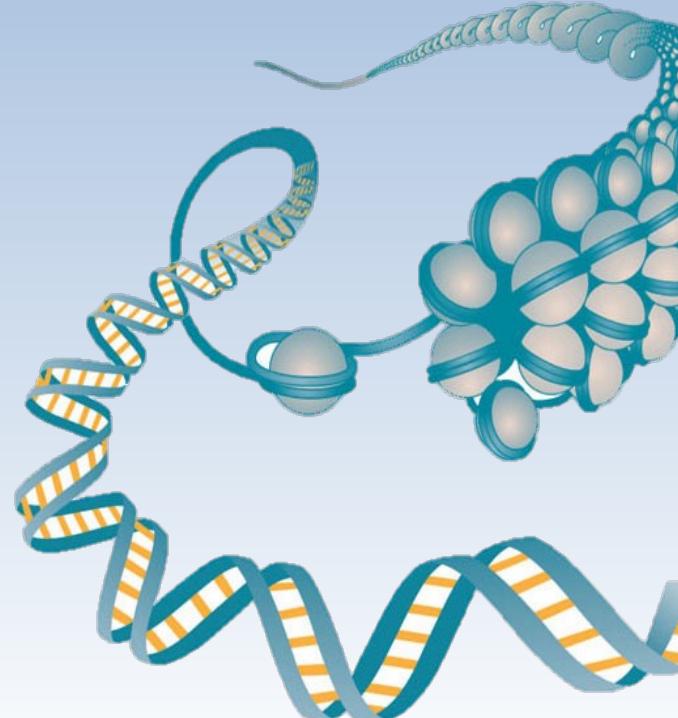
# I. Epigenetics: Essential Notions



## Basic Facts



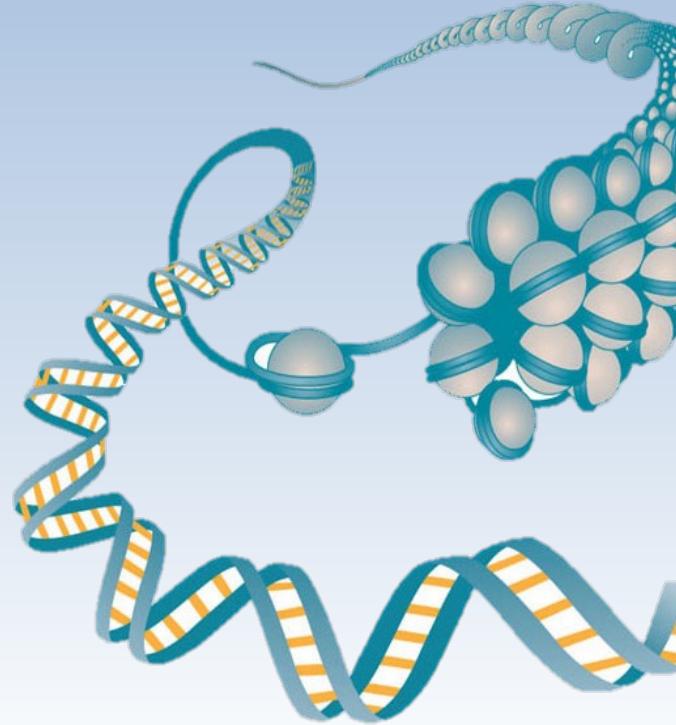
## Epigenetics Actors



## Epigenetics pathologies



# Basics Facts



# Notions of Epigenetics

**Genetic= Cellular manual**

In recent years epigenetic alterations have come to prominence in cancer research in particular by hypermethylation of CpG islands located in the promoter regions of tumor-suppressor genes is now firmly established as an important mechanism for gene inactivation in cancer. One of the most remarkable achievements in the field has been the identification of the methyl-CpG-binding domain family of proteins which provide mechanistic links between specific patterns of DNA methylation and histone modifications.

**Epigenetic= how to read the manual**

In recent years, epigenetic alterations have come to prominence in cancer research. In particular, hypermethylation of CpG islands located in the promoter regions of tumor-suppressor genes is now firmly established as an important mechanism for gene inactivation in cancer. One of the most remarkable achievements in the field has been the identification of the methyl-CpG-binding domain family of proteins, which provide mechanistic links between specific patterns of DNA methylation and histone modifications.

**Woman without her man is nothing**

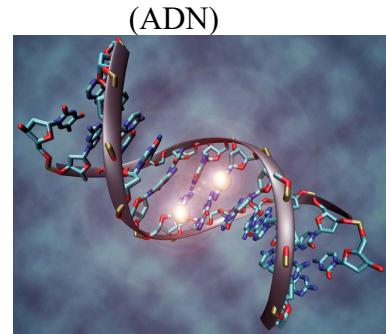
**Woman, without her, man is nothing**

**Punctuation is important!!!**

# Definitions of Epigenetics

A NEW FRONTIER!

**GENETICS**

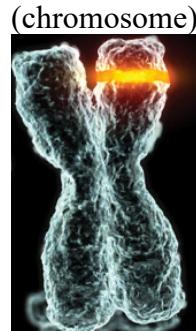


Genetic Code

→ 4 letters/  
nucleotides (ATGC)

*Dressing of our chromosomes/genes: by epigenetics*

**EPIGENETICS**

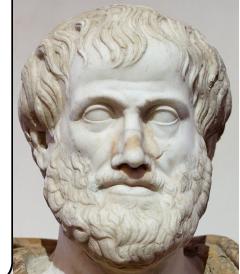


→ 5<sup>e</sup> letter/  
nucleotide (5mC)

# Definitions of Epigenetics

Aristotle, 384-322 BC:

*“... Epigenesis ... development of individual organic form from unformed”*



Conrad Waddington, 1942:

*“... is the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being”*



Arthur Riggs, 1996:

*“...is the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence”*



# Definitions of Epigenetics

More recently, Denise Barlow (Vienna):

*“... Epigenetics has always been all the weird and wonderful things that cannot be explained by genetics”*

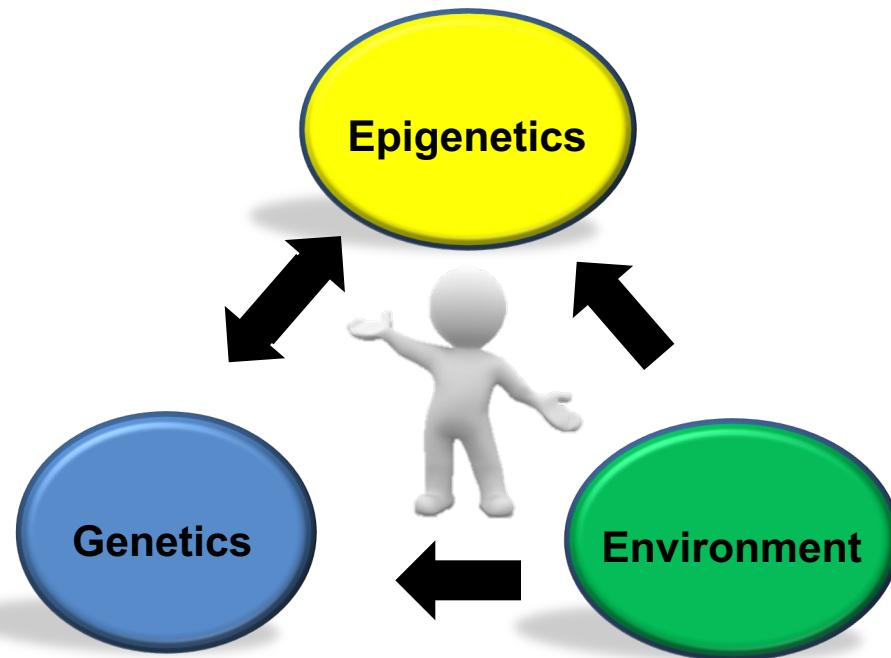


✓ Not DNA Sequence  
("epi")

✓ Heritable

✓ Influence Gene  
Function

# Influence of our environment/life style

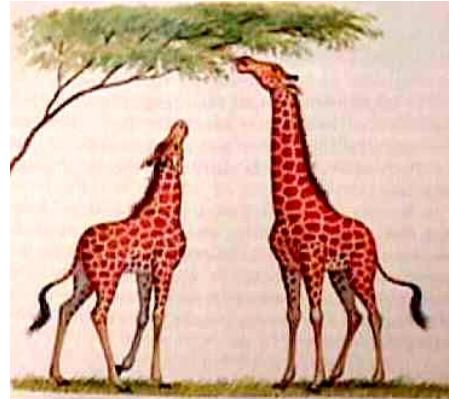


# Influence of our environment/life style



# Influence of our environment/life style

Lamarckism's revival...



# Influence of our environment/life style

## Why identical twins are not the same?

EPGENETICS

Identical twins: epigenetics makes the difference



# Influence of our environment/life style

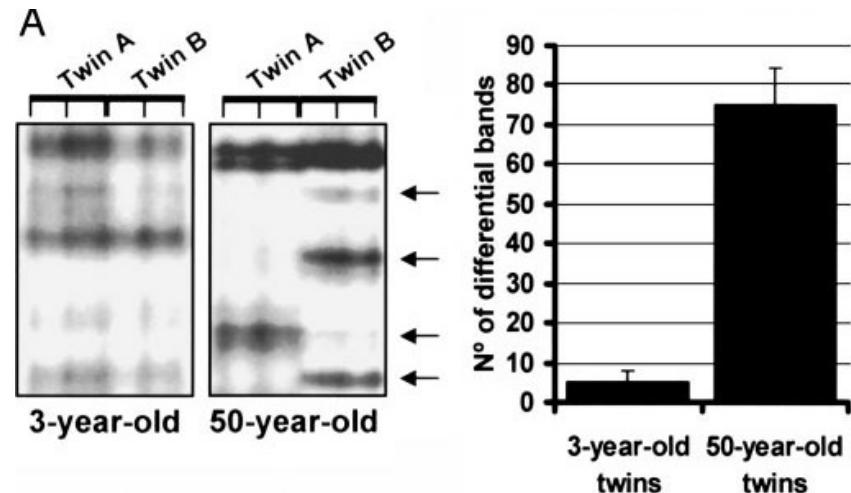
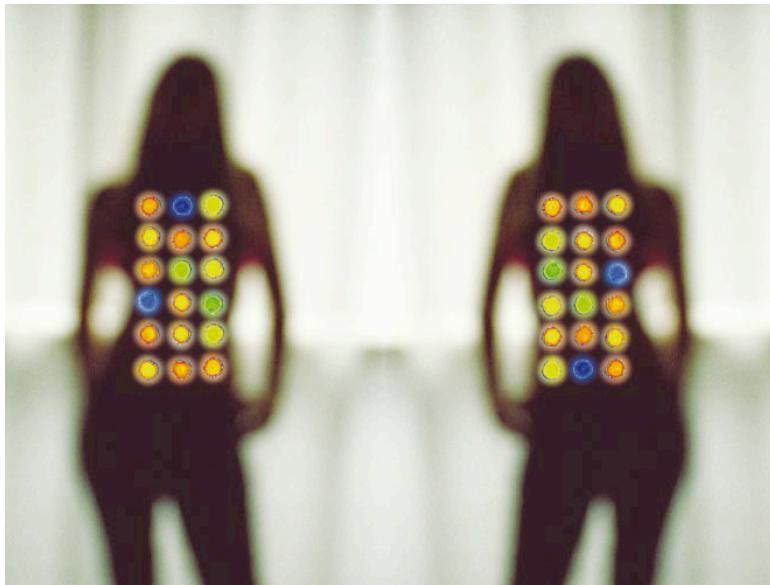
PNAS

## Epigenetic differences arise during the lifetime of monozygotic twins

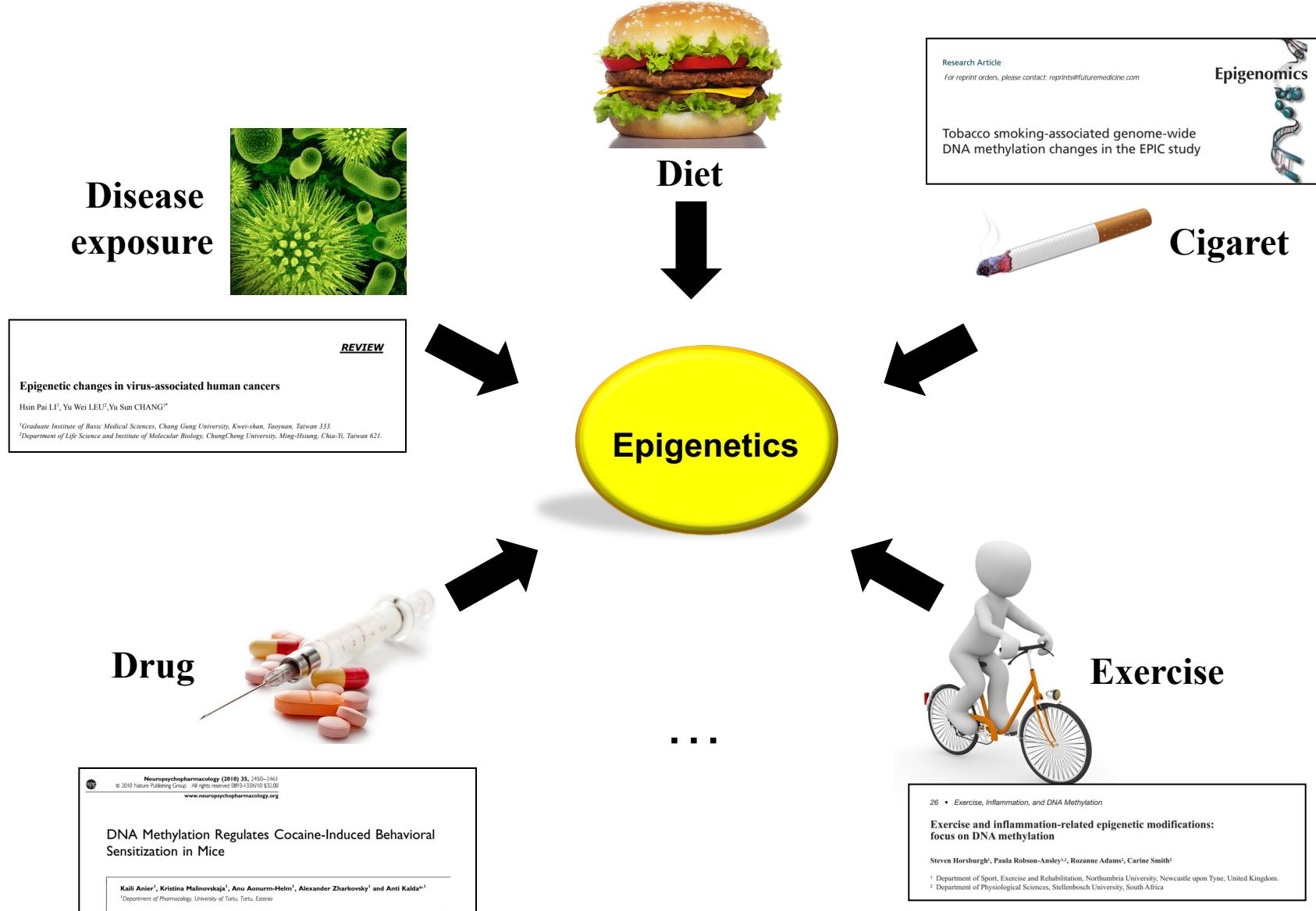
Mario F. Fraga\*, Esteban Ballestar\*, María F. Paz\*, Santiago Ropero\*, Fernando Setien\*, María L. Ballestar†,  
Damià Heine-Suñer†, Juan C. Cigudosa§, Miguel Urioste||, Javier Benítez||, Manuel Boix-Chornet†,  
Abel Sanchez-Aguilera†, Charlotte Ling||, Emma Carlsson||, Pernille Poulsen\*\*, Allan 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 Fernández 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 Diabetes Center, 2820 Gentofte, Denmark; ††Twin Research and Genetic Epidemiology Unit, St. Thomas' Hospital, London SE1 7EH, United Kingdom; and  
‡‡Human Cancer Genetics Program, Department of Molecular Virology, Immunology, and Medical Genetics, Ohio State University, Columbus, OH 43210



# Influence of our environment/life style



# Influence of our environment/life style

nature

Vol 456 | 6 November 2008

## RESEARCH HIGHLIGHTS

### Famine's shadow

*Proc. Natl Acad. Sci. USA* doi:10.1073/pnas.0806560105 (2008)

If a starving woman becomes pregnant, her child's DNA can still bear traces of her hunger more than six decades later.

Lambert Lumey of Columbia University in New York, Bastiaan Heijmans of Leiden University Medical Center in the Netherlands and their colleagues studied the methyl groups attached to a gene called *IFG2*. They measured methylation at five points along *IFG2* in people prenatally exposed to the 1944–45 Dutch famine — when a Nazi embargo led to food rationing in the west of Holland of fewer than 700 calories a day.

Compared with same-sex siblings conceived when the same mothers had more flesh on their bones, those affected early in fetal development have less methylation on *IFG2* today, implying that their cells express it more readily.



Early-life environmental conditions can cause epigenetic changes in humans that persist throughout life!

# Central roles in biology and medicine



~80 000 genes



~15 000 genes



~20 000 genes

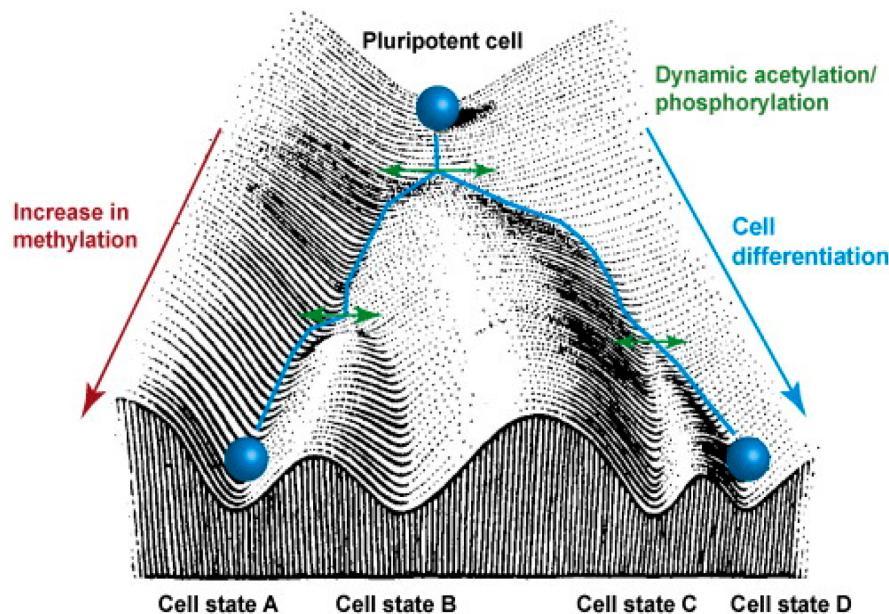
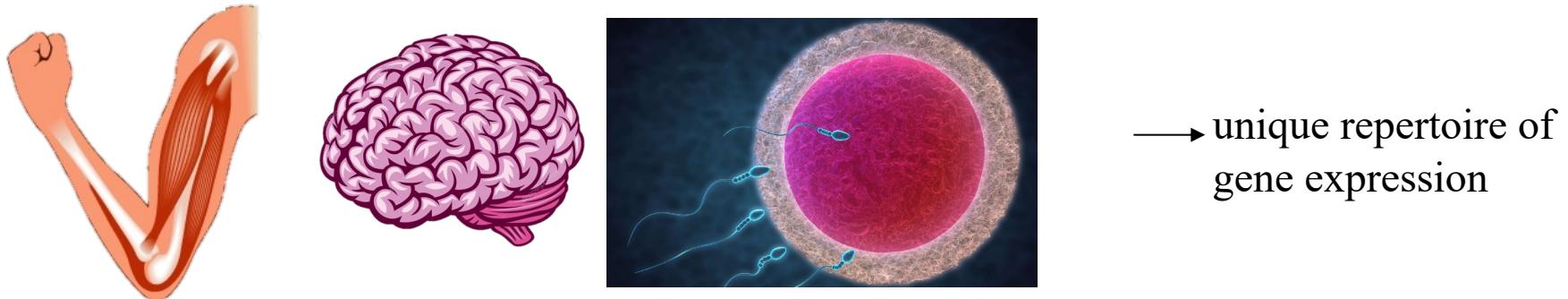
**« We are more than the sum of our genes! »**



While the average human-chimpanzee divergence is ~1% across the genome,  
at CpG sites it increases to ~15%

# Central roles in biology and medicine

Our body=more than 250 cell types with the same genome

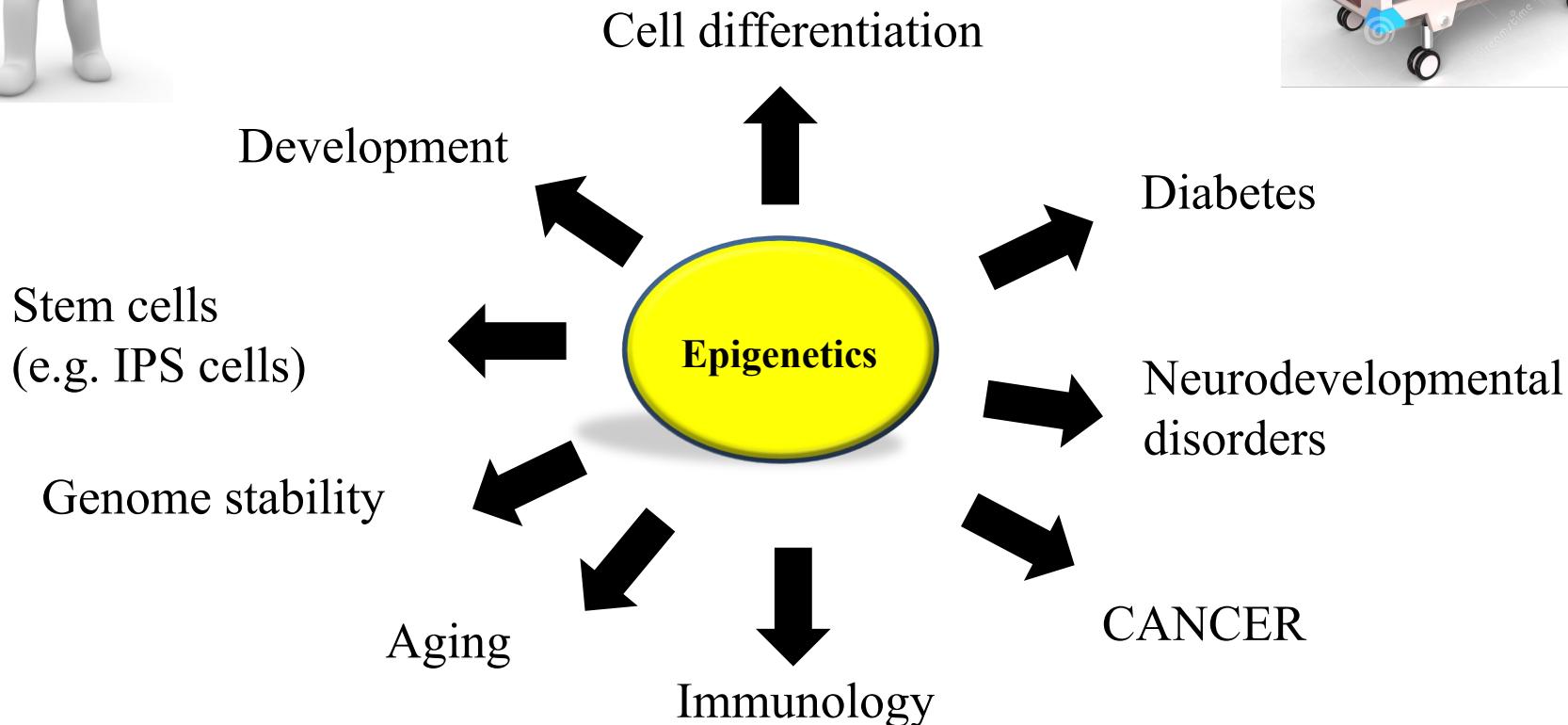


# Central roles in biology and medicine



In Health

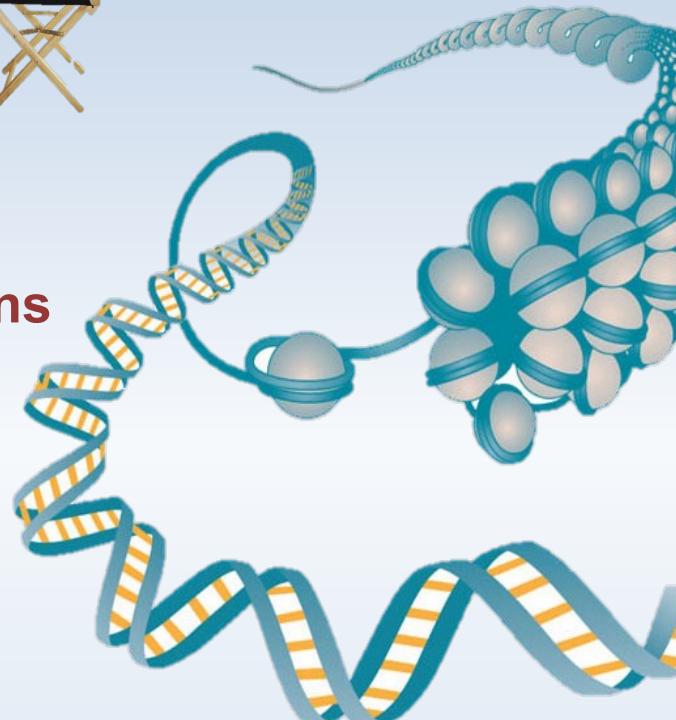
In Disease



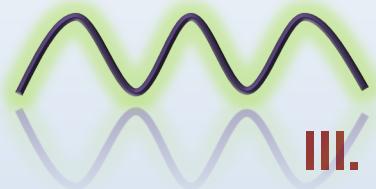
# Epigenetic Actors



I. Histone modifications



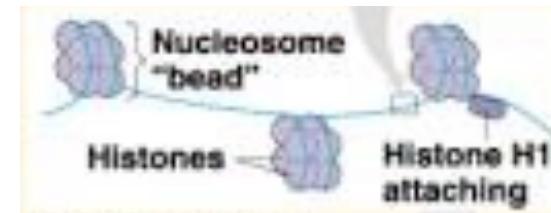
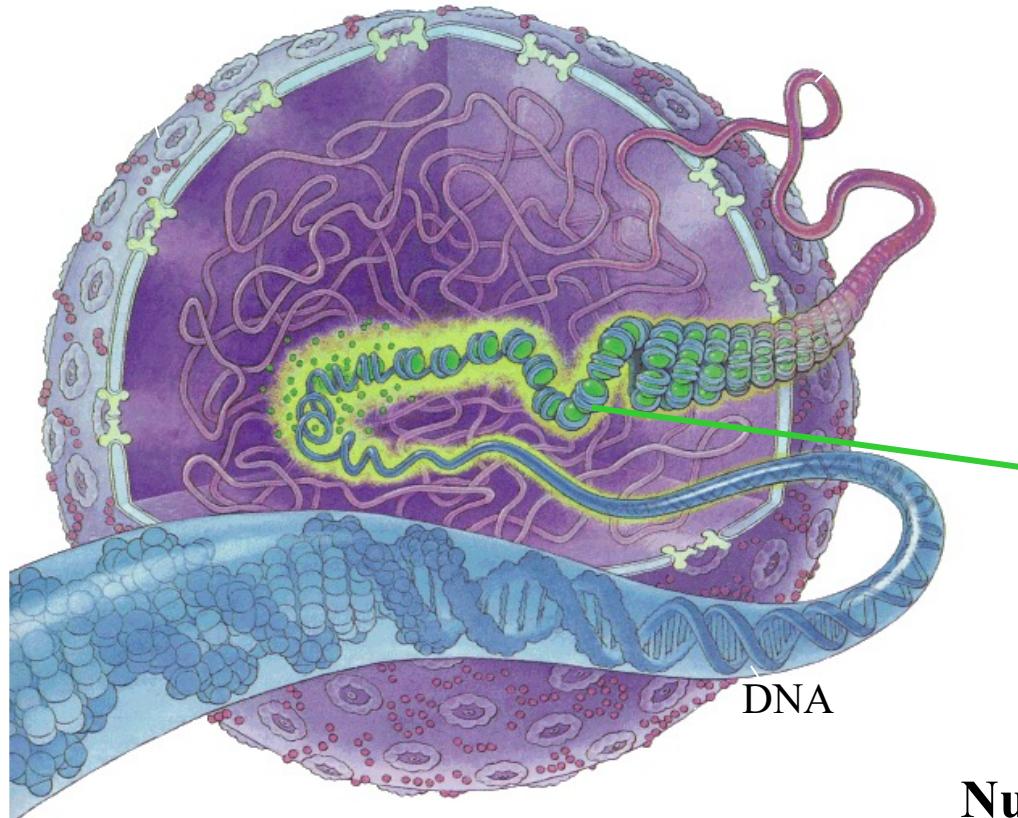
II. DNA modifications



