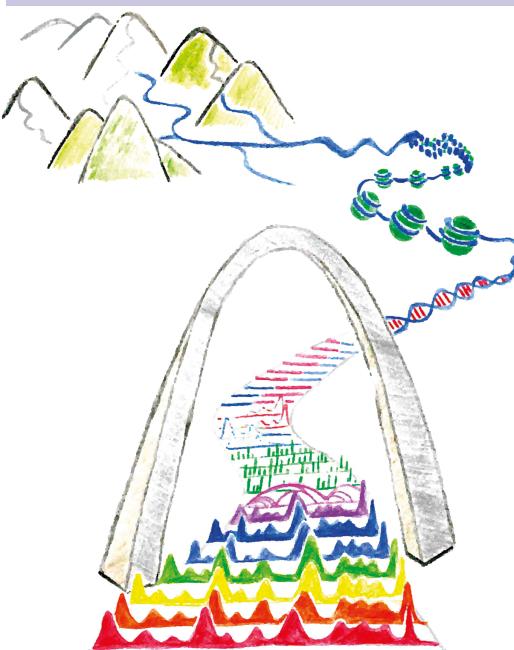


系统生物学与生物信息学  
海外学者短期讲学系列课程

Current Topics in Epigenomics

表观基因组学前沿



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Tsinghua University  
April 15-27

# **Epigenetic Mechanisms**

# Outline

- Epigenetic phenomenon
  - A tale of two mice.
  - Where is the queen bee?
  - The Monarch!
- What is epigenetics/epigenomics?
- Epigenetic mechanisms
- Epigenome in health, in disease and with environment
- Modern epigenomic technology and resources

# A tale of two mice