III. Non coding RNAs

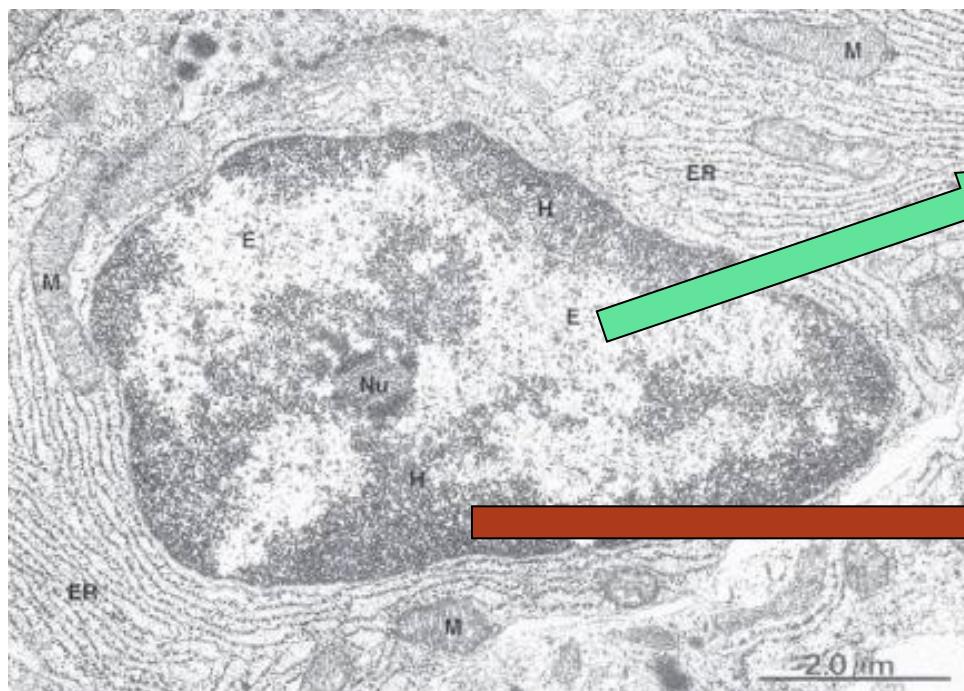
IV. New epigenetic layer

# In nucleus, chromatin compacts genome

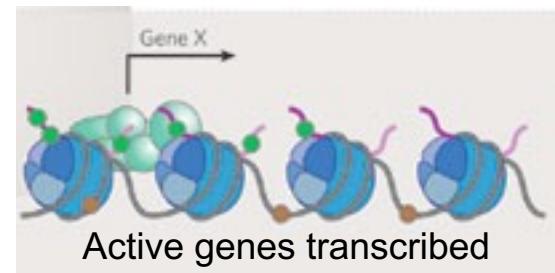


**Nucleosome:** fundamental unit of chromatin (DNA + histones)

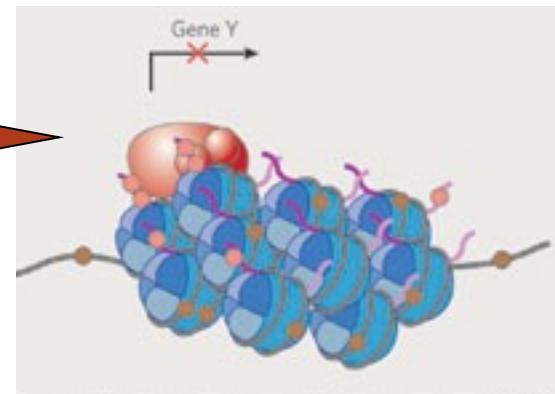
# Chromatin



Euchromatin



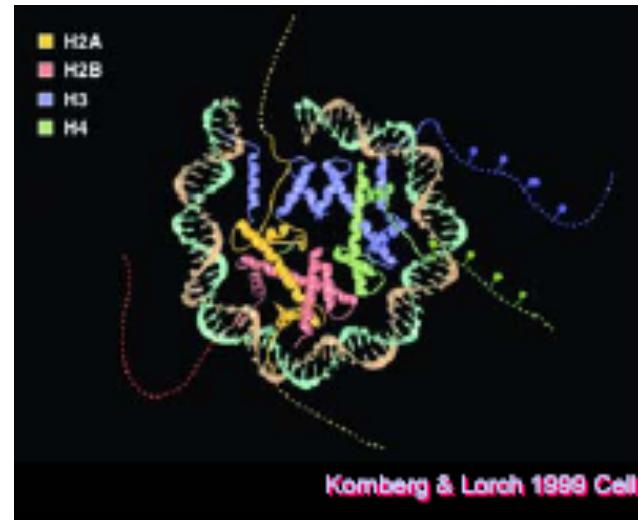
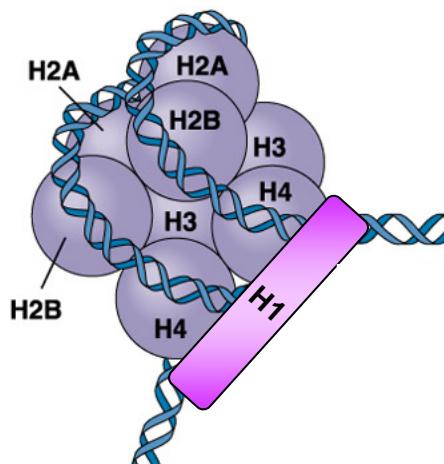
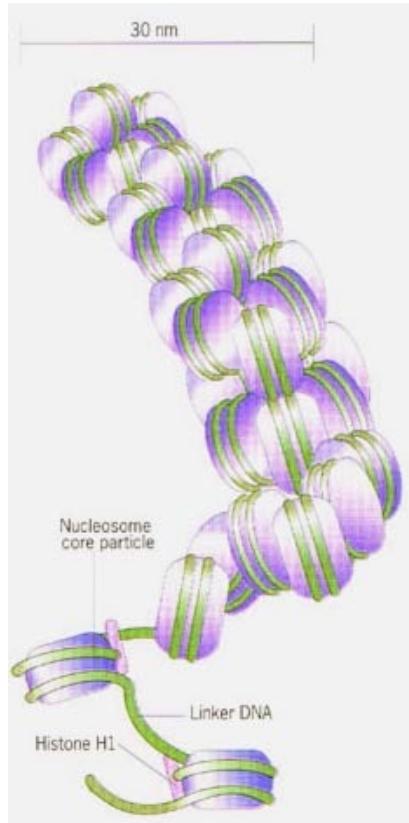
Heterochromatin



Silenced Genes, repressed

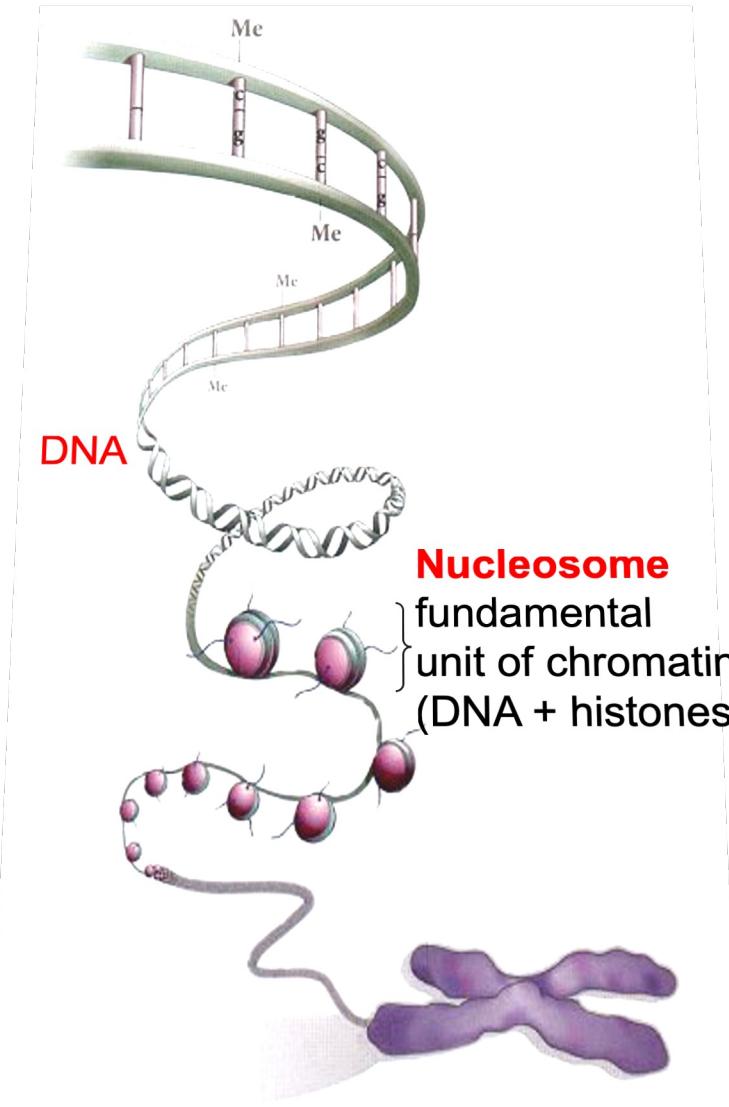
# Chromatin

## The Nucleosome



147bp of DNA rolled up around an histone octamere  
The histone H1 compacts the structure

# Chromatin



- Several degrees of chromatin compaction

- This compaction is DYNAMIC & influences gene expression:

OPEN chromatin → genes ON

CLOSED chromatin → genes OFF

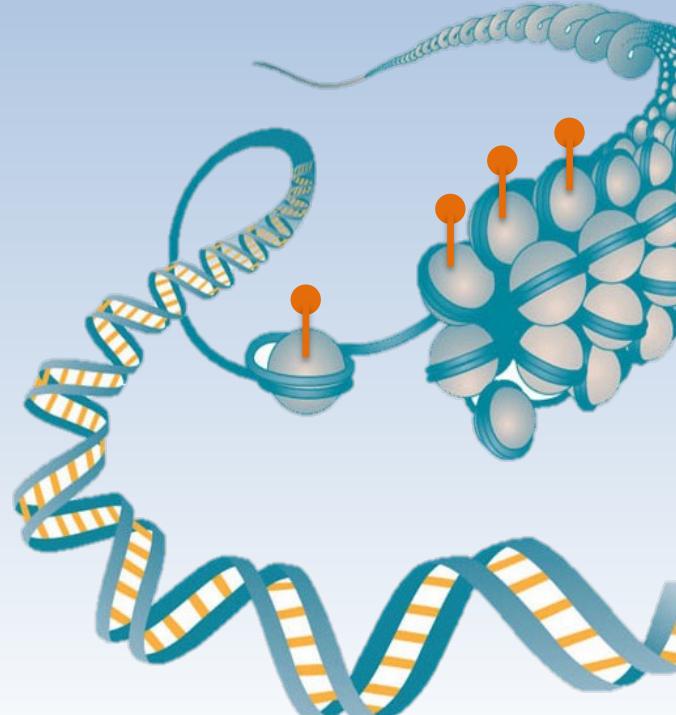
Chromatin



regulated by epigenetic modifications



*"chromatin modifying enzymes"*



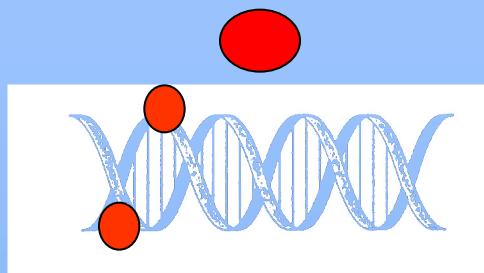
# Histone modifications

# Histone modifications

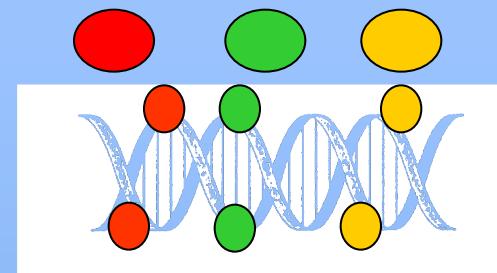
Increasing epigenetics complexity

## 1. First layer/modification

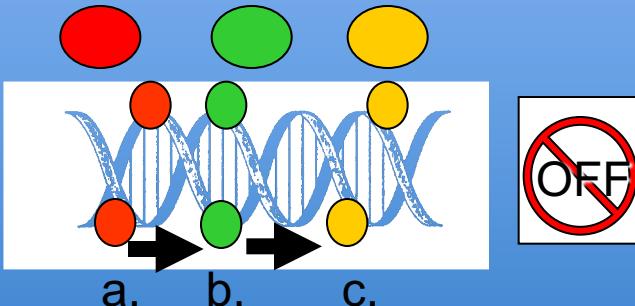
(1996)



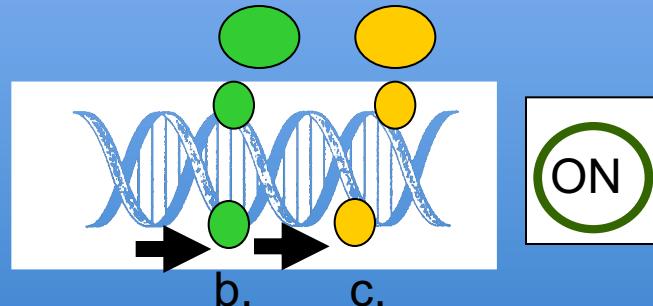
## 2. Multitude of modifications



## 3. Interconnections/Interdependancies



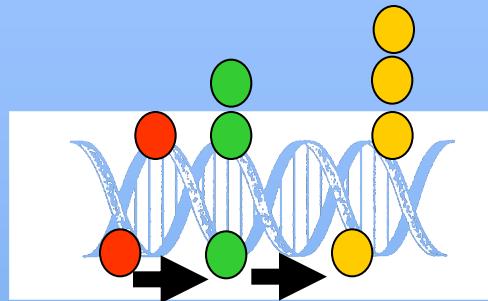
## 4. COMBINATIONS determine gene expression



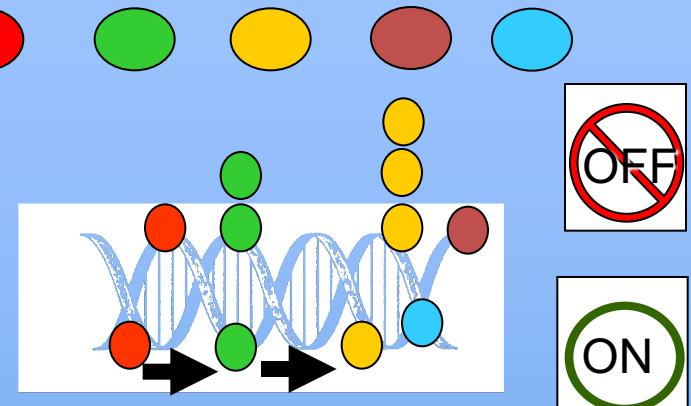
# Histone modifications

Increasing epigenetics complexity

5. Mono,di,tri-methylation (2003)



6. New modifications (2004-2008, ...)



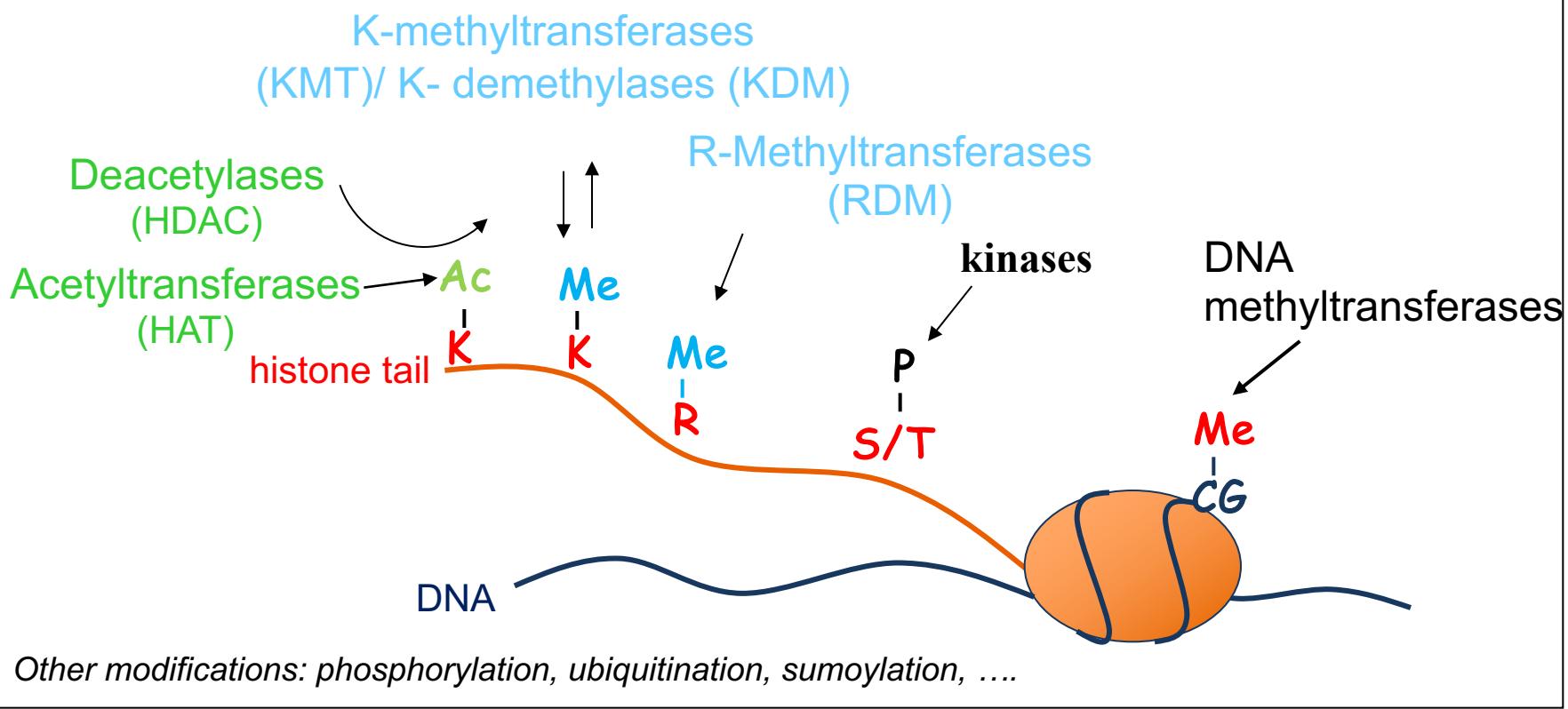
GENE EXPRESSION / EXTEND INFORMATION

HISTONE CODE

HOW TO PREDICT HISTONE COMBINATION AND GENE EXPRESSION  
OUTPUT/BIOLOGICAL CONSEQUENCES?

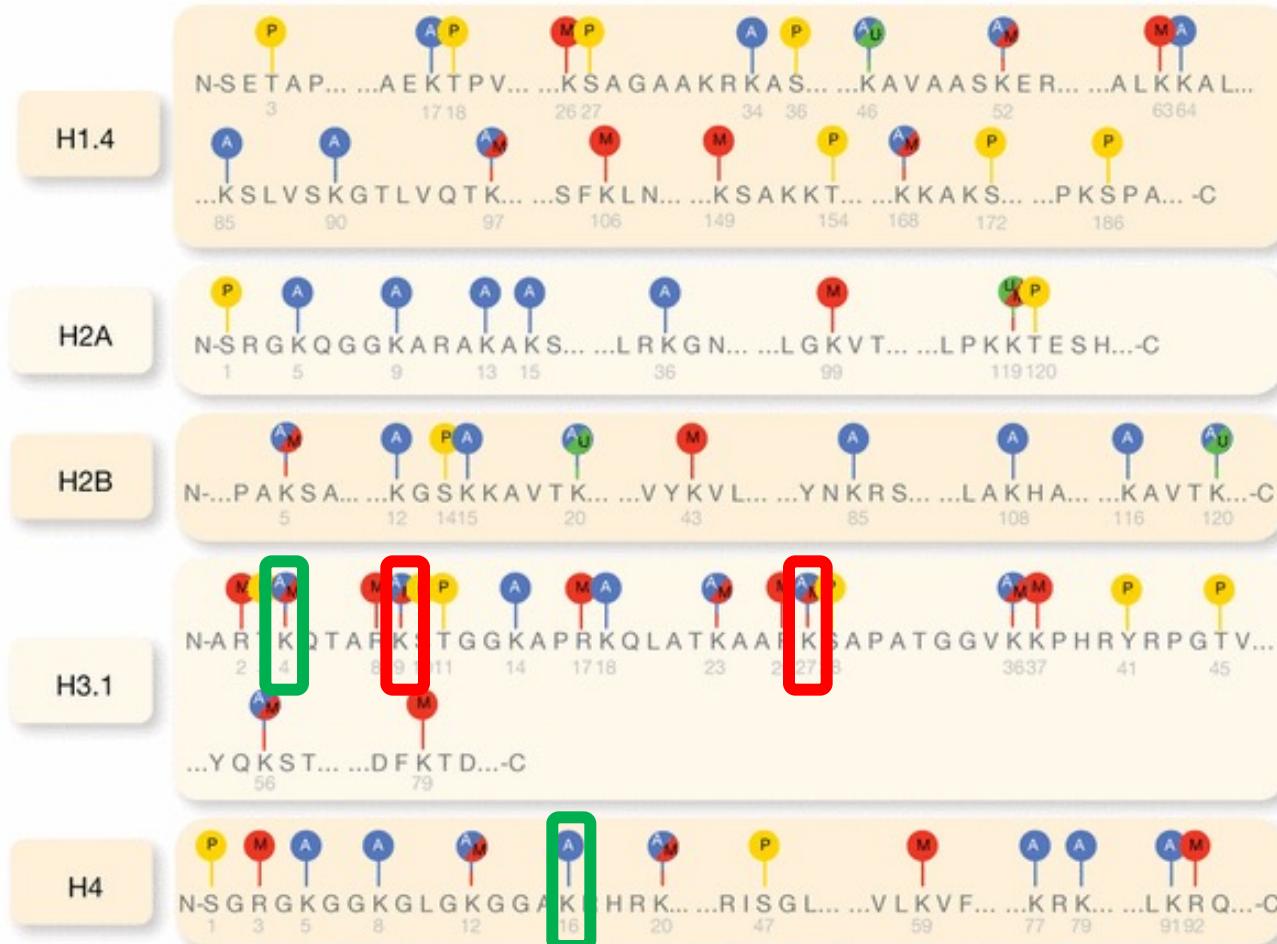
# Histone modifications

## Enzymes modifying chromatin



- Associated with gene activation and/or repression
- **INTERPLAY** between these chromatin associated modifications

# Histone modifications



## Activation

H3K4me3

H4K16ac

H4K9ac

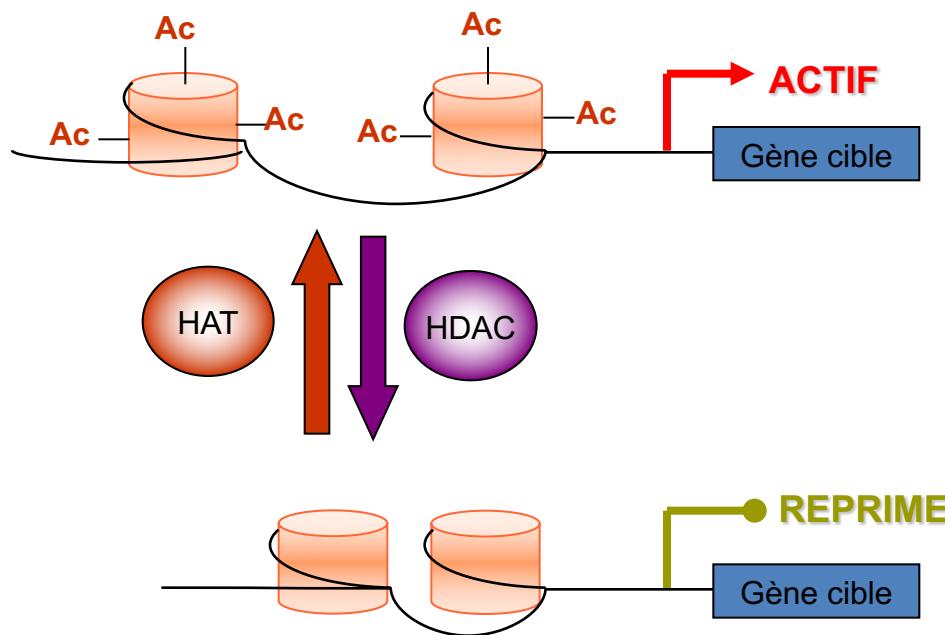
## Repression

H3K9me3

H3K27me3

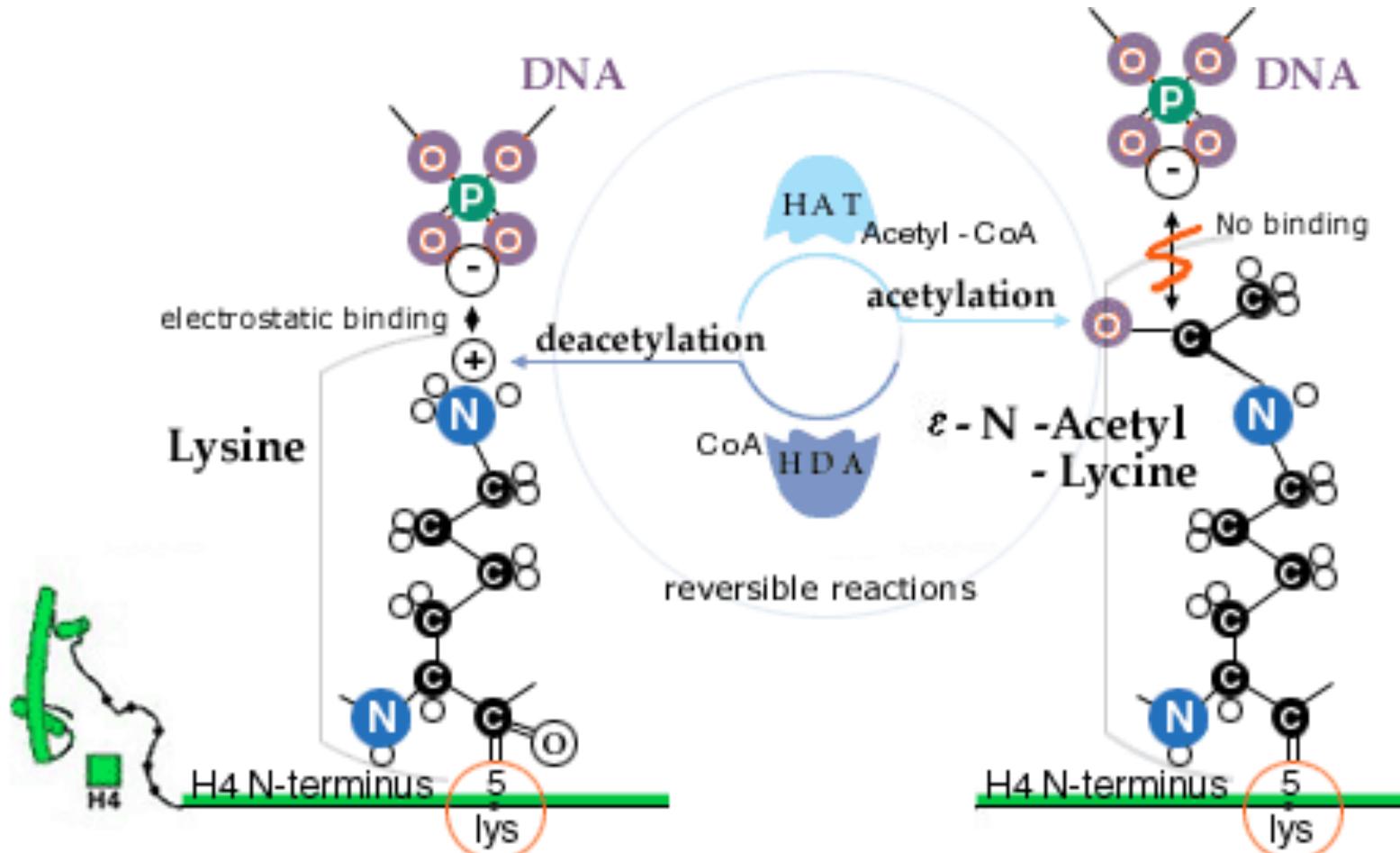
# Histone modifications

## Acetylation and deacetylation



# Histone modifications

## Acetylation and deacetylation

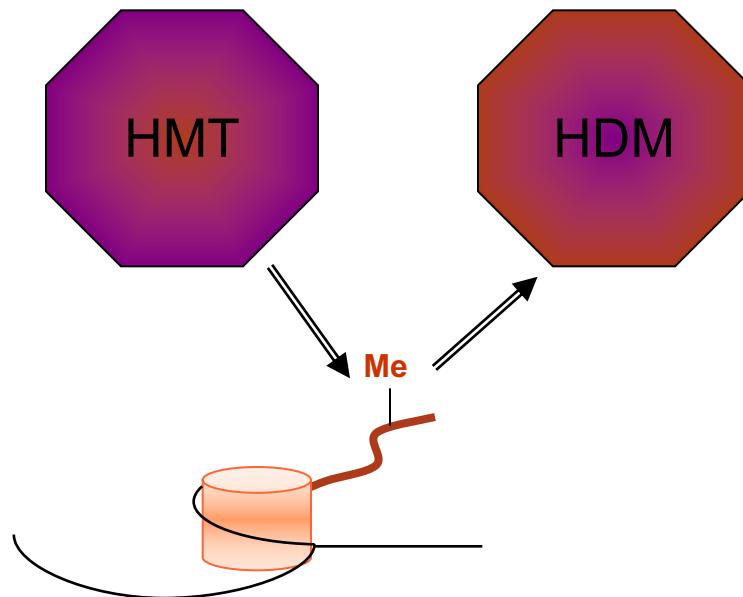


# Histone modifications

## Methylation and demethylation

Histone methyltransferases  
(HMT)

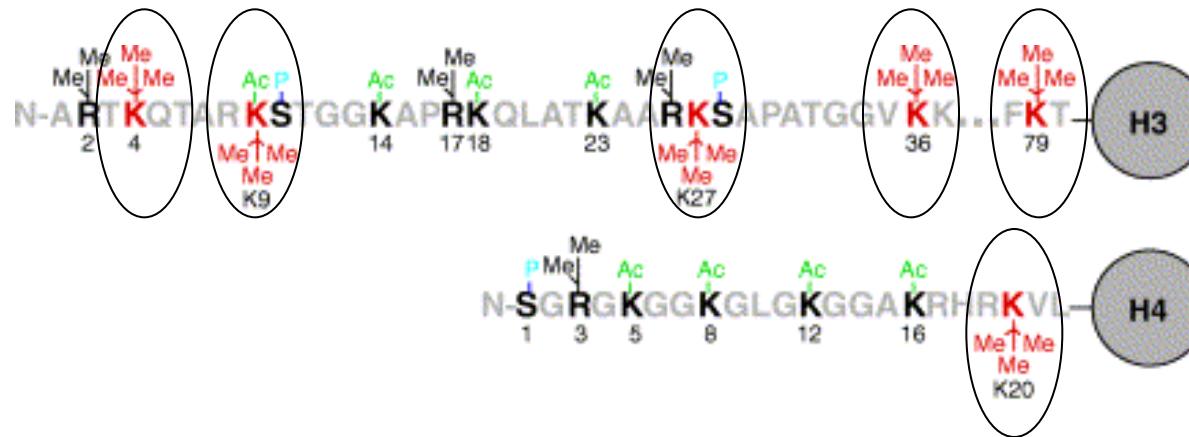
Histone demethyltransferases  
(HDM)



# Histone modifications

## Methylation et demethylation

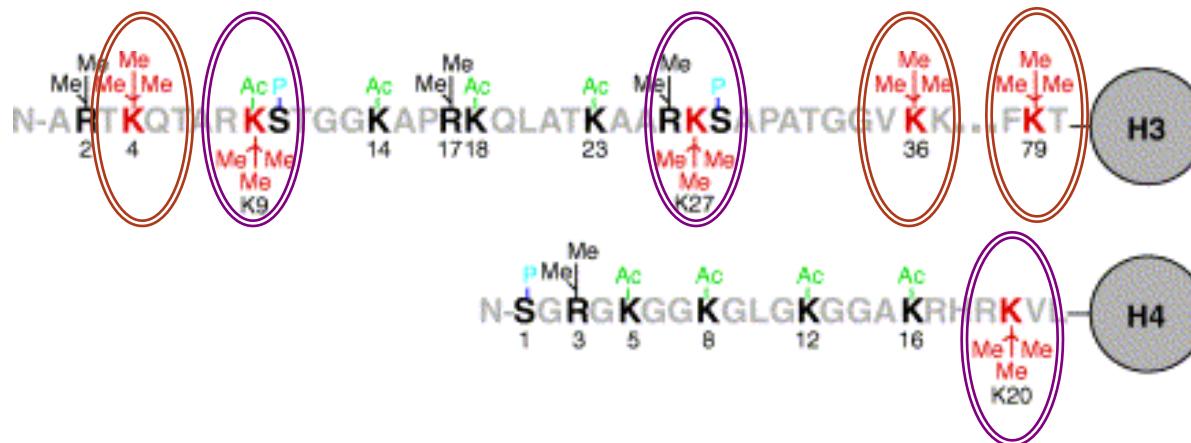
Lysine methylation (Lys, K) : mono, di, tri



# Histone modifications

## Methylation et demethylation

Lysine methylation (Lys, K) : mono, di, tri



Transcriptionnal Activation

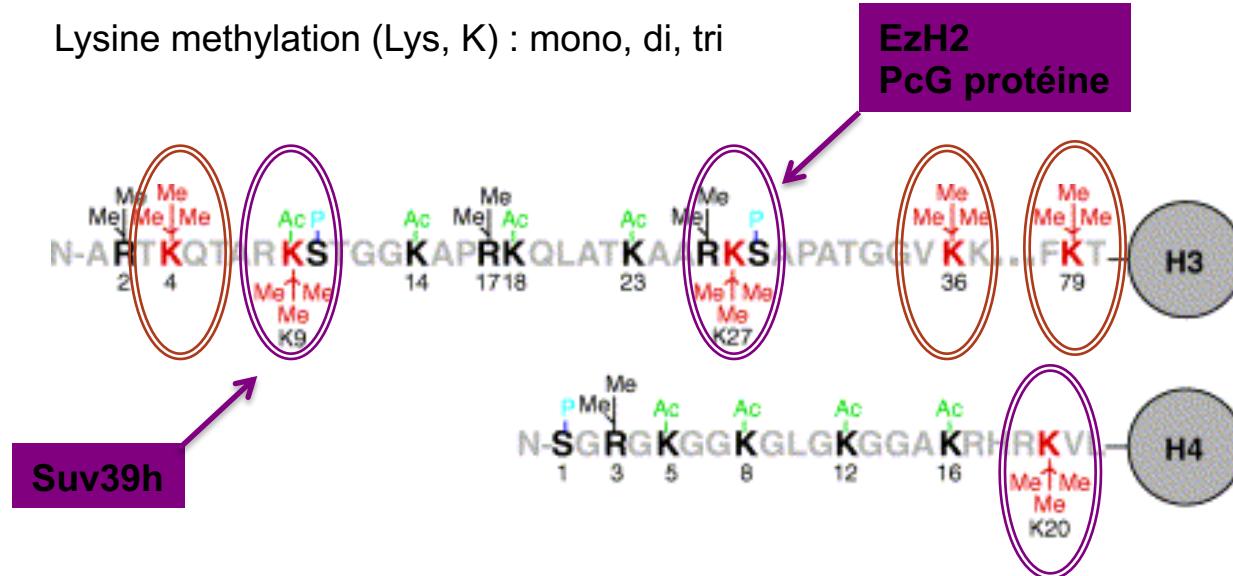


Transcriptionnal Repression

# Histone modifications

## Methylation et demethylation

Lysine methylation (Lys, K) : mono, di, tri



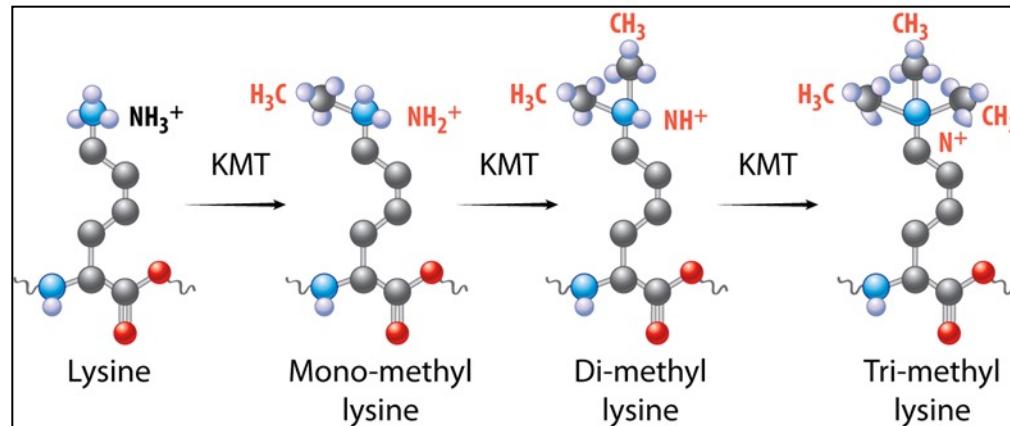
Transcriptionnal Repression

Transcriptionnal Activation

# Histone modifications

## Methylation et demethylation

Lysine methylation (Lys, K) : mono, di, tri

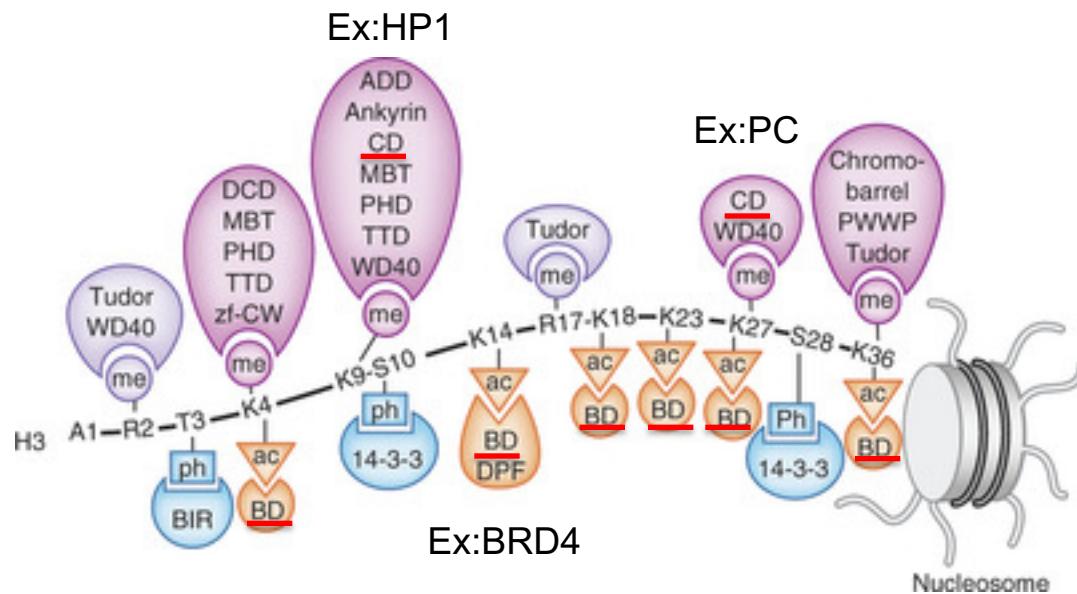


# Histone modifications

## Proteins-Reader of histone modifications

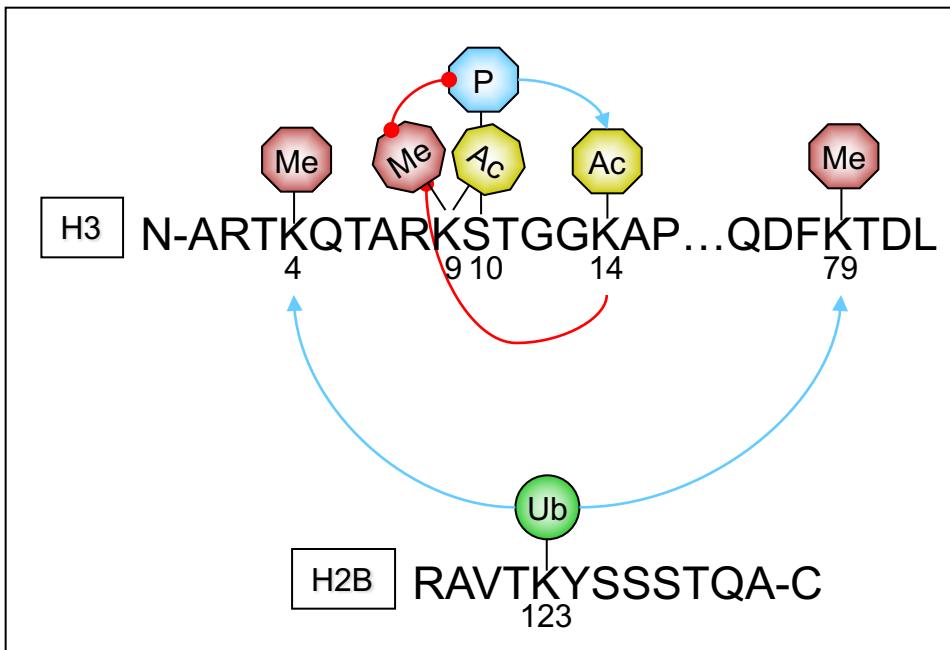
CD= Chromodomain: Binding of methylated lysine

BD= Bromodomain: Binding of acetylated lysine



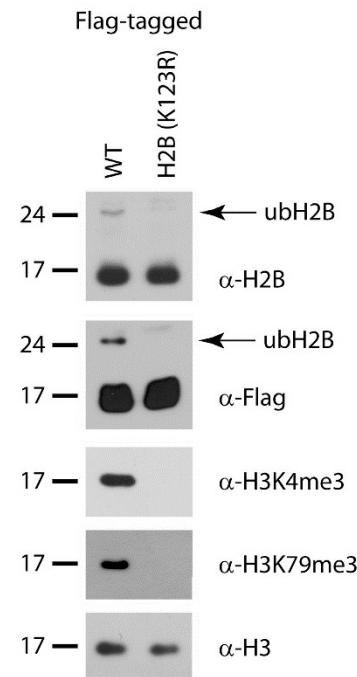
# Histone modifications

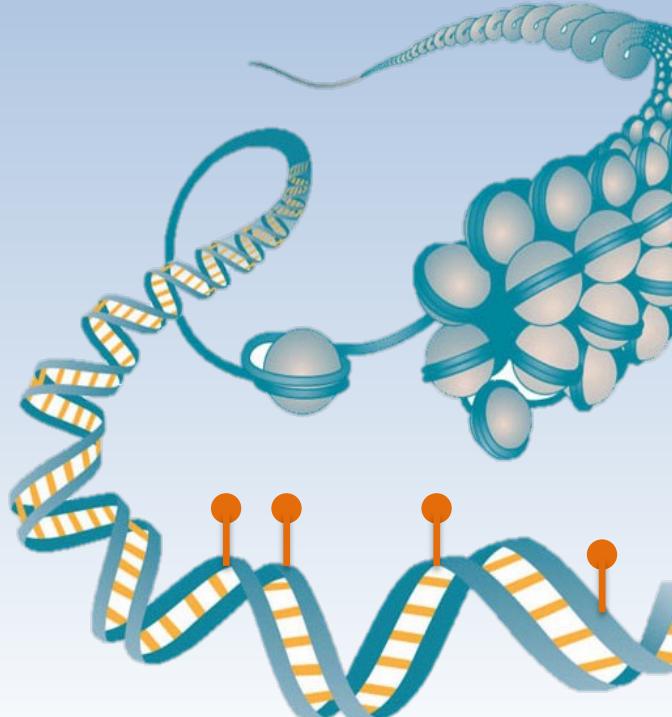
## Mutual influences



Histone H2BK123 monoubiquitination is the critical determinant for H3K4 and H3K79 trimethylation by COMPASS and Dot1

Shima Nakanishi,<sup>1</sup> Jung Shin Lee,<sup>1</sup> Kathryn E. Gardner,<sup>2</sup> Jennifer M. Gardner,<sup>1</sup> Yoh-hei Takahashi,<sup>1</sup> Mahesh B. Chandrasekharan,<sup>3</sup> Zu-Wen Sun,<sup>3</sup> Mary Ann Osley,<sup>4</sup> Brian D. Strahl,<sup>2</sup> Sue L. Jaspersen,<sup>1,5</sup> and Ali Shilatifard<sup>1</sup>

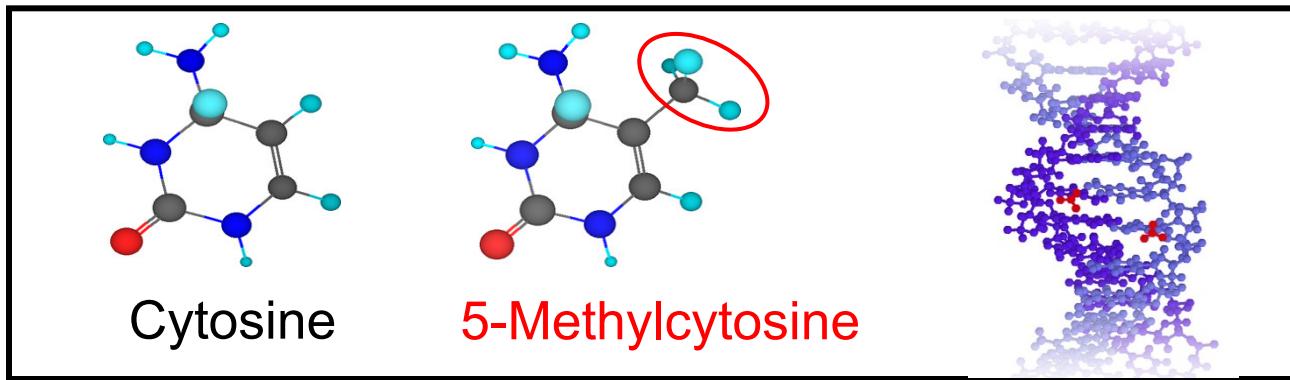




# DNA modifications

# DNA methylation

## Major features:

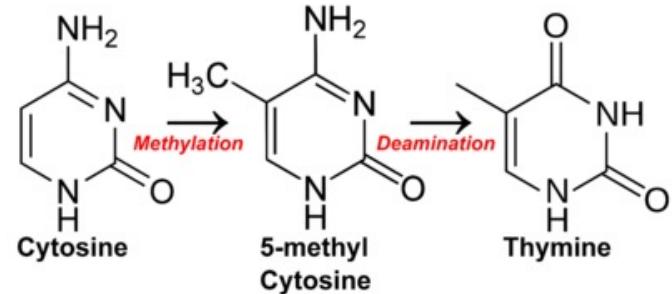


- CG dinucleotides
- only DNA modification known until 2009 (hmC, fC, CaC,...)

# DNA methylation

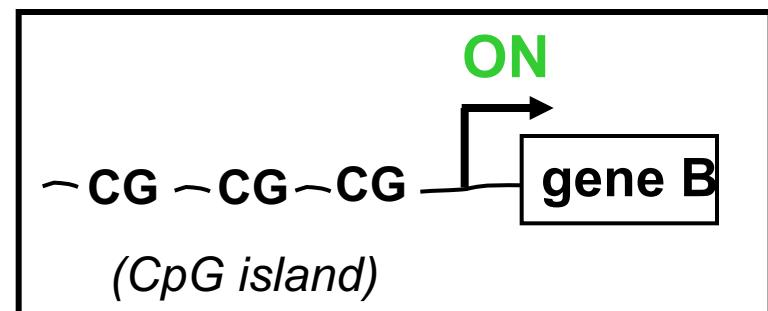
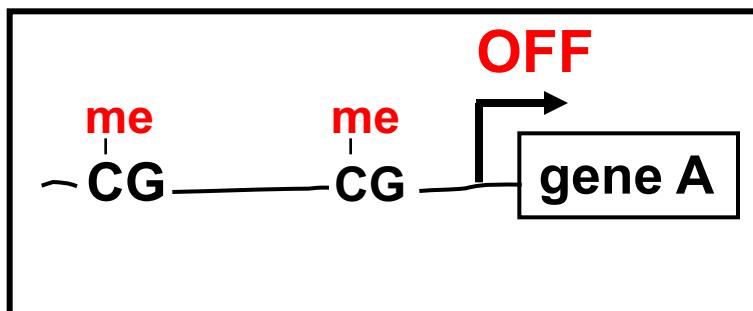
- Not random

- CpGs under represented (prediction)
- mCpG high mutagenic potential (thymine)



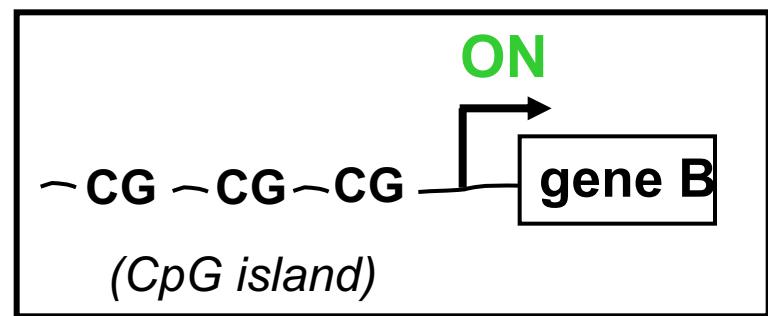
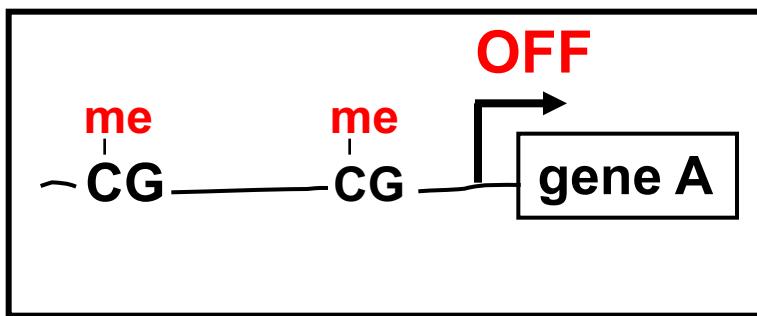
- CpG island

- CG rich (>50% C+G), 500 to 2000 bp, in promoters
- NOT METHYLATED



# DNA methylation

- Gene silencing

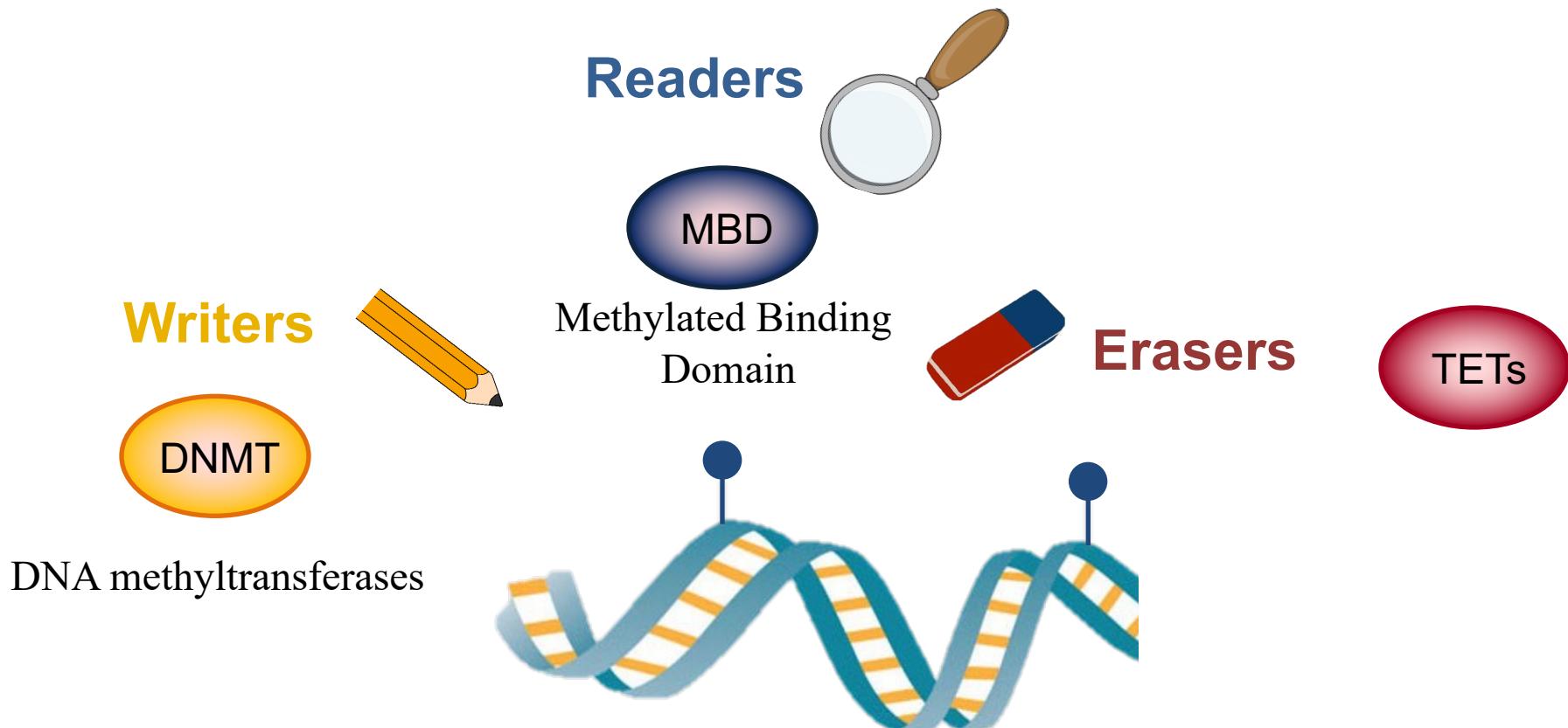


Promoters

**Gene bodies:** Role in activation and elongation

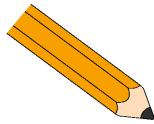
# DNA methylation

## Proteins implicated in DNA methylation



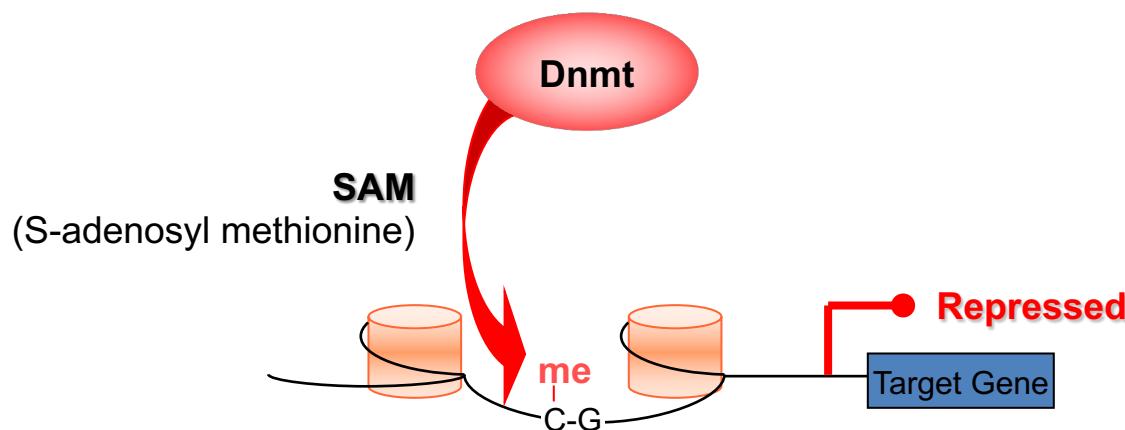
# DNA methylation

Writers



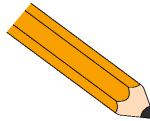
## Proteins involved in DNA methylation

**DNMTs** : DNA methyltransferases



# DNA methylation

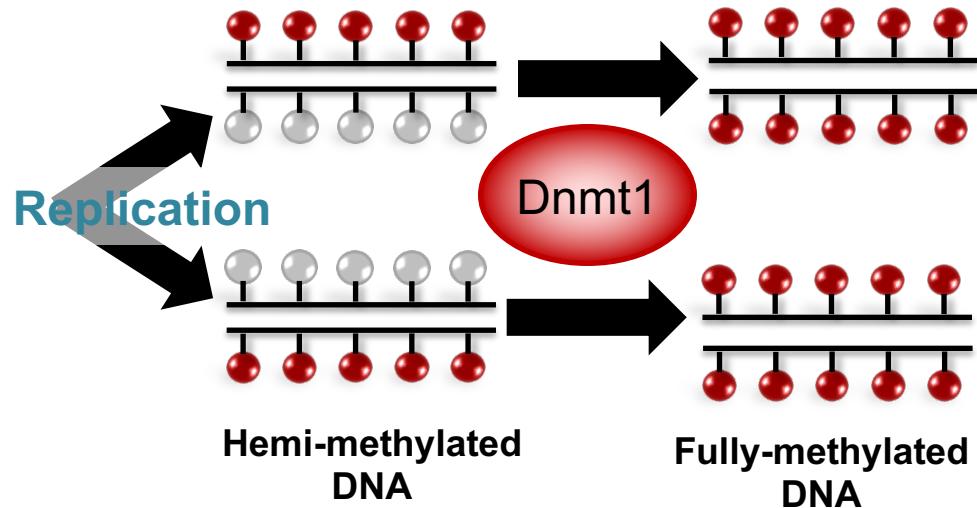
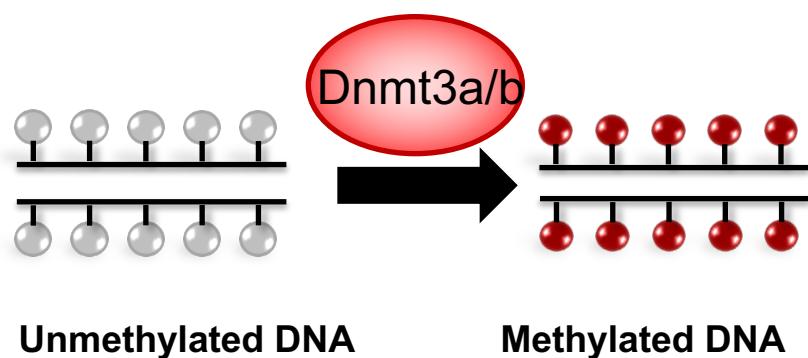
Writers



## DNA methyltransferases (DNMTs)

*de novo* DNA Methyltransferase: Dnmt3a/3b

Establishment of new methylation profile



**DNA Methyltransferase of Maintenance: Dnmt1**

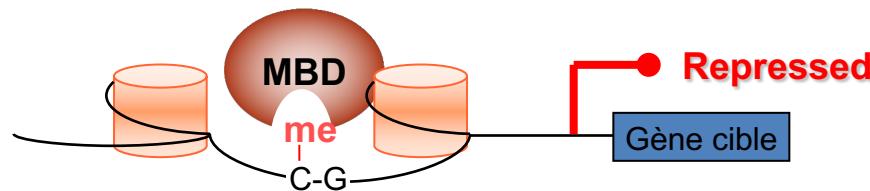
“copy” of DNA methylation during DNA replication

# DNA methylation



Readers

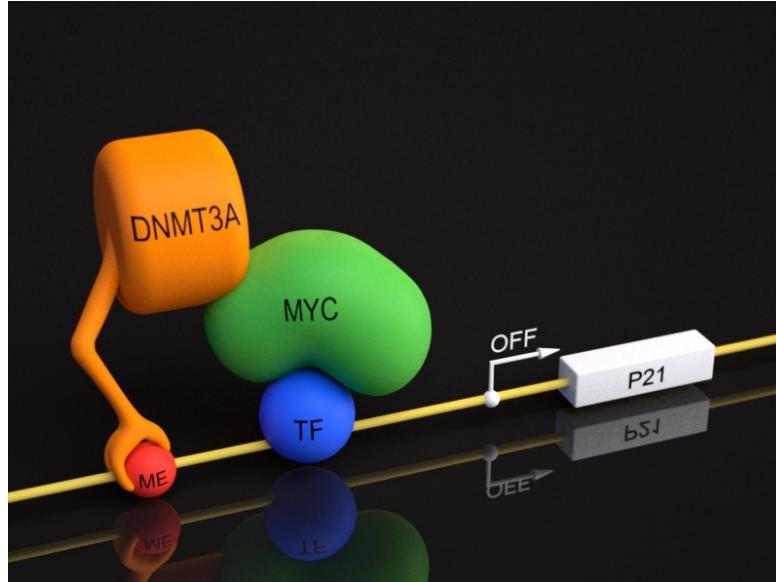
## Methyl-CpG Binding Domain Proteins (MBD)



- Maintenance of repressed state
- Recruitment of other repressors

# Targeting of DNA methylation

By Interaction with transcriptional factors

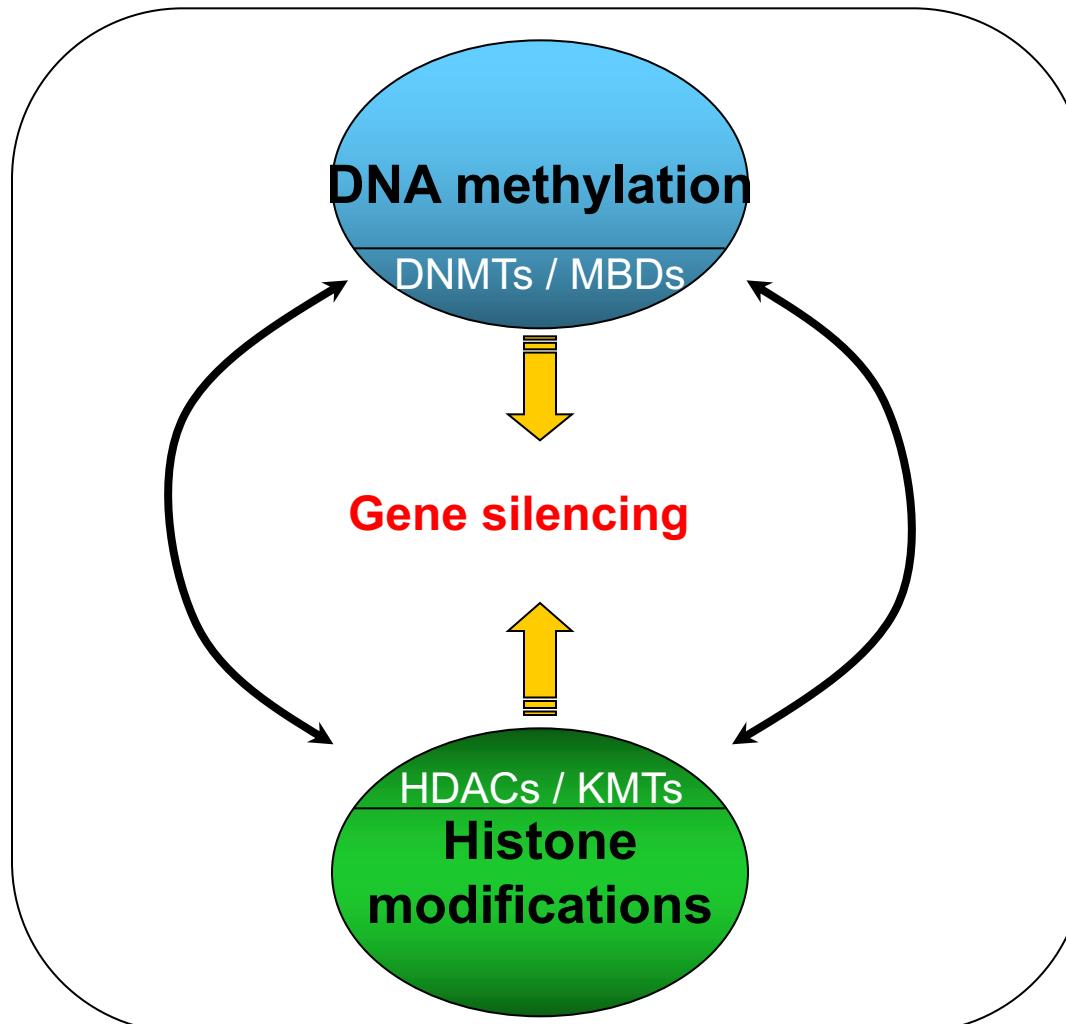


e.g. PML/RAR in leukemia  
Myc in various cancers

(Di Croce et al., *Science*)  
(Brenner et al., *EMBO J.*)

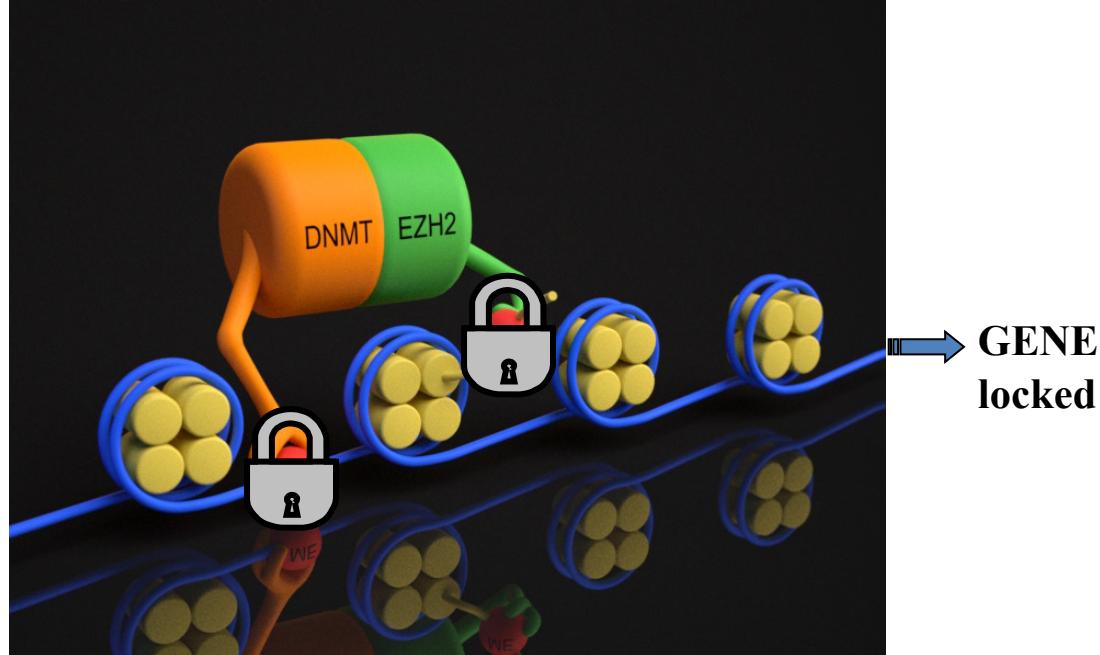
# Repression by DNA methylation

By connection with other repressive machineries



# The DNMT KMT connection

chromatin



(double security, double locking)

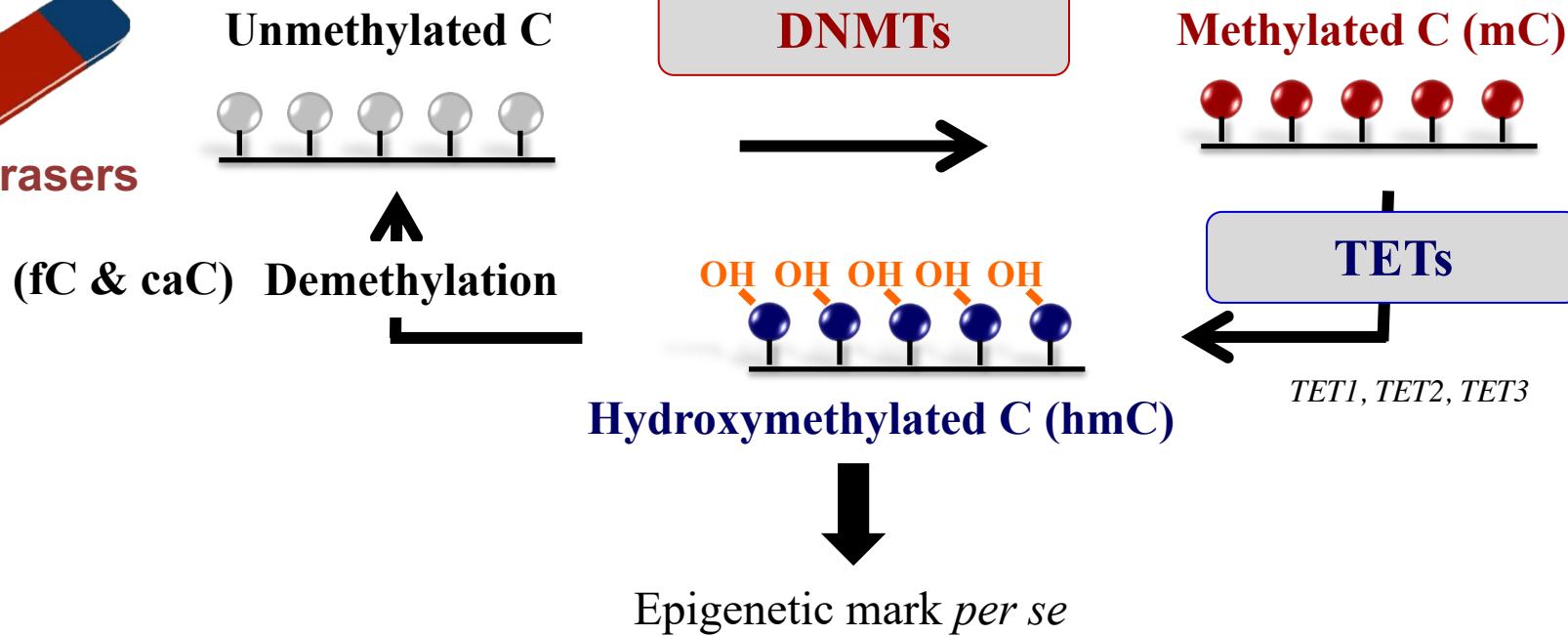
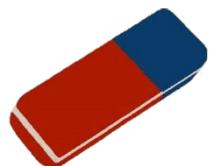
**nature**

LETTERS

## The Polycomb group protein EZH2 directly controls DNA methylation

Emmanuelle Viré<sup>1</sup>, Carmen Brenner<sup>1</sup>, Rachel Deplus<sup>1</sup>, Loïc Blanchon<sup>1</sup>, Mario Fraga<sup>2</sup>, Céline Didelot<sup>1</sup>, Lluís Morey<sup>3</sup>, Aleyde Van Eynde<sup>4</sup>, David Bernard<sup>1</sup>, Jean-Marie Vanderwinden<sup>5</sup>, Mathieu Bollen<sup>4</sup>, Manel Esteller<sup>2</sup>, Luciano Di Croce<sup>3</sup>, Yvan de Launoit<sup>1,6</sup> & François Fuks<sup>1</sup>

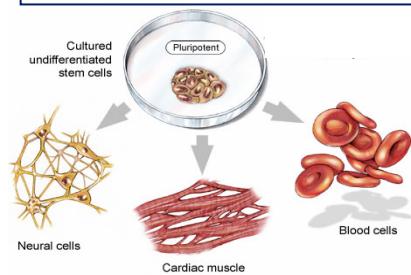
# DNA hydroxymethylation



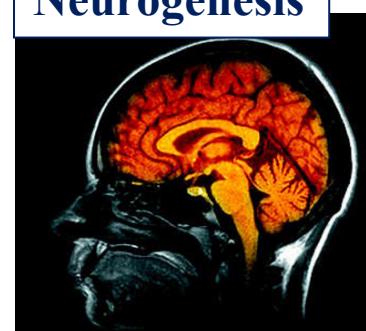
## Development



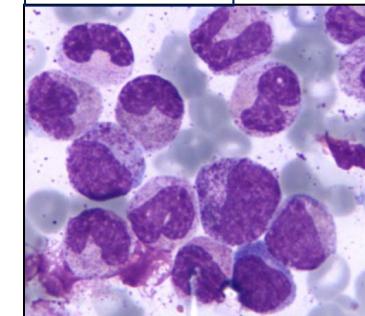
## Cell pluripotency

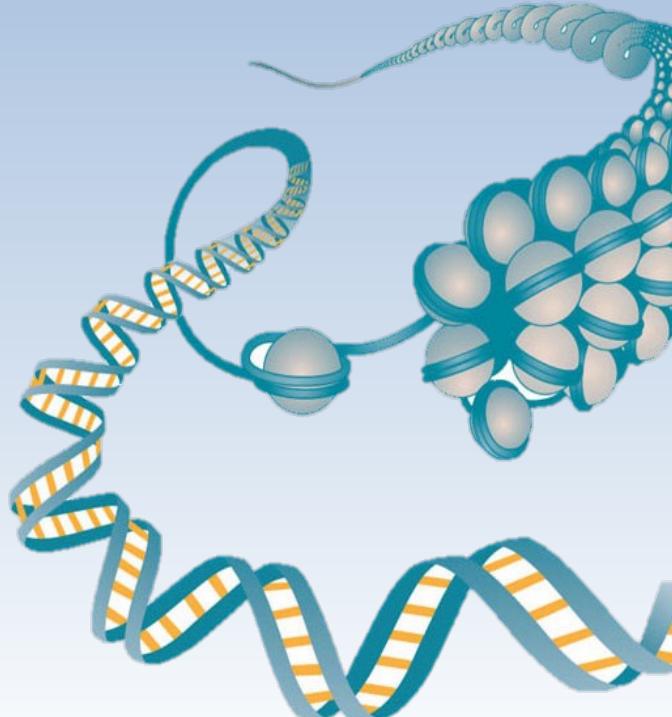


## Neurogenesis

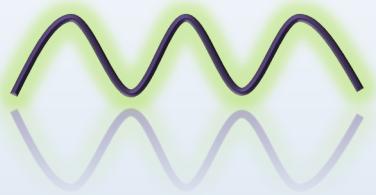


## Cancers

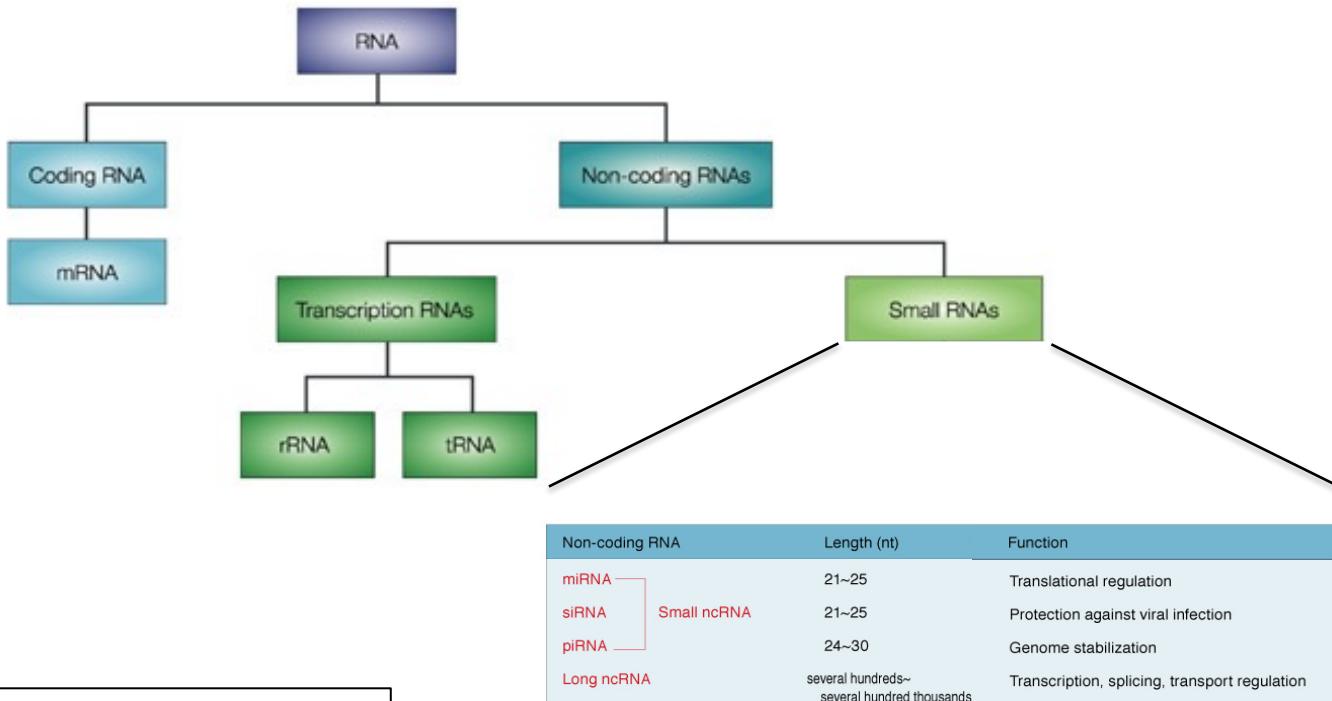




# Non coding RNAs



# Non coding RNAs



**frontiers**  
in Immunology

**REVIEW**  
Published: 29 January 2019  
doi: 10.3389/fimmu.2019.00164

**Review Article**  
**Roles of Identified Long Noncoding RNA in Disease**

**Yan Li,<sup>1</sup> Keyang Xu,<sup>2</sup> Kechen Xu,<sup>3</sup> Sixiang Chen,<sup>2</sup> Yifang Cao<sup>2</sup>**

<sup>1</sup>The First Clinical Medical College, Chengdu University of Traditional Chinese Medicine, China  
<sup>2</sup>Zhejiang Chinese Medical University, Hangzhou, 310053 Zhejiang, China  
<sup>3</sup>The Fourth Affiliated Hospital, Zhejiang University School of Medicine, Yiwu, 322000 Zhejiang, China  
<sup>4</sup>The First Hospital of Jiaxing, Jiaxing, 314001 Zhejiang, China

**SCIENTIFIC REPORTS**

**OPEN** Relationships of Non-coding RNA with diabetes and depression

Tian An<sup>1</sup>, Jing Zhang<sup>2</sup>, Yue Ma<sup>3</sup>, Juan Lian<sup>1</sup>, Yan-Xiang Wu<sup>1</sup>, Bo-Han Lv<sup>1</sup>, Meng-Hua Ma<sup>2</sup>, Jun-Hua Meng<sup>2</sup>, Yun-Tao Zhou<sup>2</sup>, Zhi-Yong Zhang<sup>2</sup>, Qing Liu<sup>3</sup>, Si-Hua Gao<sup>1</sup> & Guang-Jian Jiang<sup>1</sup>

**ARTICLE**  
published: 25 March 2014  
doi: 10.3389/fgene.2014.00057

**SCIENTIFIC**

**OPEN** Silencing an insulin-induced lncRNA, LncASIR, impairs the transcriptional response to insulin signalling in adipocytes

Received: 2 August 2018  
Accepted: 26 March 2019  
Published online: 04 April 2019

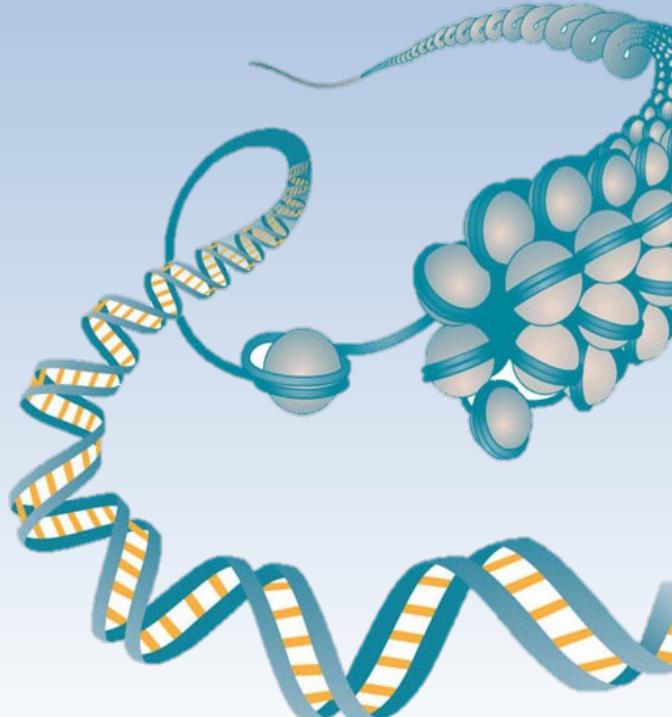
Ufuk Degirmenci<sup>2,3</sup>, Jia Li<sup>1</sup>, Yen Ching Lim<sup>1</sup>, Diana Teh Chee Siang<sup>1</sup>, Shibo Lin<sup>4</sup>, Hui Liang<sup>4</sup> & Lei Sun<sup>1,2</sup>

**Regulation of metabolism by long, non-coding RNAs**

**Jan-Wilhelm Komfeld<sup>1,2</sup> and Jens C. Brüning<sup>1,2,3,4 \*</sup>**

<sup>1</sup> Cologne Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases, Köln, Germany  
<sup>2</sup> Max-Planck-Institute for Neurological Research, Köln, Germany  
<sup>3</sup> Department of Mouse Genetics and Metabolism and Center for Molecular Medicine Cologne, Institute for Genetics at the University Hospital of Cologne, University of Cologne, Cologne, Germany  
<sup>4</sup> Center for Endocrinology, Diabetes and Preventive Medicine, University Hospital Cologne, University of Cologne, Cologne, Germany

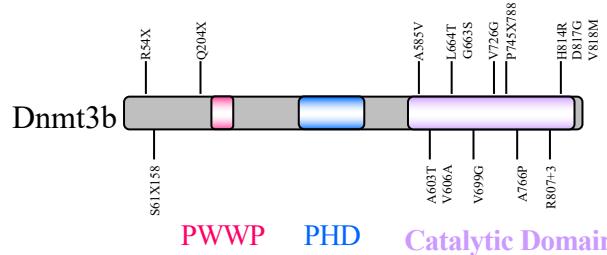
# Epigenetic pathologies



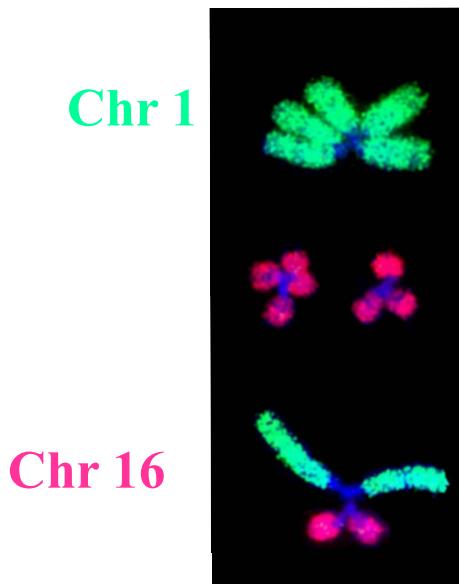
# Epigenetic pathologies

## ICF Syndrom

- **Immunodeficiency** frequent respiratory infections
- **Centromeric instability**
- **Facial dysmorph**



Chromatin decondensation  
at centromere: breaking point

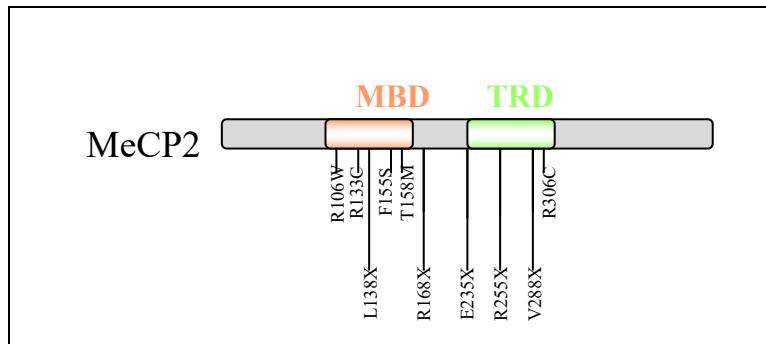


Deletion-duplications  
(multiradial conformation)

# Epigenetic pathologies

## Rett Syndrom

- Neurological troubles
- Loss of coordinated moves (stereotypic moves)
- Language loss
- Difficulty to walk
- Difficulty to breath



- No global chromatin desorganization
- No repression of MeCP2 target genes (bdnf)

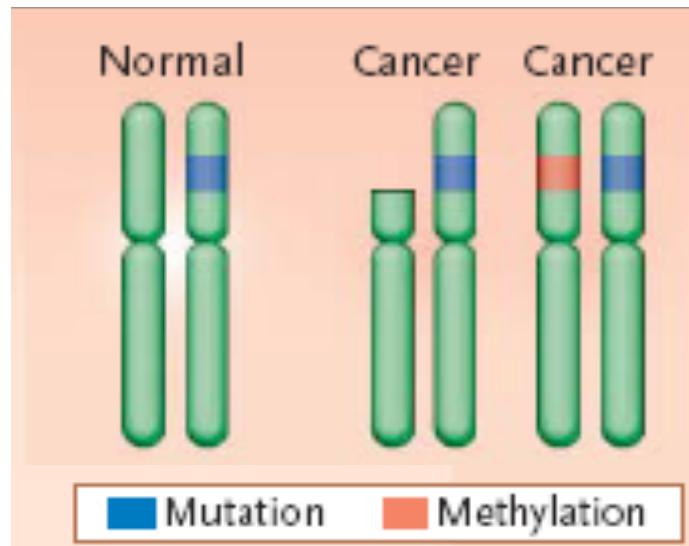
## II. Epigenetics & Cancer



# Cancers: Genetics AND Epigenetics

## Cancers

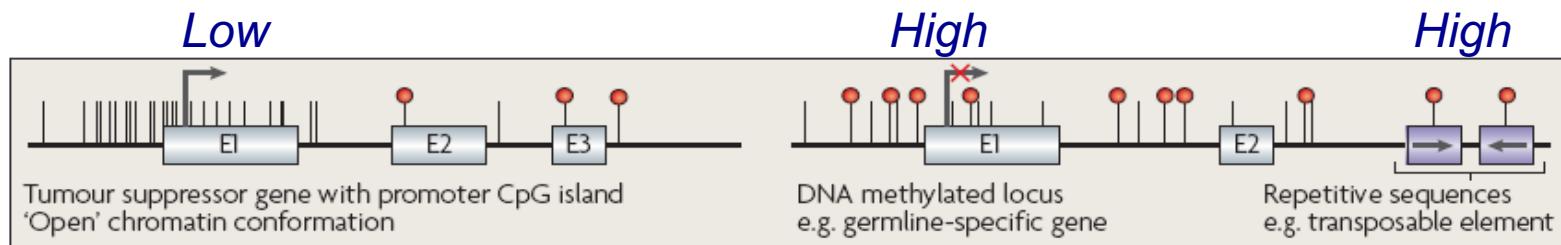
Genetic and epigenetic disease



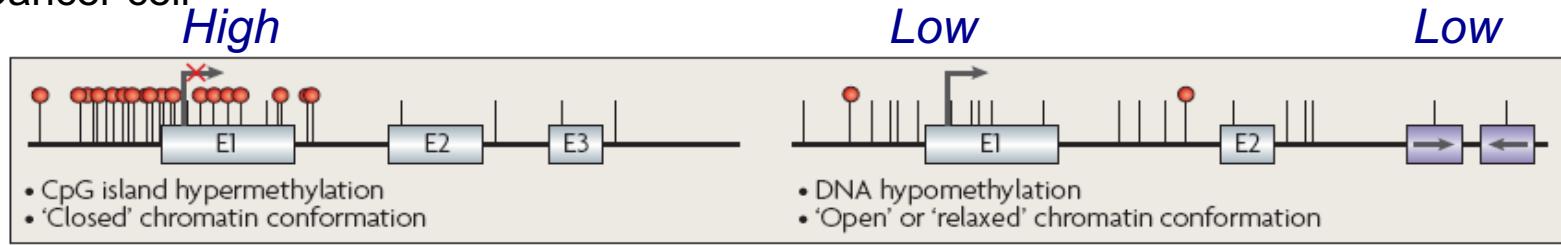
(Knudson hypothesis)

# Epigenetics in cancer

Normal cell



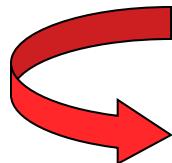
Cancer cell



# Epigenetics in cancer

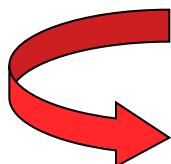
ALL cancers: aberrant DNA methylation profiles

- **Hypomethylation** of silencing genes (repetitive sequences, pluripotent genes,...)



Chromosomal instability  
Activation of forbidden genes

- **Hypermethylation** of tumor suppressor genes



Repression of « control » genes

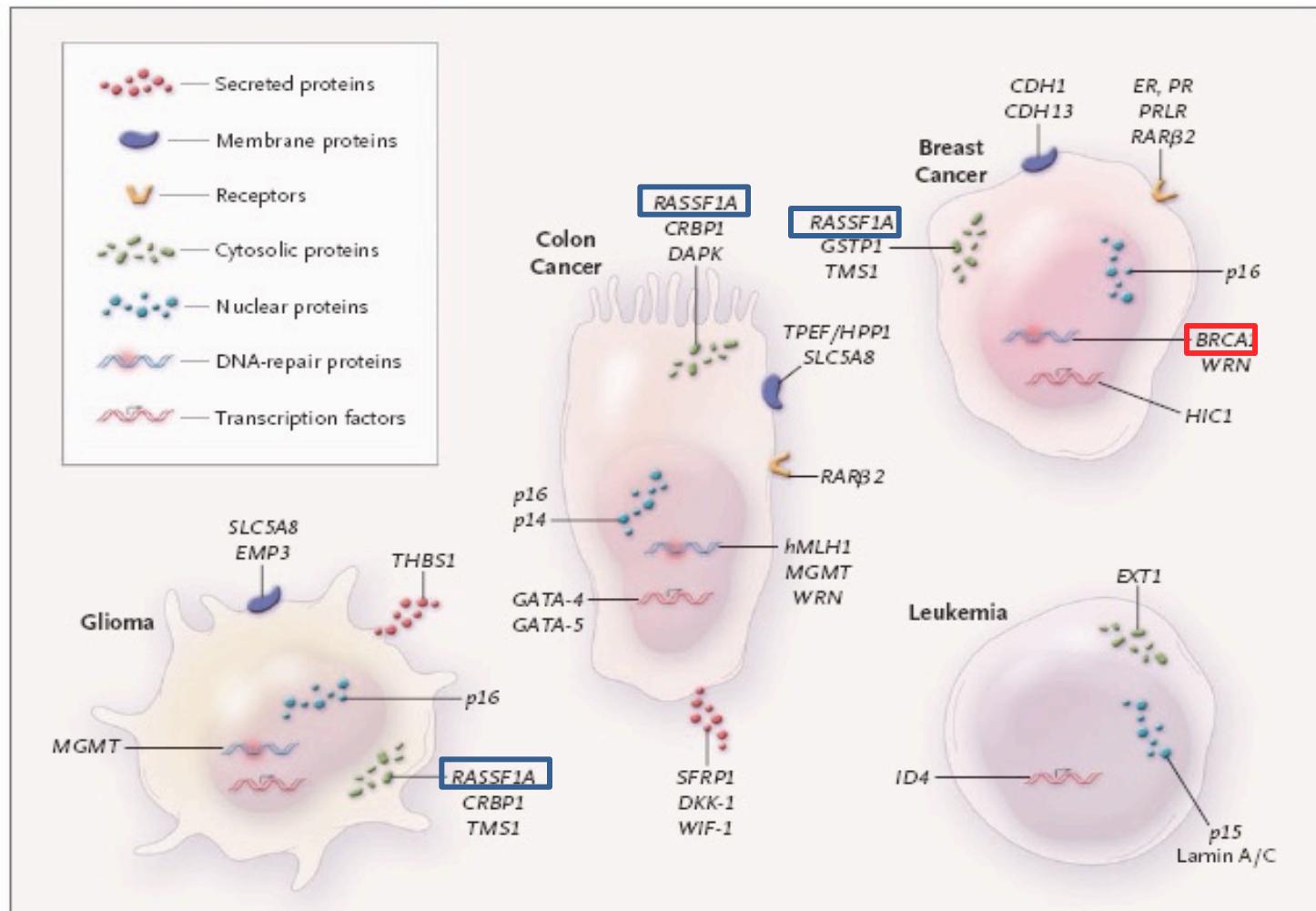
Control of cell cycle: p21,...

Control of apoptosis: p53...

Control of DNA repair: MGMT...

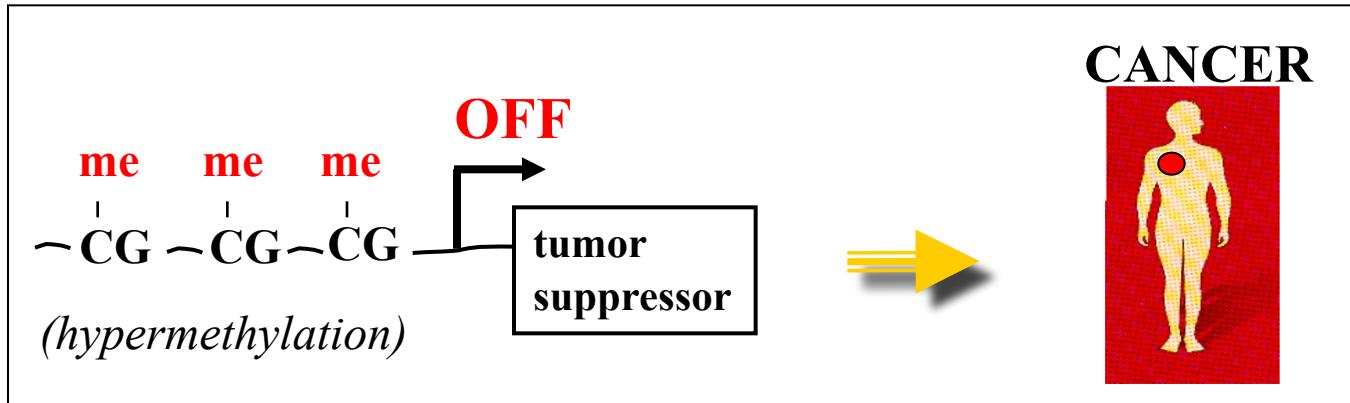
# Epigenetics in cancer

- Silencing of tumor suppressor genes: e.g. Rb, p16, RAR $\beta$ , MGMT
- Some specific of one cancer, others in many cancers



# Epigenetic Biomarkers

## Increasing clinical implications



## DIAGNOSTIC

- Diagnosis at early stage
- Molecular classification of tumors
- Treatment response



# Epigenetic Biomarkers

## Cancers: Detection in fluids

Disease	DNA source	Markers
Bladder cancer	Plasma	<i>CDKN2A (ARF)</i>
	Plasma	<i>CDKN2A (INK4A)</i>
	Serum	<i>CDKN2A (INK4A)</i>
Breast cancer	Plasma	<i>CDKN2A (INK4A)</i>
	Plasma	<i>CDKN2A (INK4A)</i>
Colorectal cancer	Serum	<i>MLH1</i>
	Serum	<i>CDKN2A (INK4A)</i>
	Serum	<i>CDKN2A (INK4A)</i>
	Plasma	<i>CDKN2A (INK4A)</i>
Oesophageal cancer	Plasma (AC)	<i>APC</i>
	Plasma (SCC)	<i>APC</i>
	Serum (SCC)	<i>CDKN2A (INK4A)</i>
Gastric cancer	Serum	<i>CDH1</i>
	Serum	<i>CDKN2A (INK4A)</i>
	Serum	<i>CDKN2B (INK4B)</i>
	Serum	<i>DAPK1</i>
	Serum	<i>GSTP1</i>
	Serum	Panel of five
Head and neck cancer	Serum	<i>CDKN2A (INK4A)</i>
	Serum	<i>DAPK1</i>
	Serum	<i>MGMT</i>
	Serum	Panel of three
	Plasma (nasopharyngeal)	<i>DAPK1</i>
Liver cancer	Plasma/serum	<i>CDKN2A (INK4A)</i>
	Plasma/serum	<i>CDKN2B (INK4B)</i>
	Plasma/serum	Panel of two
Lung cancer	Serum (NSCLC)	<i>CDKN2A (INK4A)</i>
	Serum (NSCLC)	<i>DAPK1</i>
	Serum (NSCLC)	<i>GSTP1</i>
	Serum (NSCLC)	<i>MGMT</i>
	Serum (NSCLC)	Panel of four
	Plasma	<i>CDKN2A (INK4A)</i>
	Plasma/serum	<i>APC</i>
	Plasma	<i>CDKN2A (INK4A)</i>
Prostate cancer	Plasma/serum	<i>CDKN2A (INK4A)</i>
	Plasma	<i>GSTP1</i>
		<i>GSTP1</i>

# Epigenetic Biomarkers

## Commercially-available DNA methylation test kits for cancer

Gene(s)	Type of Biomarker	Type of Cancer	Diagnostic Test Kit: Brand Name (Manufacturer)	References
<i>VIM</i>	diagnostic	Colorectal	Cologuard (Exact Sciences)	[128] <sup>1</sup>
<i>SEPT9</i>	diagnostic	Colorectal	Epi proColon (Epigenomics) ColoVantage (Quest Diagnostics) RealTime mS9 (Abbott)	[129] <sup>1</sup>
<i>SHOX2</i>	diagnostic	Lung	Epi prolong (Epigenomics)	[130–135] <sup>2</sup>
<i>GSTPI/APC/RASSF1A</i>	diagnostic	Prostate	ConfirmMDx (MDx Health)	[136–138] <sup>1</sup>
<i>MGMT</i>	predictive	Glioblastoma	PredictMDx Glioblastoma (MDx Health) SALSA MS-MLPA probemix ME011 Mismatch Repair genes (MRC-Holland) PyroMark MGMT Kit (Qiagen)	[121,139,140] <sup>1</sup>

# Epigenetic Therapy

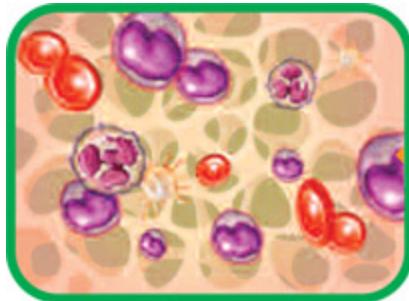
**Table 1** Selected Epigenetic Drugs

Drug	Compound	Study Phase
DNMT inhibitors	Azacitidine (Vidaza)	US FDA-approved in MDS
	Decitabine (Dacogen)	US FDA-approved in MDS
	S110	Phase I
	CP-4200 (elaidic azacytidine)	Preclinical
	Nanaomycin A	Preclinical
HDAC inhibitors	Vorinostat (Zolinza)	US FDA-approved in CTCL
	Romidepsin (Istodax)	US FDA-approved in CTCL
	Panobinostat	Phase II
	Belinostat	Phase I/II
	Valproic acid	Phase II
	Belinostat	Phase II/III
HMT inhibitors	Deazaneoplanocin A (DZNep)	Preclinical
	Quinazoline derivatives	Preclinical
	Ellagic Acid	Preclinical
Histone demethylase inhibitors	Polyamine analogues	Preclinical
	Hydroxamate analogues	Preclinical
HAT inhibitors	Spermidinyl-CoA derivatives	Preclinical
	Hydrazinocurcumin	Preclinical
	Pyrazolone-containing small molecules	Preclinical

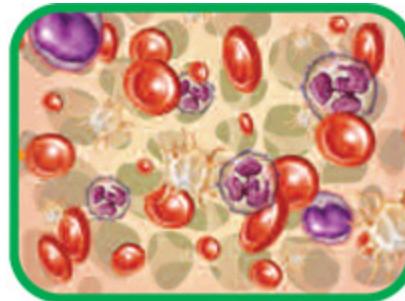
# Epigenetic Therapy

**Already a reality!**

Leukemia: Myelodysplasie (MDS)

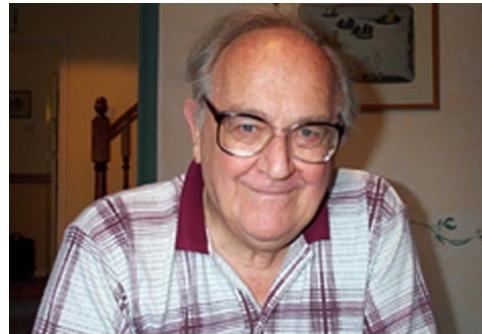


Bone marrow in a person with MDS



Healthy bone marrow

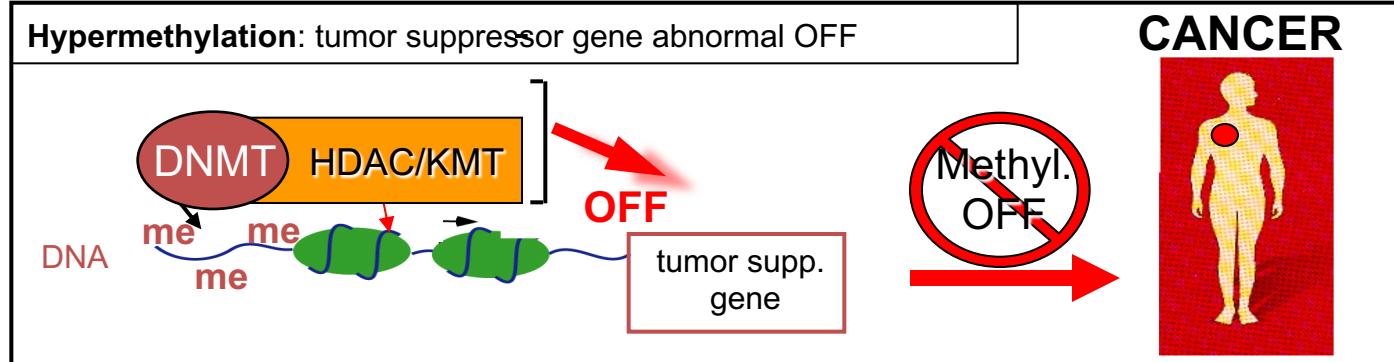
Rather in « older » men (60-70 years)



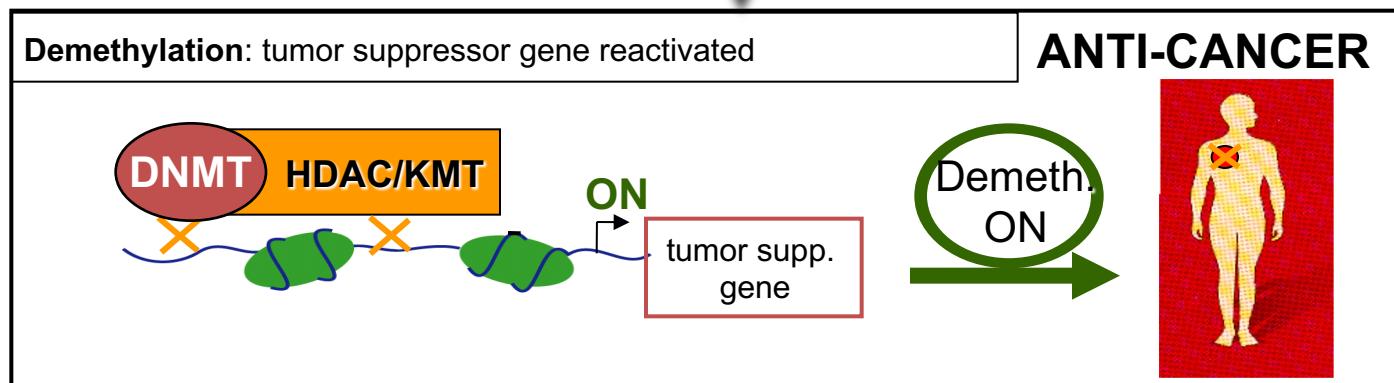
Treatment with **azacytidine**  
**DNMT inhibitor**

# Epigenetic Therapy

## Strategy: DNA methylation-histone modifications



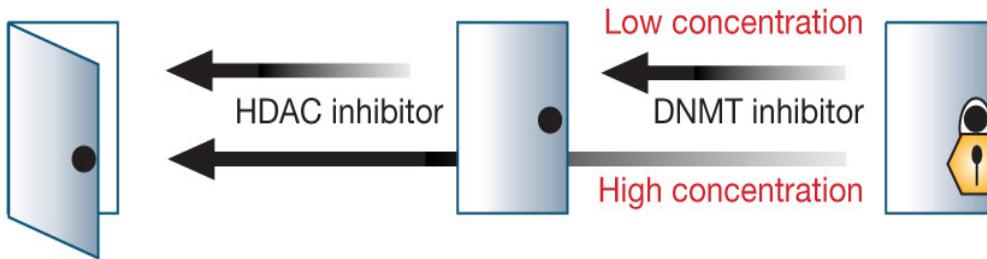
*DNMT + HDAC + KMT inhibitors*



# Epigenetic Therapy

## Use of combined epigenetic drugs

Application in anti-cancer therapy of DNMT-HDAC connexion



NIH Public Access  
Author Manuscript

*Semin Hematol.* Author manuscript; available in PMC 2009 January 1.

Published in final edited form as:  
*Semin Hematol.* 2008 January ; 45(1): 23–30.

**DNA Methyltransferase and Histone Deacetylase Inhibitors in the Treatment of Myelodysplastic Syndromes**

Elizabeth A. Griffiths, MD and

Published in final edited form as:  
*Future Oncol.* 2011 February ; 7(2): 263–283. doi:10.2217/fon.11.2.

**Rational therapeutic combinations with histone deacetylase inhibitors for the treatment of cancer**

K Ted Thurn<sup>1,\*</sup>, Scott Thomas<sup>1,\*</sup>, Amy Moore<sup>1,\*</sup>, and Pamela N Munster<sup>1</sup>

<sup>1</sup>Department of Medicine, Hematology/Oncology Division, University of California, San Francisco, CA, USA

# III. Epigenomics is coming of age



# Why of interest?

Sequencing the human genome:



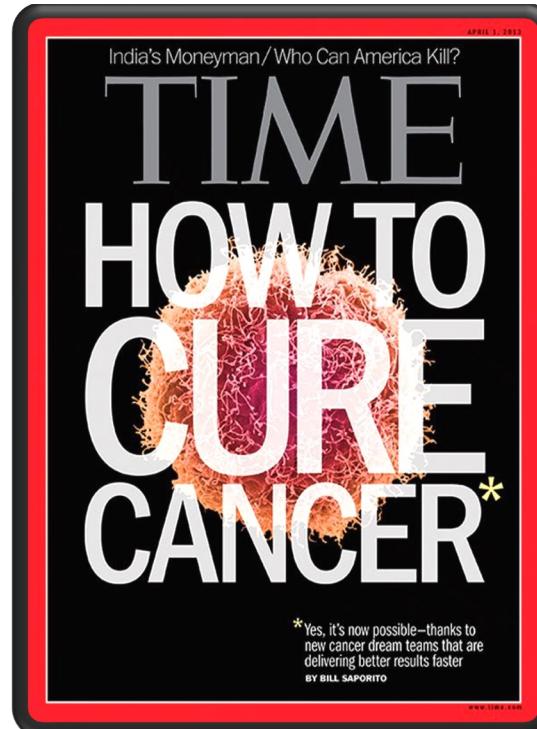
“Is that all?”

# Epigenomics

nature

## Time for the epigenome

The complexity of genetic regulation is one of the great wonders of nature, but it represents a daunting challenge to unravel. The International Human Epigenome Consortium is an appropriate response.

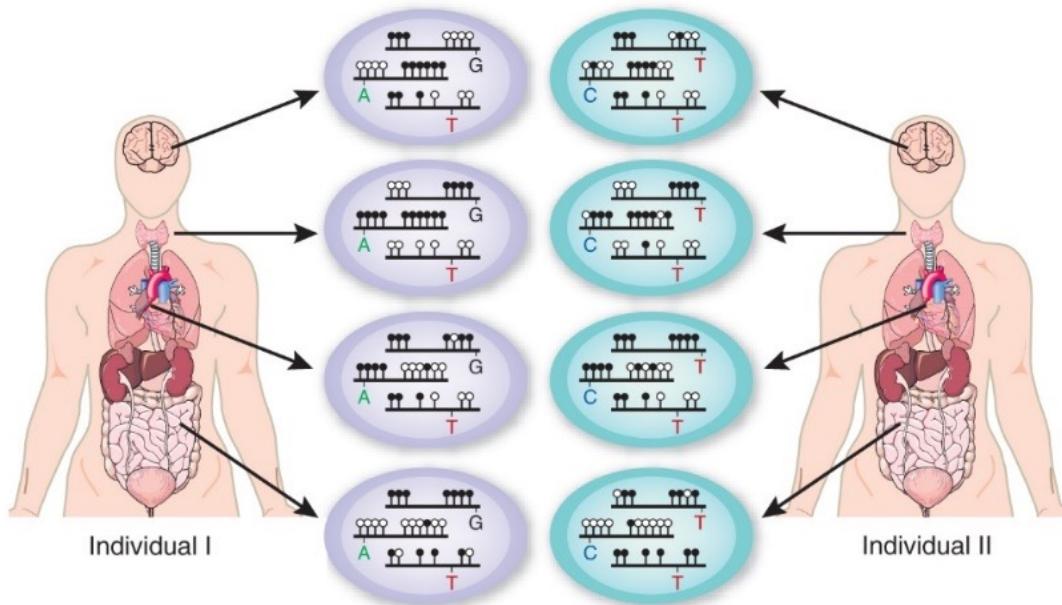


# Epigenomics

Epigenetics: study of epigenetic modifications of a specific gene

**Epigenomics:** study of epigenetic modifications of all the genome

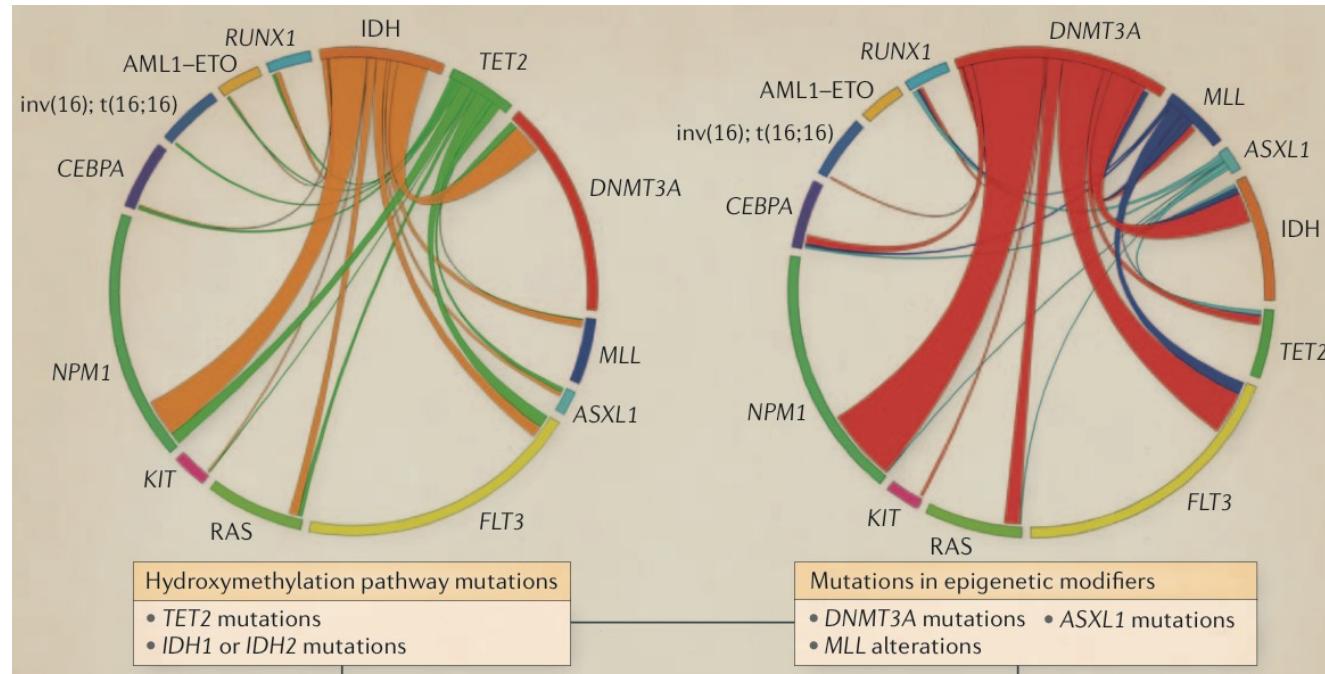
Not ONE  
but  
MANY  
EPIGENOMES



Inter- and  
intra-  
individual  
differences

# NGS & Cancer Epigenomics: A « BIG SURPRISE »

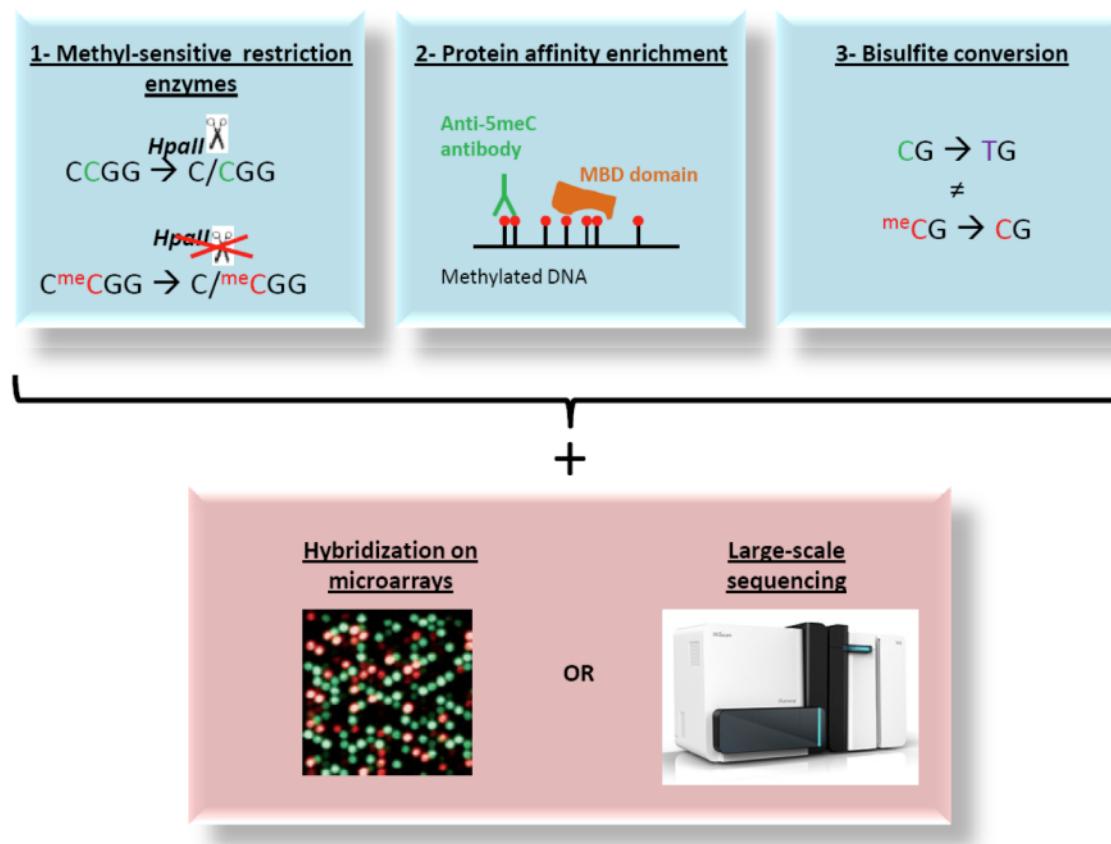
*When Genetics meets Epigenetics*



**Cancer mutations: increasing number in epigenetic genes**

# Epigenomic technologies

## DNA methylome: various technologies



DNA methylation discrimination

Read out

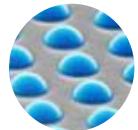
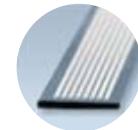
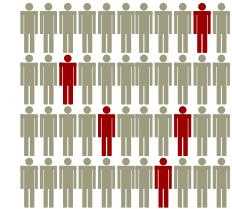
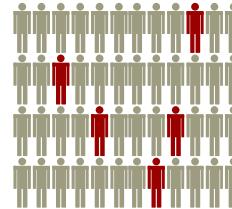
# Epigenomic technologies

## DNA methylome: various technologies

Pretreatment	Array-based analysis	NGS-based analysis
Enzyme digestion	<ul style="list-style-type: none"><li>• DMH</li><li>• MCAM</li><li>• HELP</li><li>• MethylScope</li><li>• CHARM</li><li>• MMASS</li></ul>	<ul style="list-style-type: none"><li>• Methyl-seq</li><li>• MCA-seq</li><li>• HELP-seq</li><li>• MSCC</li></ul>
Affinity enrichment	<ul style="list-style-type: none"><li>• MeDIP</li><li>• mDIP</li><li>• mCIP</li><li>• MIRA</li></ul>	<ul style="list-style-type: none"><li>• MeDIP-seq</li><li>• MIRA-seq</li></ul>
Sodium bisulphite	<ul style="list-style-type: none"><li>• BiMP</li><li>• GoldenGate</li><li>• Infinium</li></ul>	<ul style="list-style-type: none"><li>• RRBS</li><li>• BC-seq</li><li>• BSPP</li><li>• WGSBS</li></ul>

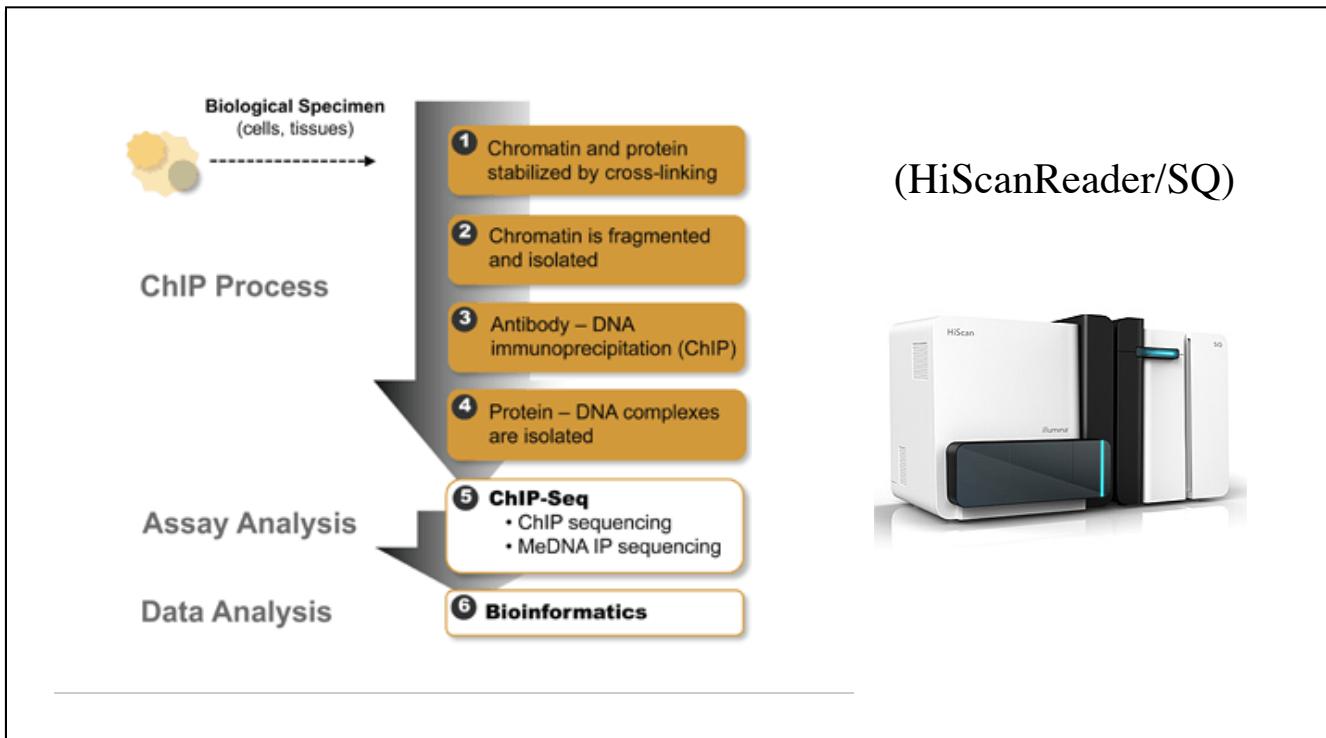
# Infinium technologies

- Many clinical samples:
- Fast
- Reproducible
- Moderate cost
- Low amount of genomic DNA
- DNA methylation and gene expression



# Epigenomic technologies

## Histone marks profiling: ChIP-Seq



# ULB Epigenomics core facility/**EPICS**



(Head: F. Fuks)

**ULB** LABORATORY OF CANCER EPIGENETICS

**EPICS**  
EPIGENOMIC  
CREATIVE SOLUTIONS

HOME | I. EPIGENOMICS | II. TRANSCRIPTOMICS | III. BIOINFO | NGS MACHINES | ENQUIRY/QUOTES | SAMPLE |

## EPIGENOMIC CREATIVE SOLUTIONS

Your Complete & Expert Solution for Epigenomics/Transcriptomics & Bioinformatics

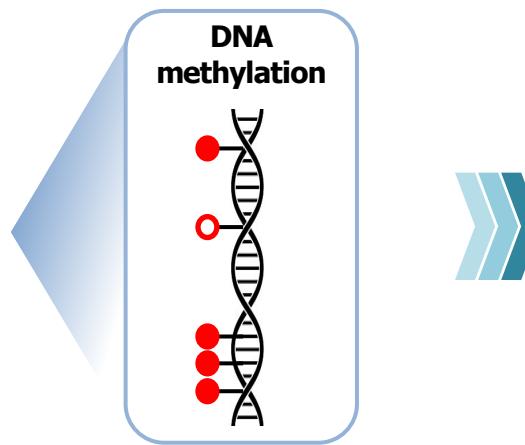
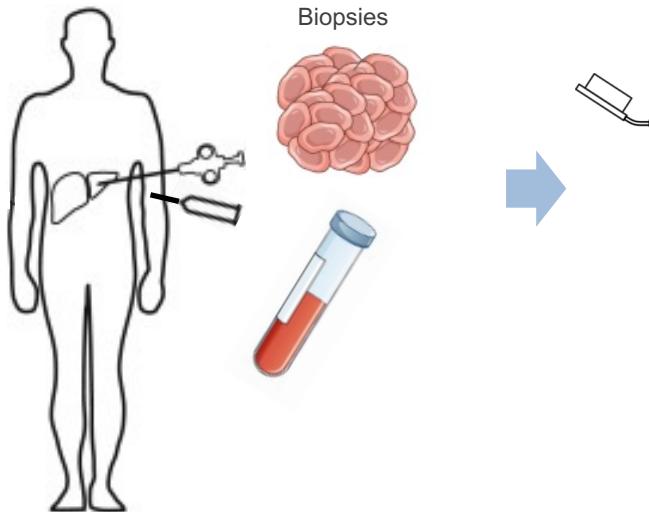
I. Epigenomics      II. Transcriptomics      III. Bioinformatics

(e.g. DNA methylome, ChIP-Seq,  
RNA-Seq)

# **IV. Applications / Translational Cancer Epigenomics**



# DNA methylation profiling



## Clinical applications:

**Detection**

**Prognosis**

**Therapy**

**Monitoring**

**Etiology**

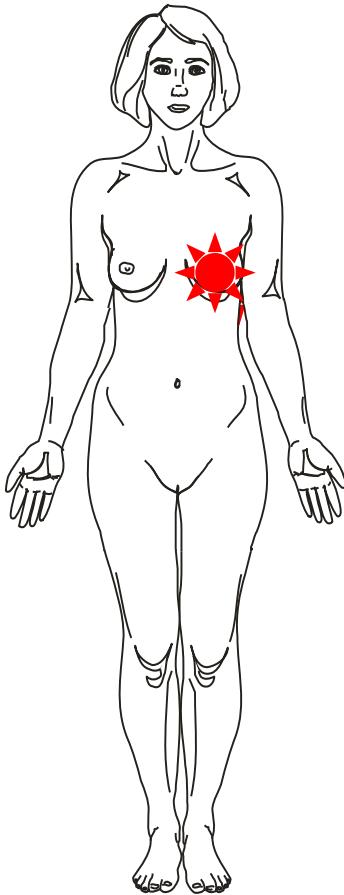
**Prevention**

**Biology**

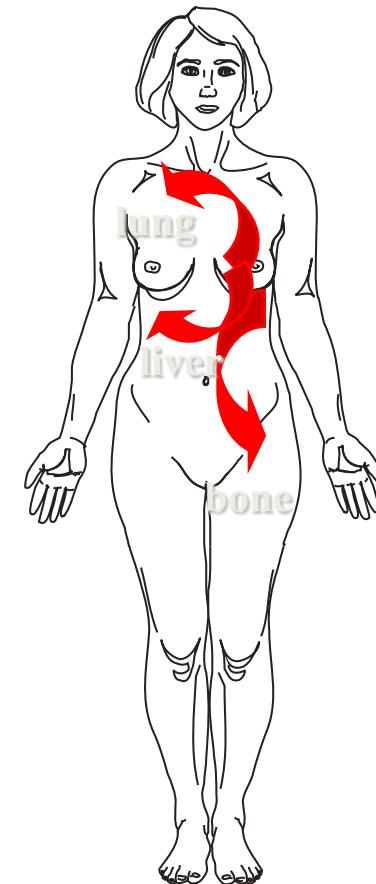
- Stable (DNA)
- Early occurring
- Sensitive techniques

# Breast cancer

**Breast cancers = « several diseases »**



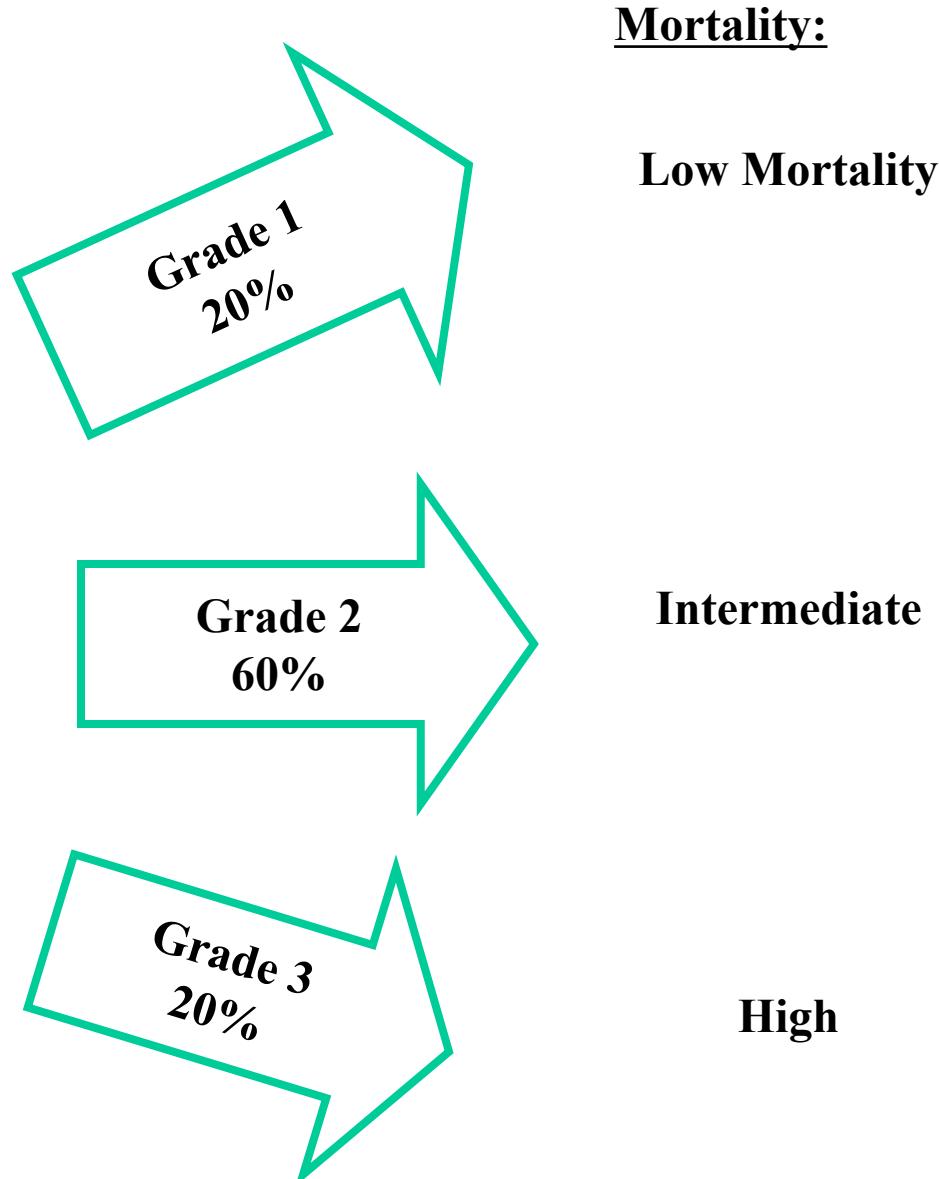
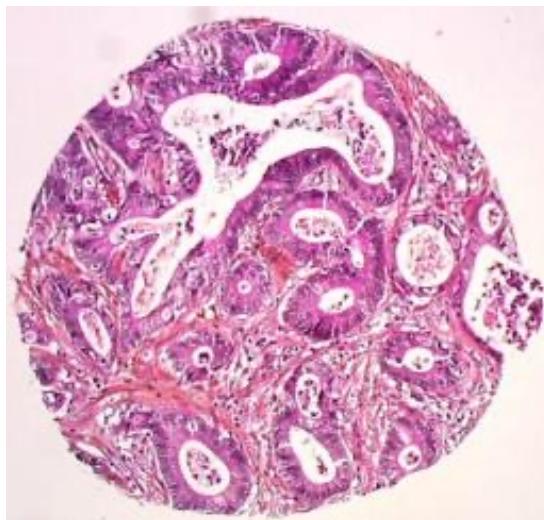
**Women with similar  
clinico-pathological  
characteristics  
can have very different  
clinical outcome**



**Metastasis**

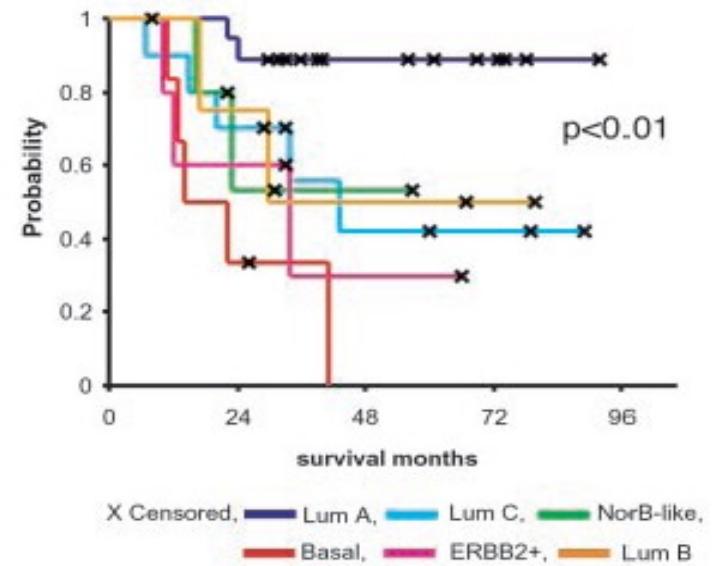
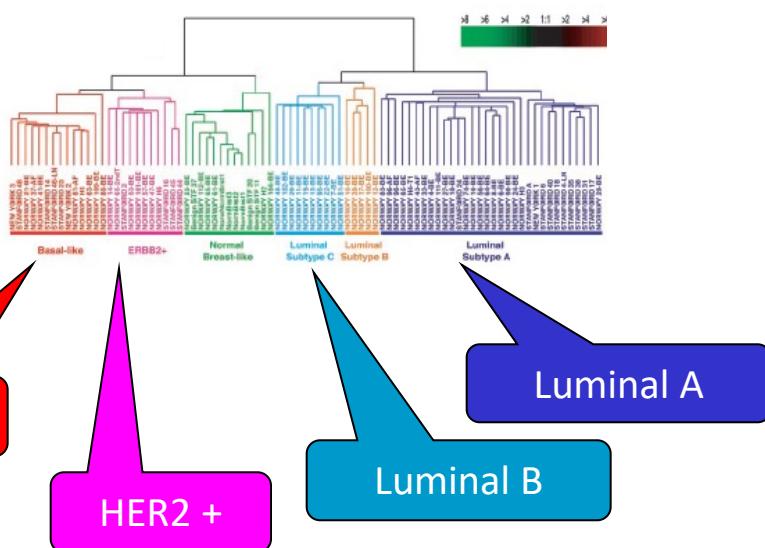
# Breast cancer

## Histological Grade



# Breast cancer

## Gene expression (*microarray*)



4 «expression subtypes»

# Breast cancer

## DNA methylation profiling reveals a predominant immune component in breast cancers

EMBO  
Molecular Medicine

Sarah Dedeurwaerder<sup>1†</sup>, Christine Desmedt<sup>2†</sup>, Emilie Calonne<sup>1</sup>, Sandeep K. Singhal<sup>2</sup>, Benjamin Haibe-Kains<sup>2,3</sup>, Matthieu Defrance<sup>1</sup>, Stefan Michiels<sup>2</sup>, Michael Volkmar<sup>1</sup>, Rachel Deplus<sup>1</sup>, Judith Luciani<sup>1</sup>, Françoise Lallemand<sup>2</sup>, Denis Larsimont<sup>4</sup>, Jérôme Toussaint<sup>2</sup>, Sandy Haussy<sup>2</sup>, Françoise Rothé<sup>2</sup>, Ghislaine Rouas<sup>2</sup>, Otto Metzger<sup>2</sup>, Samira Majjaj<sup>2</sup>, Kamal Saini<sup>2</sup>, Pascale Putmans<sup>1</sup>, Gérald Hames<sup>5</sup>, Nicolas van Baren<sup>6</sup>, Pierre G. Coulie<sup>5</sup>, Martine Piccart<sup>7</sup>, Christos Sotiriou<sup>2\*\*\*†</sup>, François Fuks<sup>1\*†</sup>

248 tissue samples

**MAIN SET OF PATIENTS:**  
**123 breast tissues**



**(VALIDATION SET OF PATIENTS:**  
**125 breast tissues)**

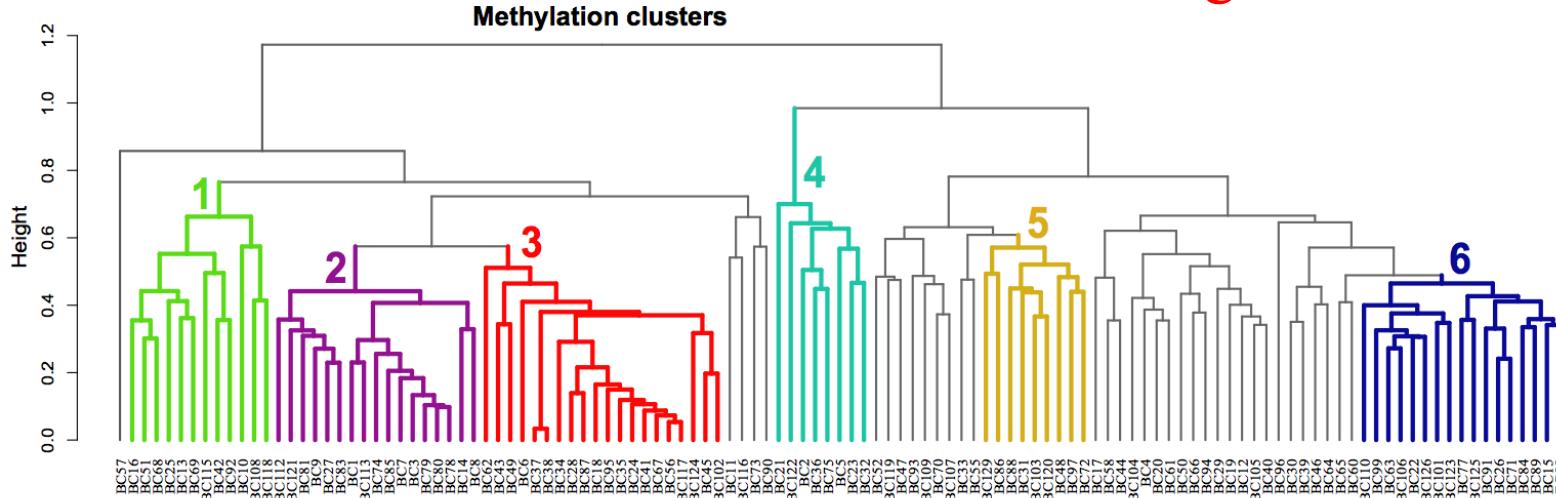
Infinium Methylation



# Breast cancer

## Six DNA methylation groups of breast tumours

Refining tumor *taxonomy*

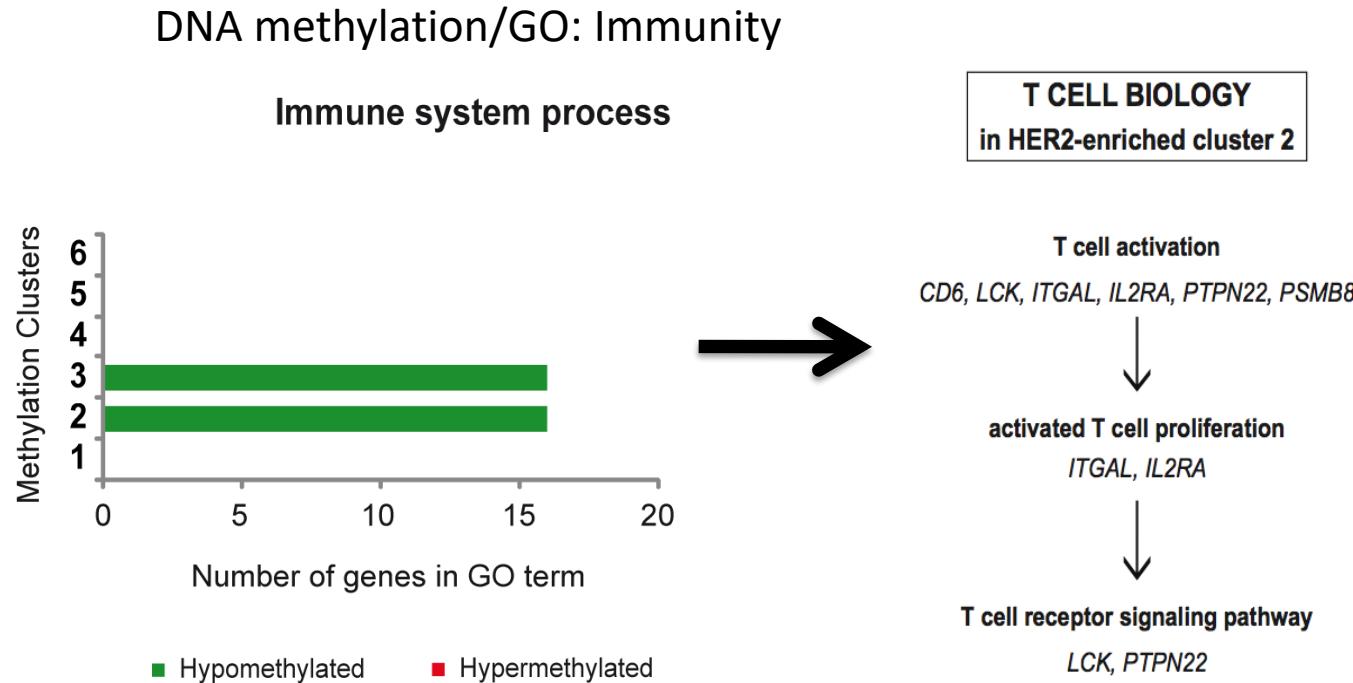


- 3 known expression subtypes: 2 ≈ HER2 ; 3 ≈ Basal-like ; 6 ≈ Luminal A
- 3 NEW subtypes: 1, 4 and 5

Similar data in independent validation set (125 samples)

# Breast cancer

## Methylation clusters show distinct biological functions



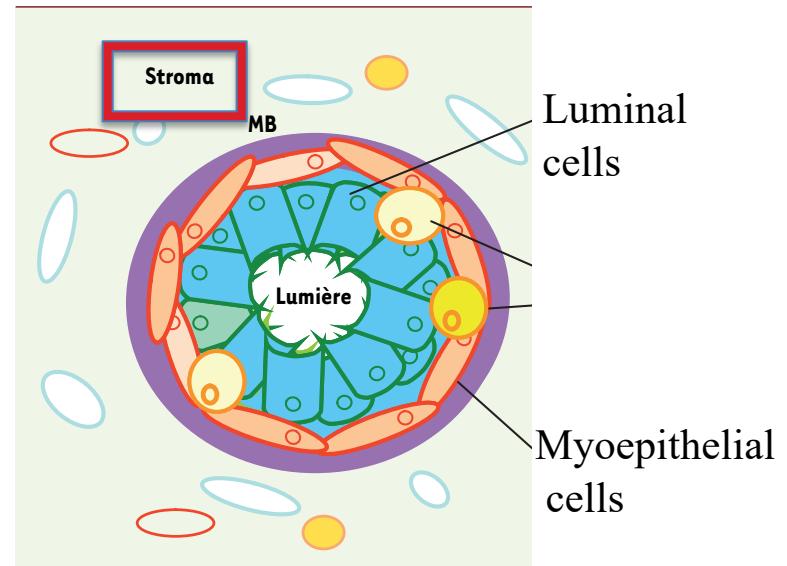
Is methylation of immune genes in clusters 2 and 3 due to tumor infiltration by immune cells (e.g. lymphocytes)?

# Breast cancer

## Tumor Microenvironment

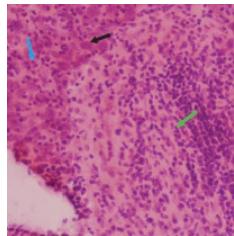
### Whole tumor breast tissues:

1. Epithelial tumor cells (mainly)
2. Cells from the surrounding stroma, including immune cells



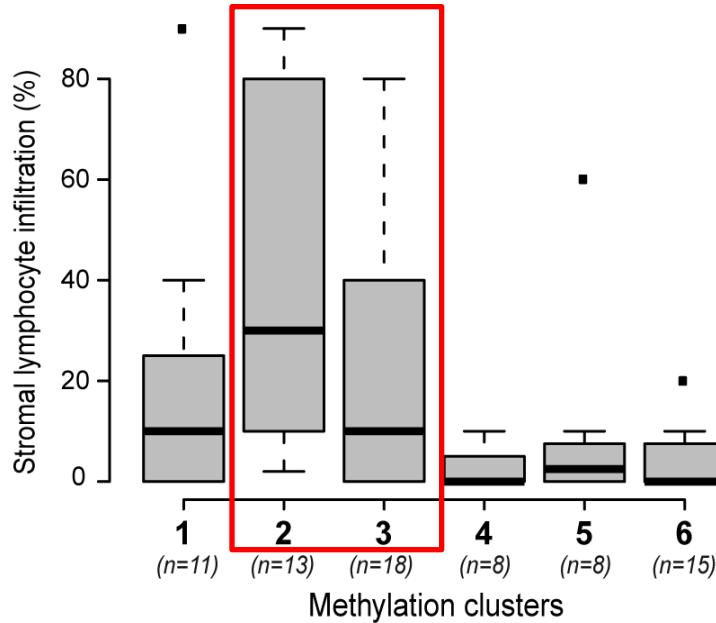
# Breast cancer

## Hypomethylation of immune genes in clusters 2 and 3 reflects a lymphocyte infiltration

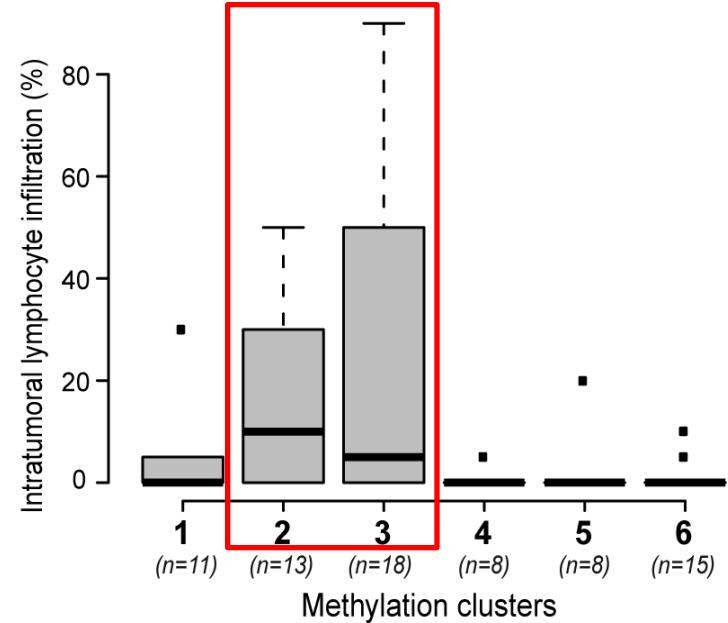


Correlating DNA methylation and pathological TIL readings

Stromal lymphocyte infiltration



Intratumoral lymphocyte infiltration



# DNA methylation profiling

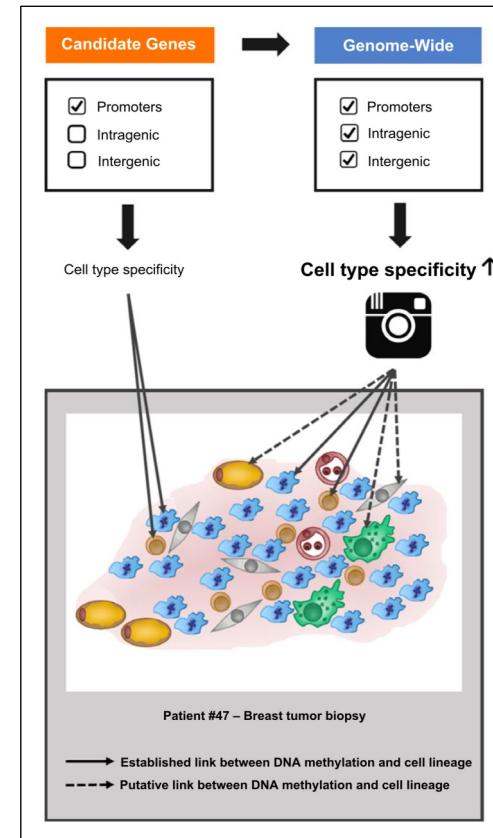
**the FEBS Journal**

**DNA methylome profiling beyond promoters – taking an epigenetic snapshot of the breast tumor microenvironment**

Jana Jeschke, Evelyne Collignon and François Fuks

Laboratory of Cancer Epigenetics, Université Libre de Bruxelles, Brussels, Belgium

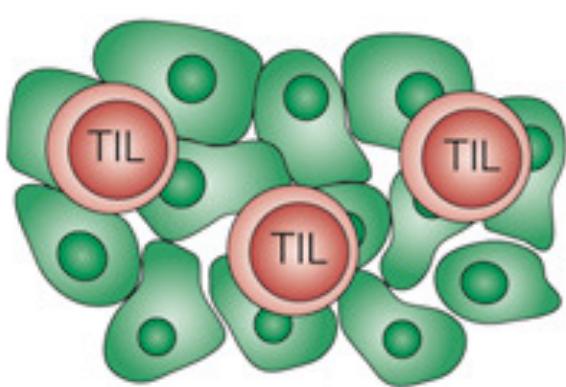
The diagram illustrates the process of DNA methylation profiling. It starts with a 'Stem cell' at the top, which undergoes 'LINEAGE SPECIFICATION' (indicated by a downward arrow) to become three different cell types: 'Cell type 1' (yellow), 'Cell type 2' (red), and 'Cell type 3' (green). Each cell type has specific DNA methylation patterns (represented by colored dots) around 'Gene X'. An arrow points from the stem cell to a 'Patient #47 – Breast tumor biopsy' sample, which contains a mix of these three cell types. A camera icon indicates the analysis of the biopsy sample. A legend at the bottom right defines the line styles: a solid line for 'Established link between DNA methylation and cell lineage' and a dashed line for 'Putative link between DNA methylation and cell lineage'.



DNA methylation  
to characterize **immune cells** in tumors

# Breast cancer

## Clinical Relevance



**TILs have prognostic and predictive value in breast cancer**

VOLUME 28 • NUMBER 1 • JANUARY 1 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

### Tumor-Associated Lymphocytes As an Independent Predictor of Response to Neoadjuvant Chemotherapy in Breast Cancer

*Carsten Denkert, Sibylle Loibl, Aurelia Noske, Marc Röller, Berit Maria Müller, Martina Komor, Jan Budczies, Silvia Darb-Esfahani, Ralf Kronenwett, Claus Hanusch, Christian von Türrn, Wilko Weichert, Knut Engels, Christine Solbach, Iris Schrader, Manfred Dietel, and Gunter von Minckwitz*

VOLUME 31 • NUMBER 7 • MARCH 1 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Prognostic and Predictive Value of Tumor-Infiltrating Lymphocytes in a Phase III Randomized Adjuvant Breast Cancer Trial in Node-Positive Breast Cancer Comparing the Addition of Docetaxel to Doxorubicin With Doxorubicin-Based Chemotherapy: BIG 02-98

*Sherene Loi, Nicolas Sirtaine, Fanny Piette, Roberto Salgado, Giuseppe Viale, Françoise Van Eeeno, Ghislaine Rouas, Prudence Francis, John P.A. Crown, Erika Hitre, Evandro de Azambuja, Emmanuel Quinaux, Angelo Di Leo, Stefan Michiels, Martine J. Piccart, and Christos Sotiriou*

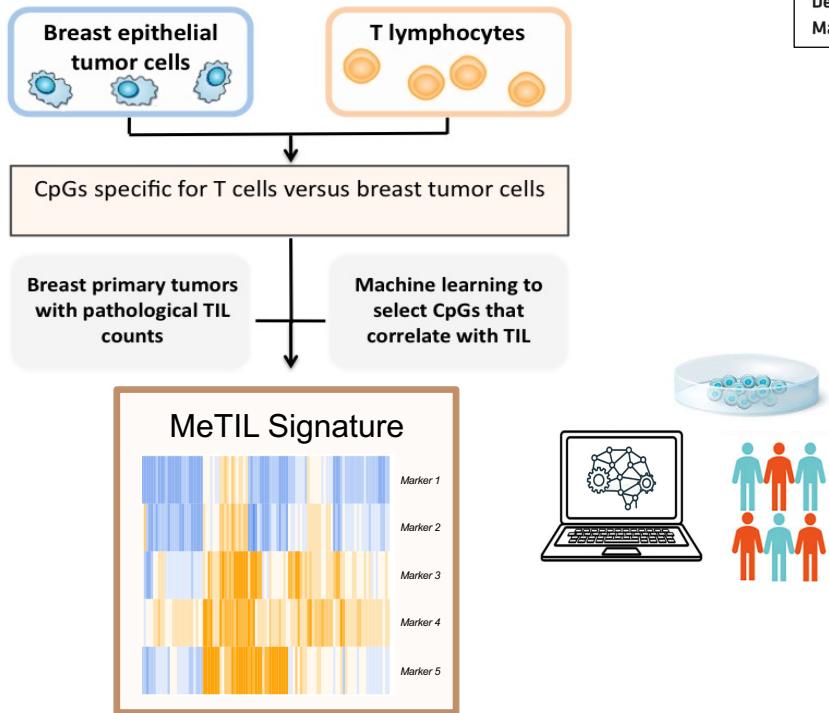
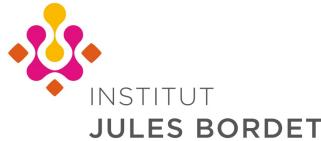
RESEARCH ARTICLE

Open Access

**Tumor-infiltrating lymphocytes predict response to anthracycline-based chemotherapy in estrogen receptor-negative breast cancer**

Nathan R West<sup>1,2</sup>, Katy Milne<sup>1</sup>, Pauline T Truong<sup>3</sup>, Nicol Macpherson<sup>4</sup>, Brad H Nelson<sup>1,2,5,6</sup> and Peter H Watson<sup>1,2,\*</sup>

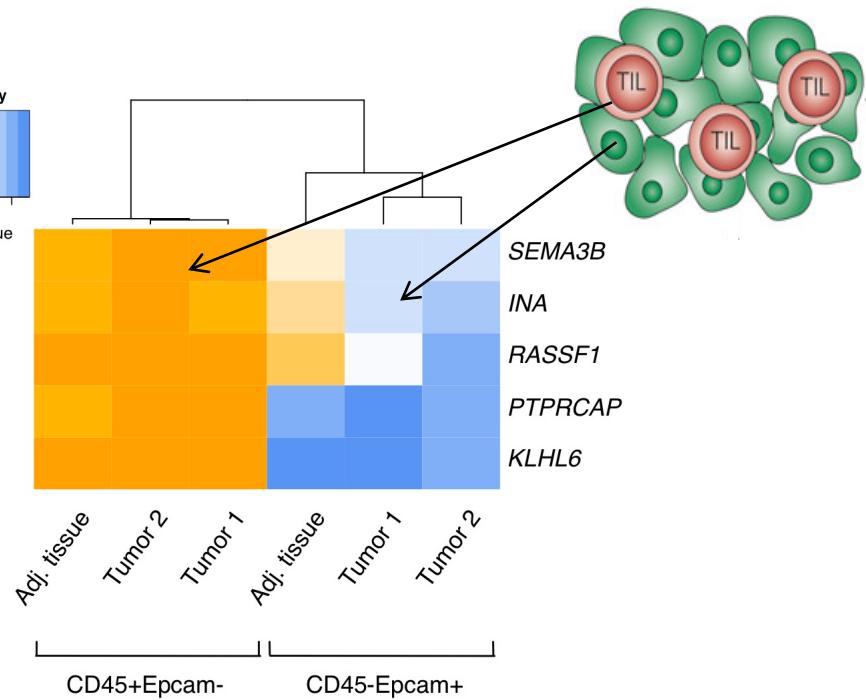
# Breast cancer



**JCI** The Journal of Clinical Investigation

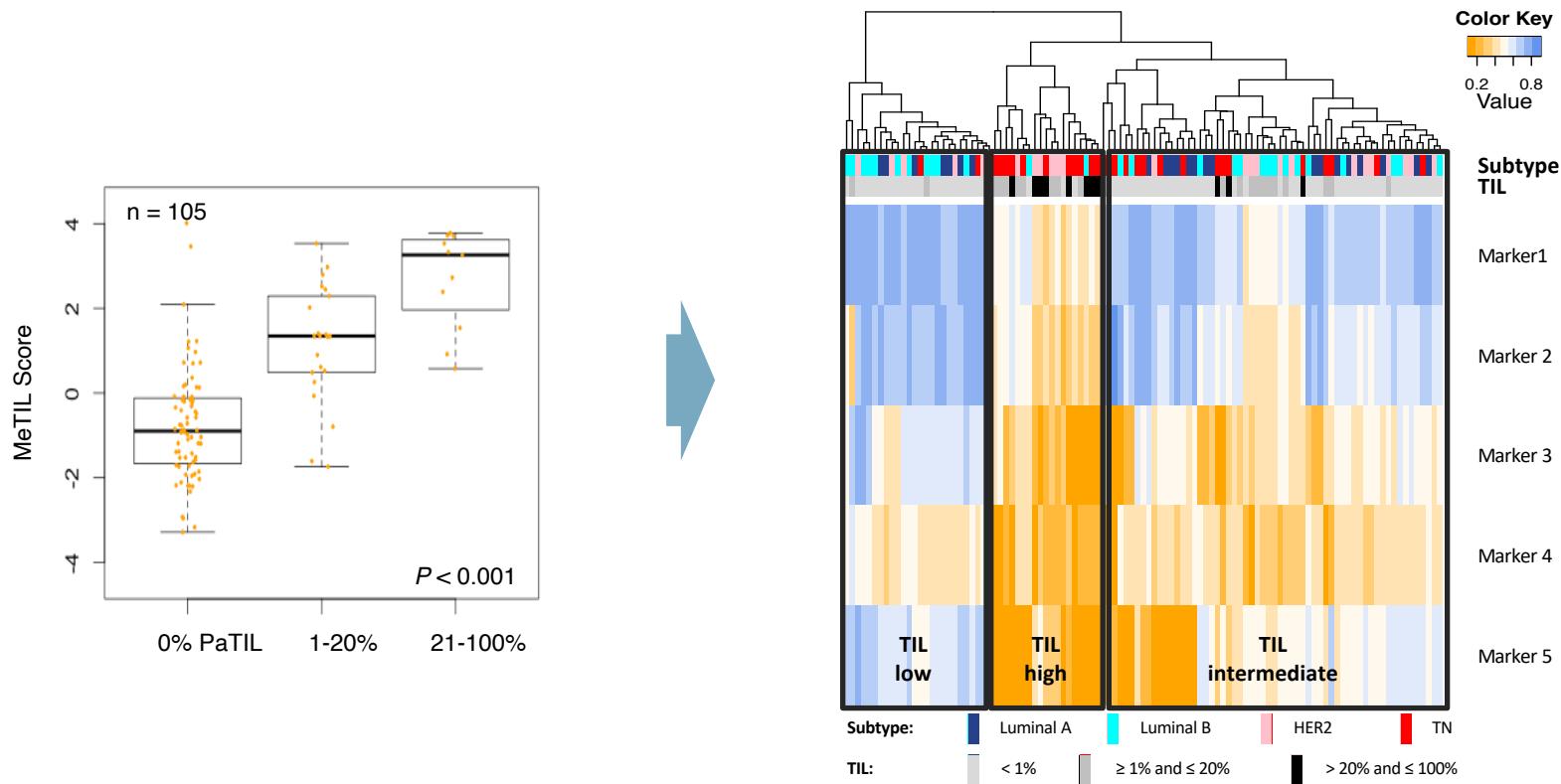
## DNA methylation-based immune response signature improves patient diagnosis in multiple cancers

Jana Jeschke,<sup>1</sup> Martin Bizet,<sup>1,2,3</sup> Christine Desmedt,<sup>4</sup> Emilie Calonne,<sup>1</sup> Sarah Dedeurwaerder,<sup>1</sup> Soizic Garaud,<sup>5</sup> Alexander Koch,<sup>6</sup> Denis Larsimont,<sup>4</sup> Roberto Salgado,<sup>4</sup> Gert Van den Eynden,<sup>4</sup> Karen Willard Gallo,<sup>5</sup> Gianluca Bontempi,<sup>2,3</sup> Matthieu Defrance,<sup>1,2</sup> Christos Sotiriou,<sup>4</sup> and François Fuks<sup>1</sup>



# Breast cancer

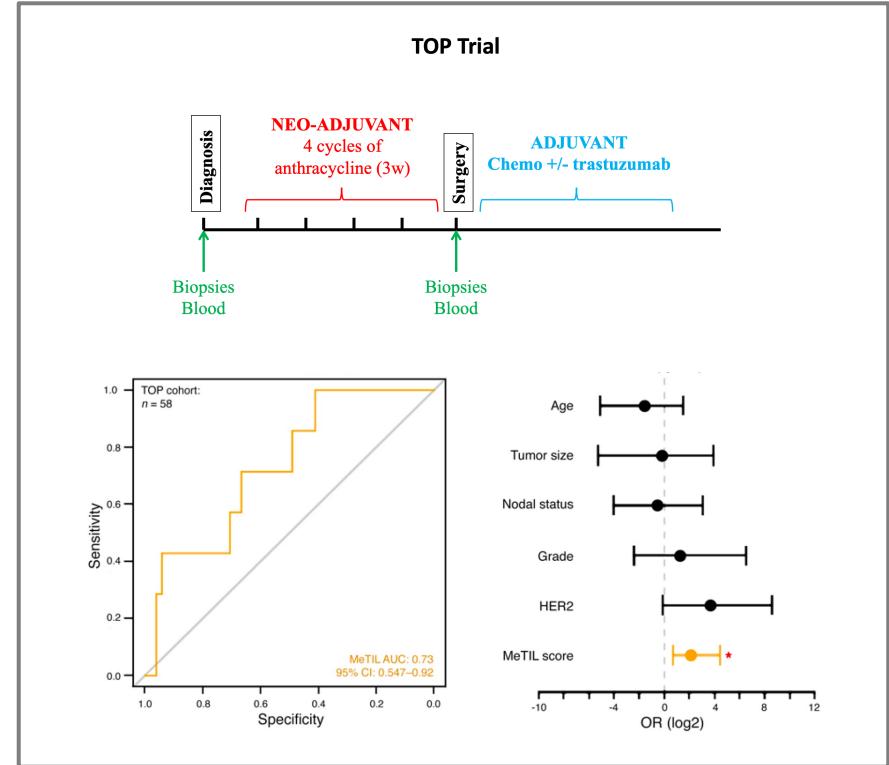
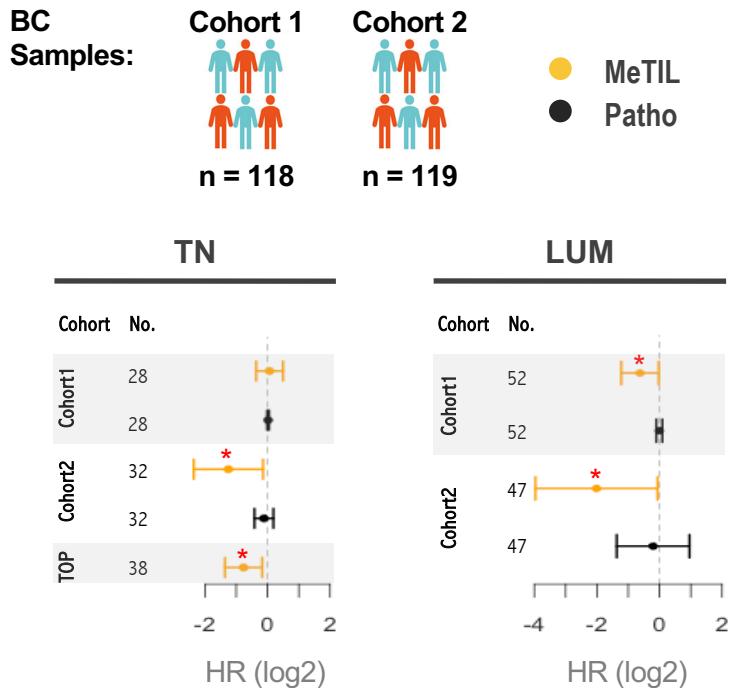
## Subgrouping with MeTIL



MeTIL groups breast tumors based on TILs

# Breast cancer

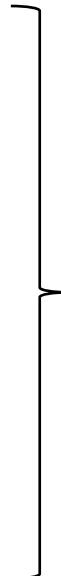
## Prognosis with MeTIL



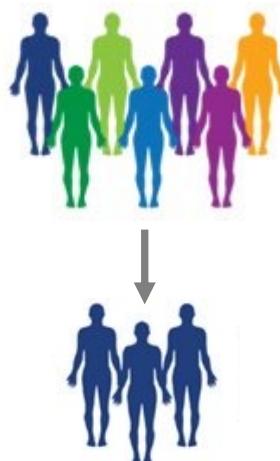
MeTIL measures TIL-based tumor **immune response** and its prognostic value

# Breast cancer

**TREATBEST Project**



MeTIL signature to stratify patients  
for cancer therapy



**TREATBEST** “TREATment of Breast cancer  
with Epigenetic Specific Tools”



Interaction with  
academica, clinic, industry!

diagenode

INSTITUT  
JULES BORDET

ULB

IDDI  
Institute for Data-Driven Innovation

ULg  
UNIVERSITÉ de Liège

# Perspectives on DNA methylation

## 1. DNA methylome: not just for cancers

### Type 2 Diabetes

The EMBO Journal (2012) 31, 1405–1426 | © 2012 European Molecular Biology Organization | Some rights reserved 0261-4189/12  
[www.embojournal.org](http://www.embojournal.org)

**DNA methylation profiling identifies epigenetic dysregulation in pancreatic islets from type 2 diabetic patients**

**Michael Volkmar<sup>1</sup>, Sarah Dedeurwaerder<sup>1</sup>, Daniel A Cunha<sup>2</sup>, Matladi N Ndlovu<sup>1</sup>, Matthieu Defrance<sup>1</sup>, Rachel Deplus<sup>1</sup>, Emilie Calonne<sup>1</sup>, Ute Volkmar<sup>3</sup>, Mariana Igoillo-Esteve<sup>2</sup>, Najib Naamane<sup>2</sup>, Silvia Del Guerra<sup>1</sup>, Matilde Masini<sup>4</sup>, Marco Bugiani<sup>4</sup>, Piero Marchetti<sup>4</sup>, Miriam Cnop<sup>2,5</sup>, Decio L Eizirik<sup>2</sup> and François Fuks<sup>1,\*</sup>**

**Introduction**  
Type 2 diabetes (T2D) has developed into a major public health concern. While previously considered as a problem primarily for western populations, the disease is rapidly gaining global importance, as today around 285 million people are affected worldwide (IDF, 2009). Lifestyle and behavioural factors play an important role in determining T2D risk. For example, experimentally induced intrauterine growth retardation as well as nutrient restriction during

### Neurological disease

**REVIEW**

**nature  
medicine**

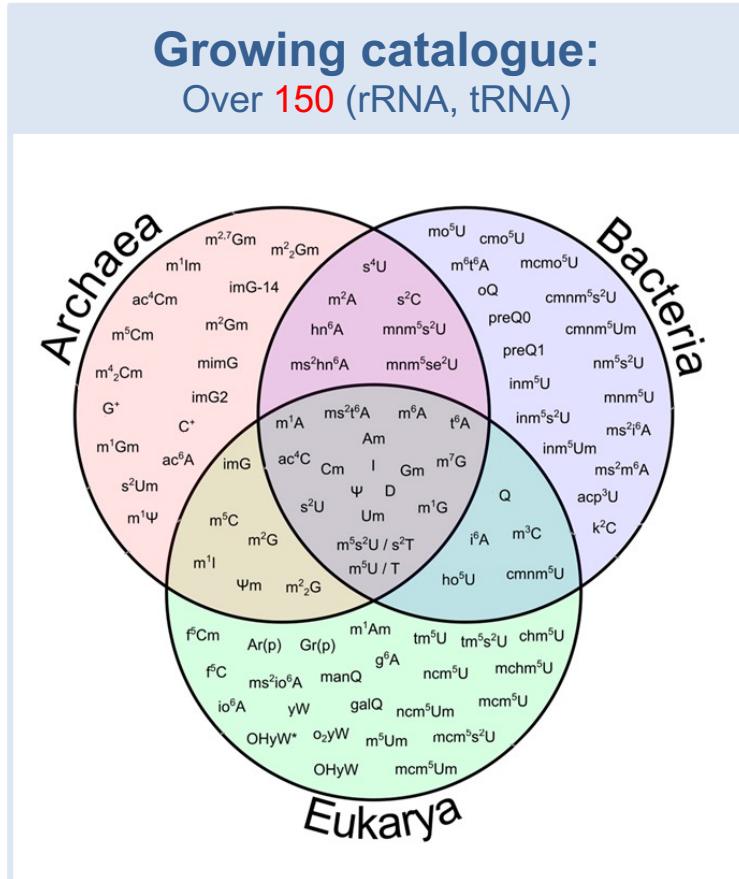
**Epigenetic mechanisms in neurological disease**

Mira Jakovcevski & Schahram Akbarian

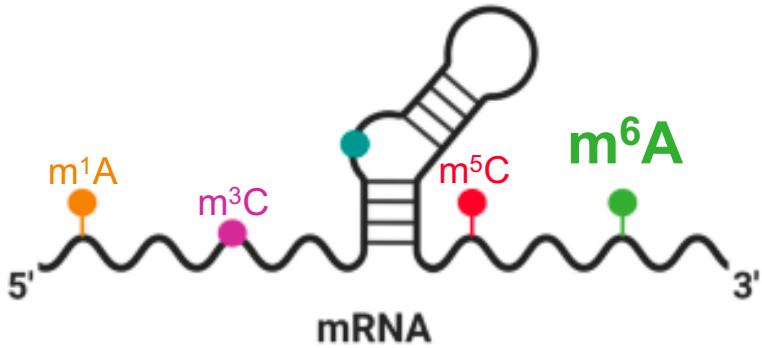
# V. A new epigenetic layer



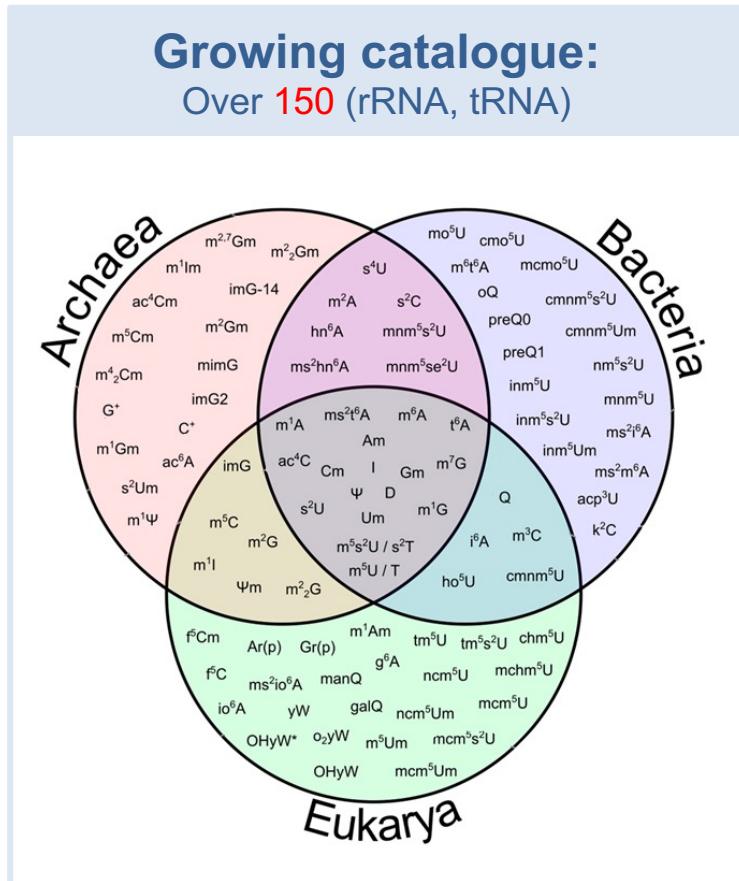
# A new epigenetic pillar: RNA modifications



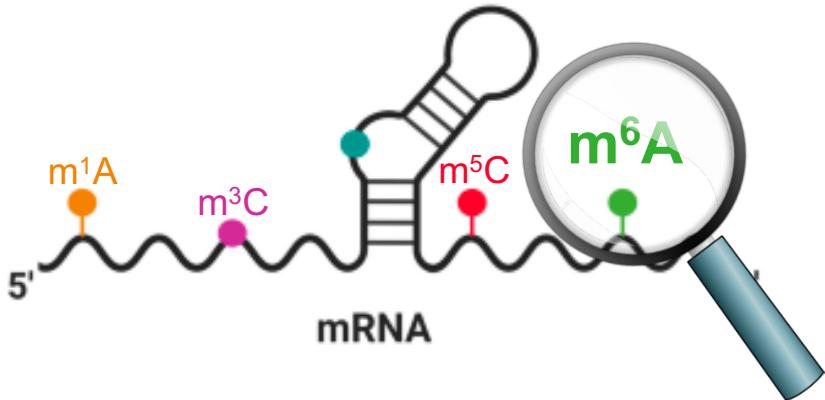
**A few found in mRNA:**



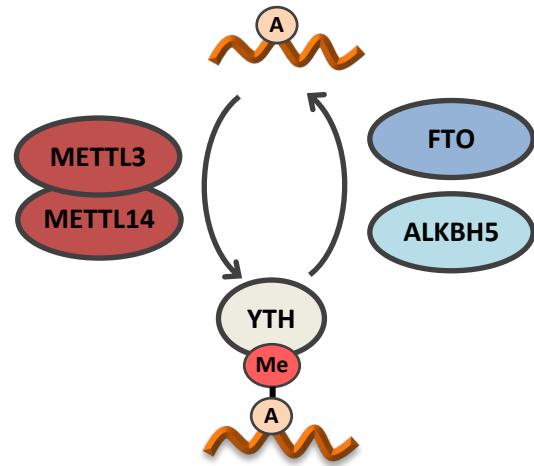
# A new epigenetic pillar: RNA modifications



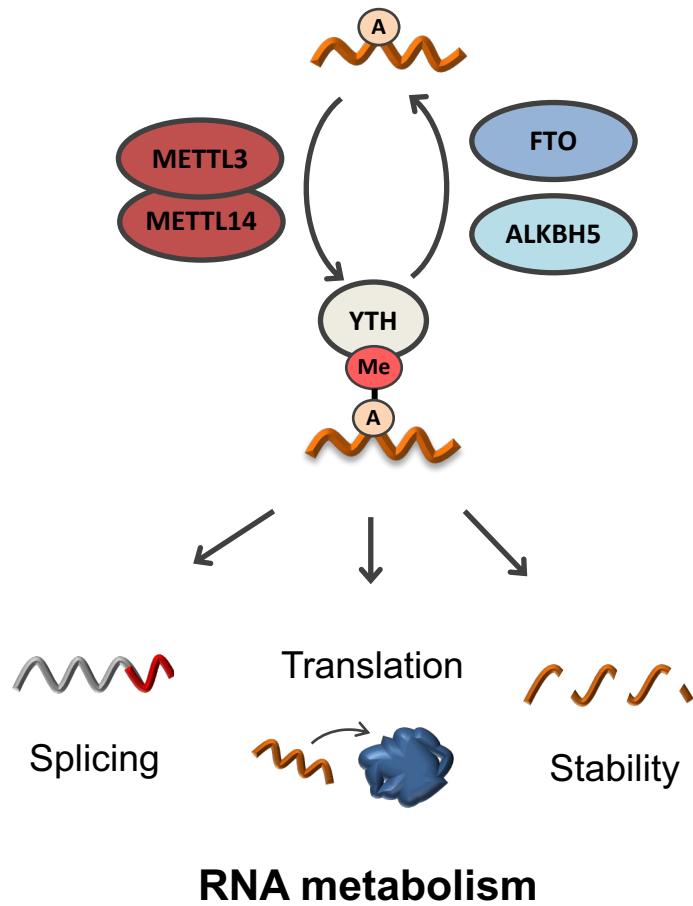
**A few found in mRNA:**



# RNA modifications: m6A



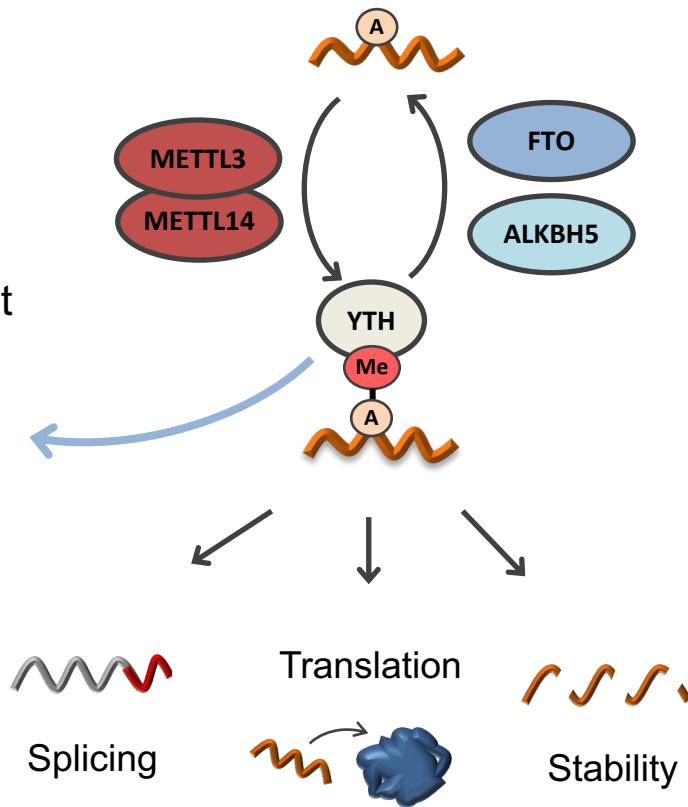
# RNA modifications: m6A



# RNA modifications: m6A

## Physiologic Roles

Stem cell pluripotency  
Embryonic development  
Neurodevelopment  
Reproductive development  
Cell homeostasis  
Innate immunity  
Adaptive immunity



RNA metabolism

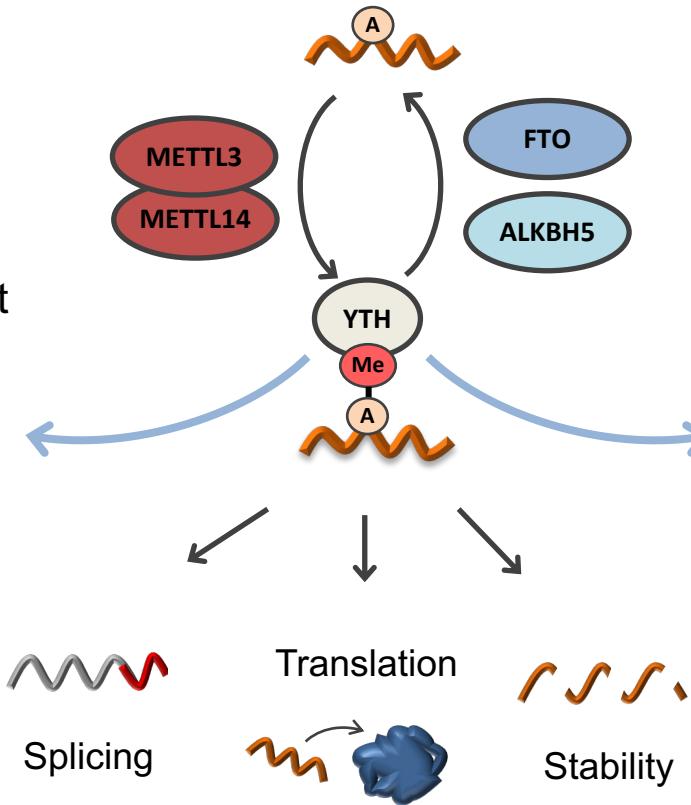
# RNA modifications: m6A

## Physiologic Roles

Stem cell pluripotency  
Embryonic development  
Neurodevelopment  
Reproductive development  
Cell homeostasis  
Innate immunity  
Adaptive immunity

## Pathologic Roles

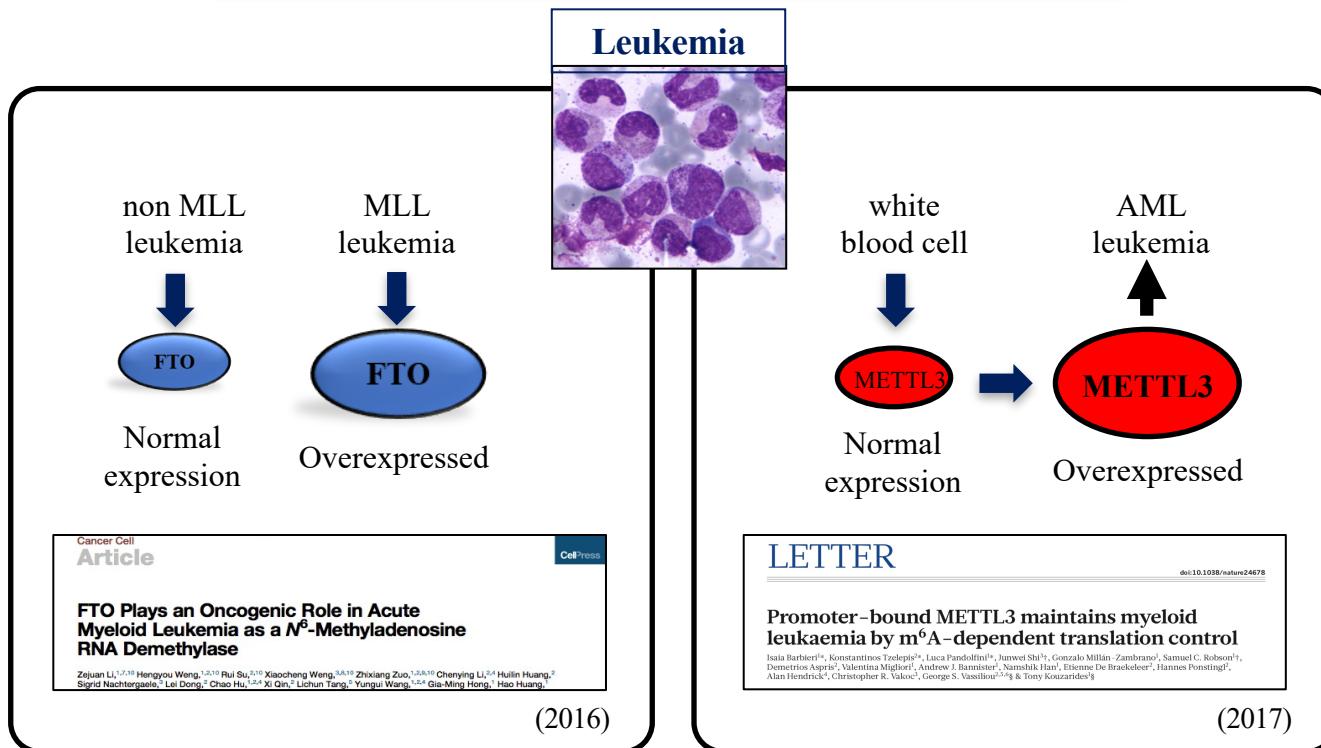
Inflammation  
Autoimmune disease  
Degeneration  
Angiogenesis  
Fibrosis  
Microorganism infection  
Regeneration  
**Cancer**



RNA metabolism

# RNA modifications: m6A

## First insights: m6A in leukemia



METTL3↑ or FTO↑ promote cancer

# RNA modifications: m6A

## m6A & Breast Cancer

**nature cancer**

**ARTICLES**  
<https://doi.org/10.1038/s43018-021-00223-7>

**Downregulation of the FTO m<sup>6</sup>A RNA demethylase promotes EMT-mediated progression of epithelial tumors and sensitivity to Wnt inhibitors**

Jana Jeschke<sup>1,2,4</sup>, Evelyne Collignon<sup>1,2,4</sup>, Clémence Al Wardi<sup>1,2,4</sup>, Mohammad Krayem<sup>2,24</sup>, Martin Bizet<sup>1</sup>, Yan Jia<sup>1,3</sup>, Soizic Garaud<sup>4</sup>, Zéna Wimana<sup>2,5</sup>, Emilie Calonne<sup>1</sup>, Bouchra Hassabi<sup>1</sup>, Renato Morandini<sup>2</sup>, Rachel Deplus<sup>1</sup>, Pascale Putmans<sup>1</sup>, Gaurav Dube<sup>1</sup>, Nitesh Kumar Singh<sup>1</sup>, Alexander Koch<sup>6</sup>, Kateryna Shostak<sup>7</sup>, Lara Rizzotto<sup>8</sup>, Robert L. Ross<sup>9</sup>, Christine Desmedt<sup>10</sup>, Yacine Bareche<sup>10</sup>, Françoise Rothé<sup>10</sup>, Jacqueline Lehmann-Che<sup>11,12</sup>, Martine Duterque-Coquillaud<sup>13</sup>, Xavier Leroy<sup>13,14</sup>, Gerben Menschaert<sup>15</sup>, Luis Teixeira<sup>11,12</sup>, Mingzhou Guo<sup>16</sup>, Patrick A. Limbach<sup>17</sup>, Pierre Close<sup>17,18</sup>, Alain Chariot<sup>21</sup>, Eleonora Leucci<sup>8,19</sup>, Ghanem Ghanem<sup>2</sup>, Bi-Feng Yuan<sup>20</sup>, Karen Willard-Gallo<sup>24</sup>, Christos Sotiriou<sup>10</sup>, Jean-Christophe Marine<sup>21,22</sup> and François Fuks<sup>1,23</sup>



**CANCER BIOLOGY**

**nature cancer**

**news & views**

**A Wnt twist in FTO's role in cancer progression**

FTO, an m<sup>6</sup>A RNA demethylase, is known mainly as an oncogene in various cancer types. FTO is now shown to act as a cancer suppressor in a subset of epithelial tumors through an interplay between epithelial-to-mesenchymal transition and Wnt signaling.

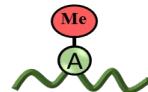
Albertas Navickas and Hani Goodarzi



INSTITUT  
JULES BORDET



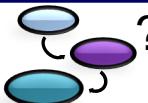
KU LEUVEN



1. Is m6A machinery altered in BC?



2. Effect on tumorigenesis?



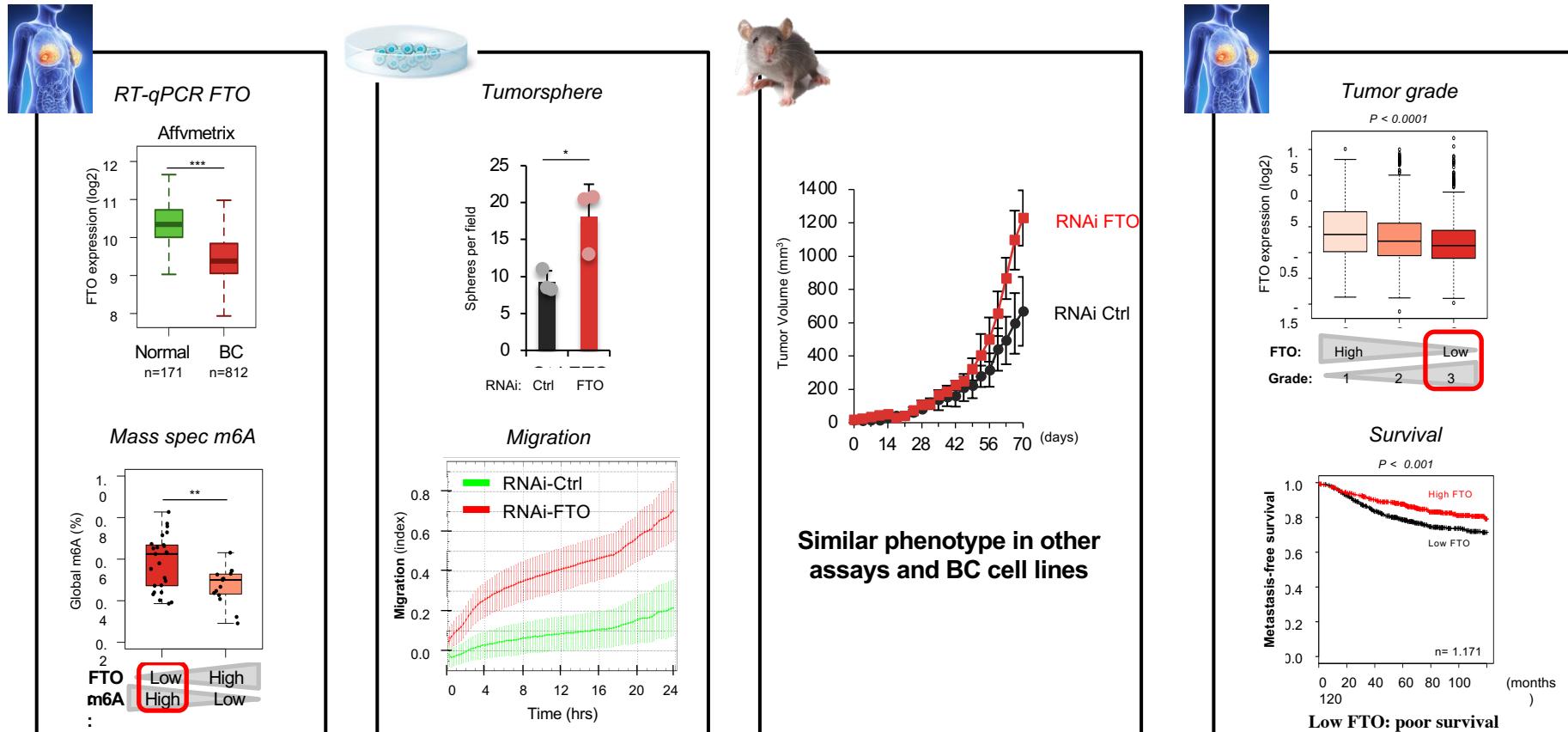
3. Mechanism(s)/Pathways?



4. Clinical relevance?

# RNA modifications: m6A

## Is m6A altered in breast cancer and does it affect tumorigenesis?

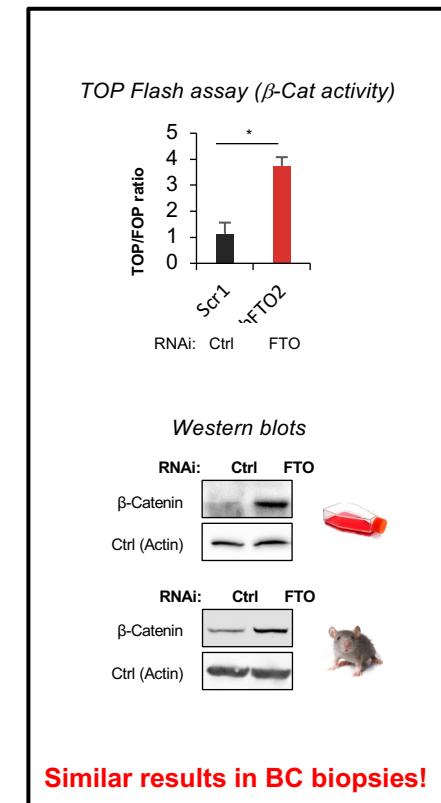
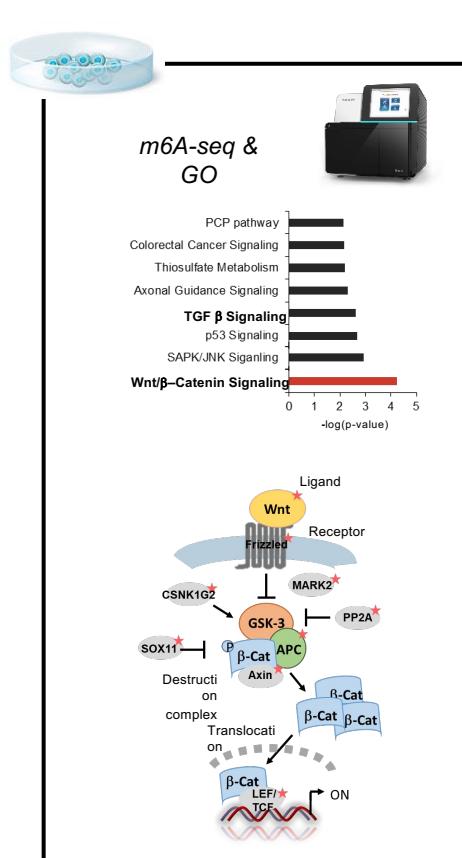
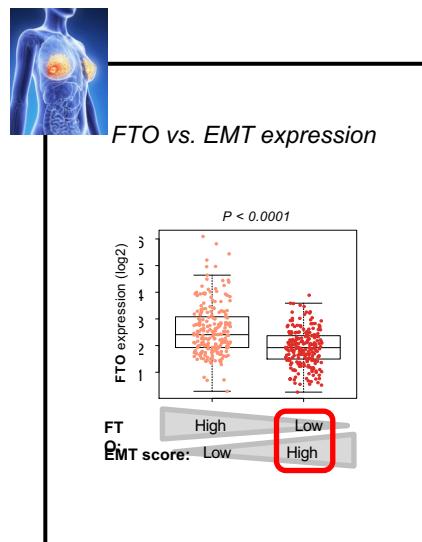
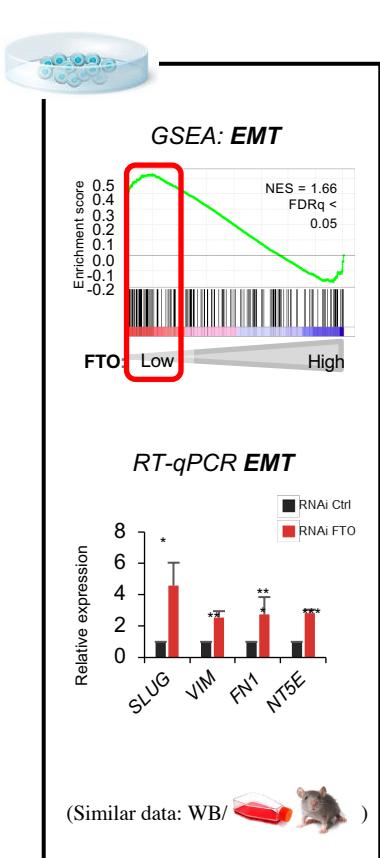


FTO is down and  
m6A up in breast  
cancer

Downregulation of FTO is associated with an aggressive tumor phenotype

# RNA modifications: m6A

## Mechanism(s)/Pathways?

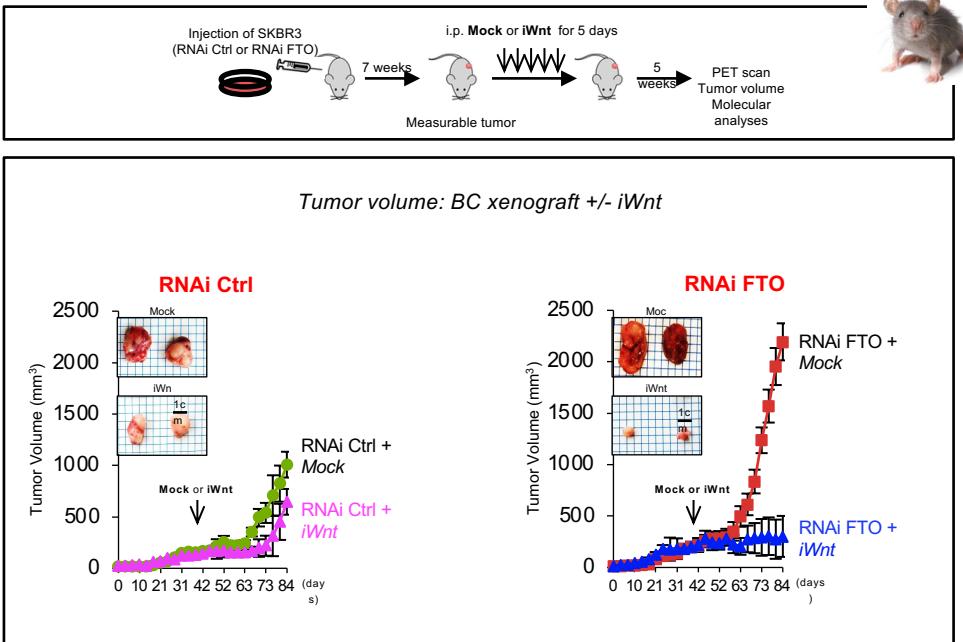
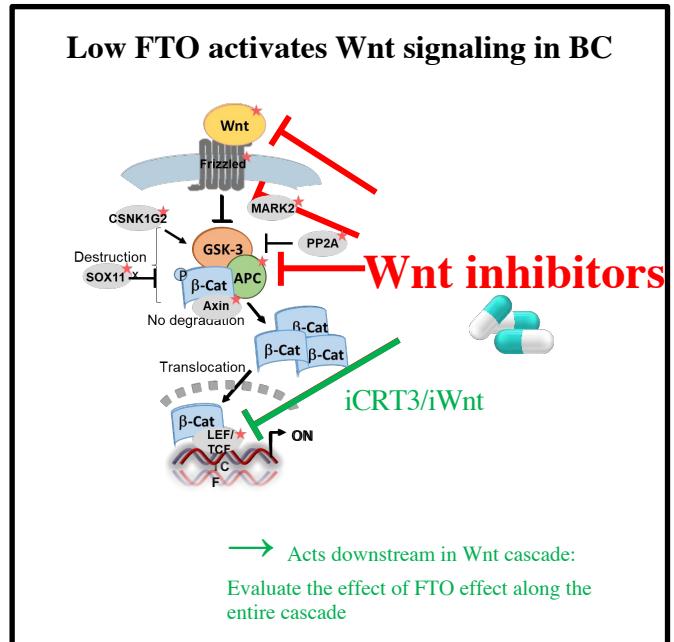


**FTO = novel regulator of EMT**

**Downregulation of FTO leads to upregulation of Wnt/b-Catenin in human breast cancer**

# RNA modifications: m6A

## Clinical Relevance?



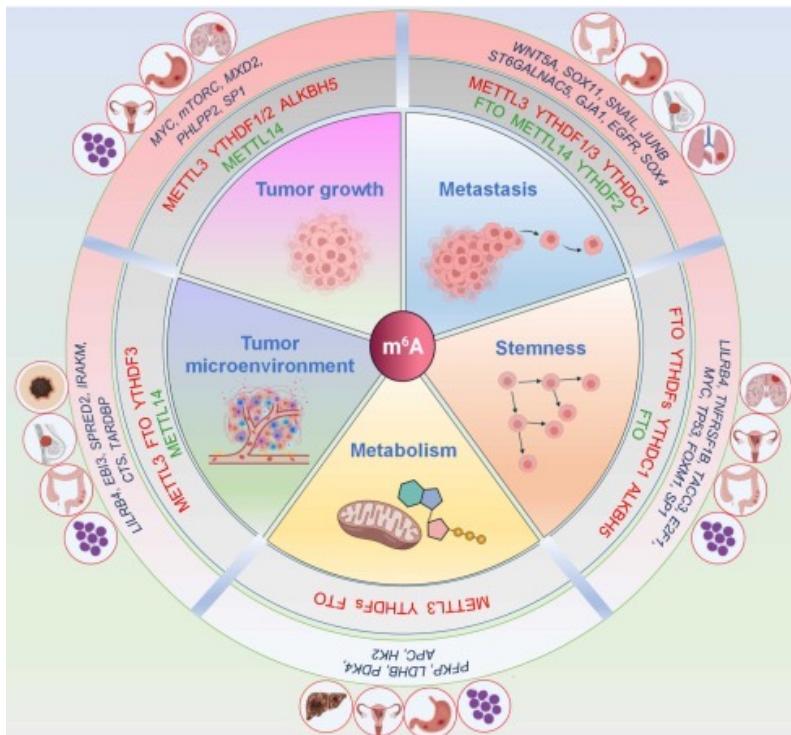
**Does FTO impact sensitivity to Wnt inhibition?**



**FTO depletion strongly potentiates the growth inhibitory effect of iWnt**

# RNA modifications: m6A

## Future Directions



### Dysregulation of m6A and its machinery:

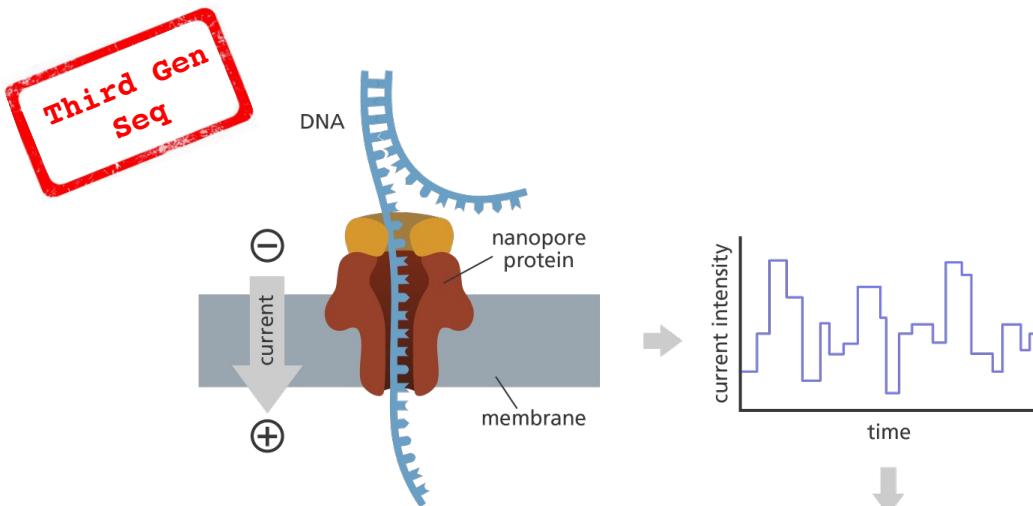
- Observed in many cancers
- Affects many key cancer pathway
- Affects key cancer pathways at the level of and downstream of gene transcription (i.e. translation)
- May have clinical value beyond that of mutation, gene expression and DNA methylation
- Most data generated in cultured cancer cells and tumors grown in mice

Primac et al. *Current opinion in genetics & development* 2022

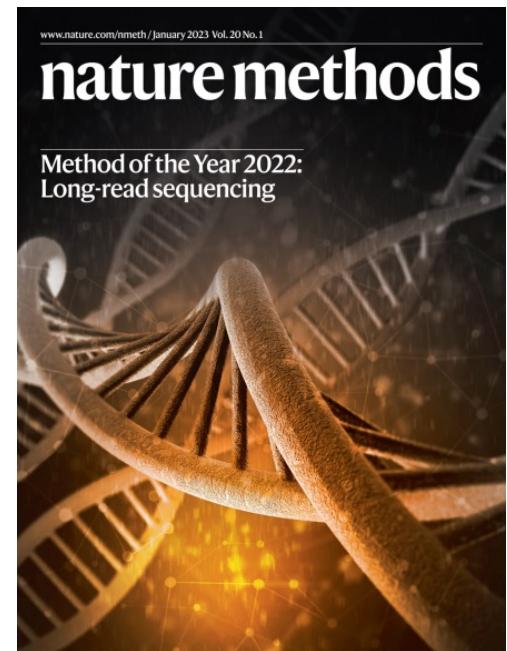


# RNA modifications: m6A

## New technologies: Oxford Nanopore Sequencing

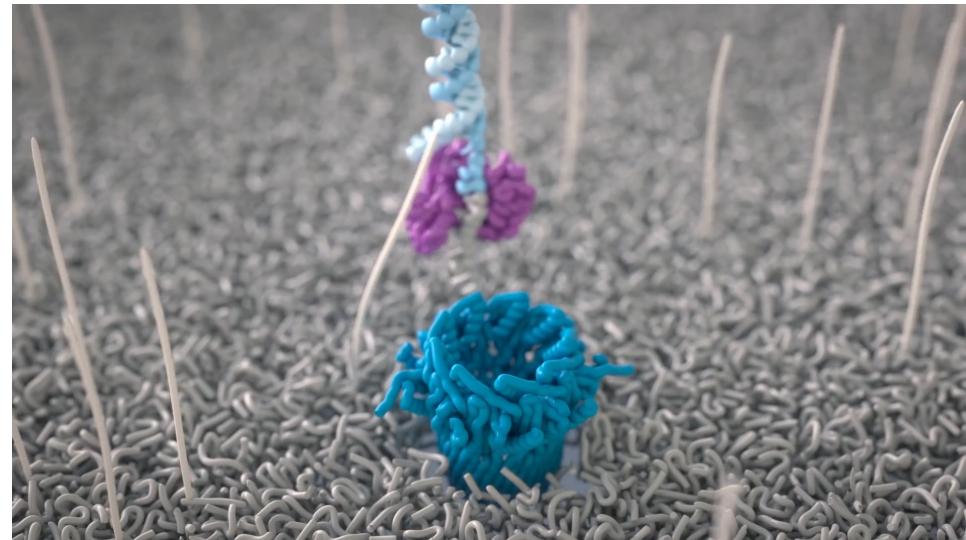


- Long reads (up to 4Mb)
- Native sequencing (no PCR bias)
- Single-molecule and real-time sequencing
- Simplified and high-speed sample prep (no PCR, library in 10 min)
- Simplified bioinformatics analysis
- Low cost, pocket-sized sequencers



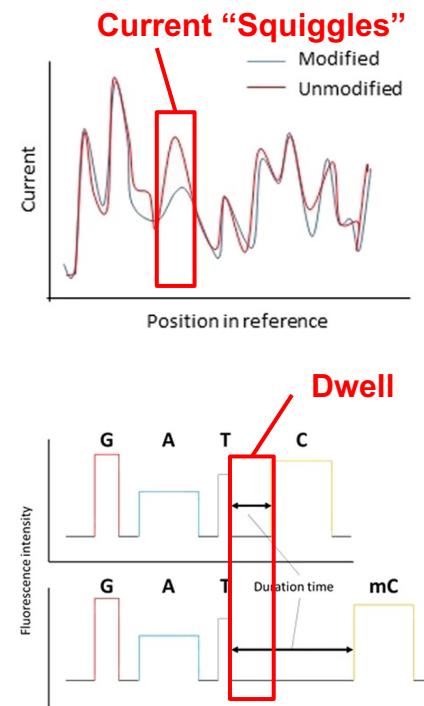
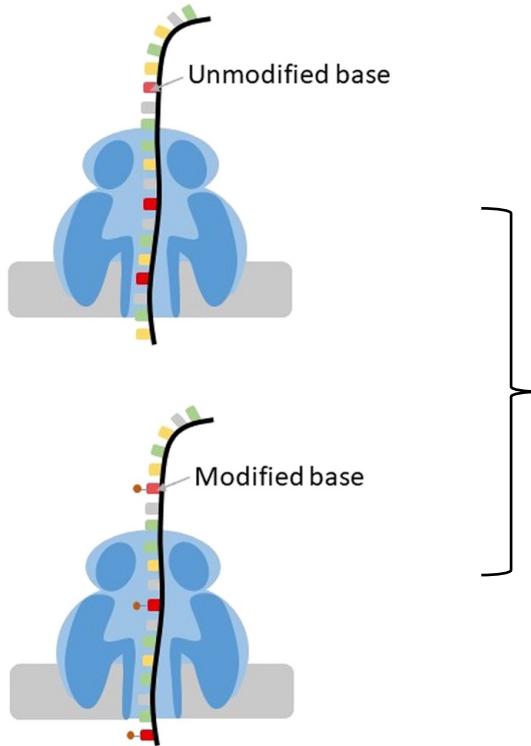
# RNA modifications: m6A

## Oxford Nanopore Sequencing



# RNA modifications: m6A

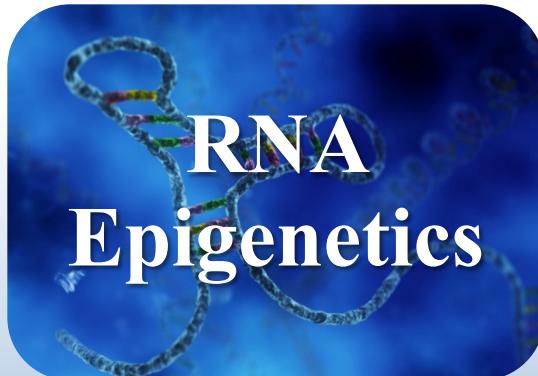
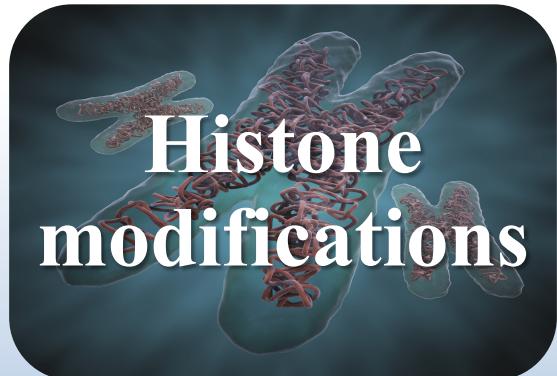
## Oxford Nanopore Sequencing



**Computational  
approach!**



Epigenetics



*Laboratoire d'Epigénétique du Cancer*  
Faculté de Médecine (Campus Erasme)  
*jana.jeschke@ulb.be*



**Please hand me  
your evaluation forms!**

EVALUATION	
<input checked="" type="checkbox"/>	_____
<input checked="" type="checkbox"/>	_____
<input checked="" type="checkbox"/>	_____
<input checked="" type="checkbox"/>	_____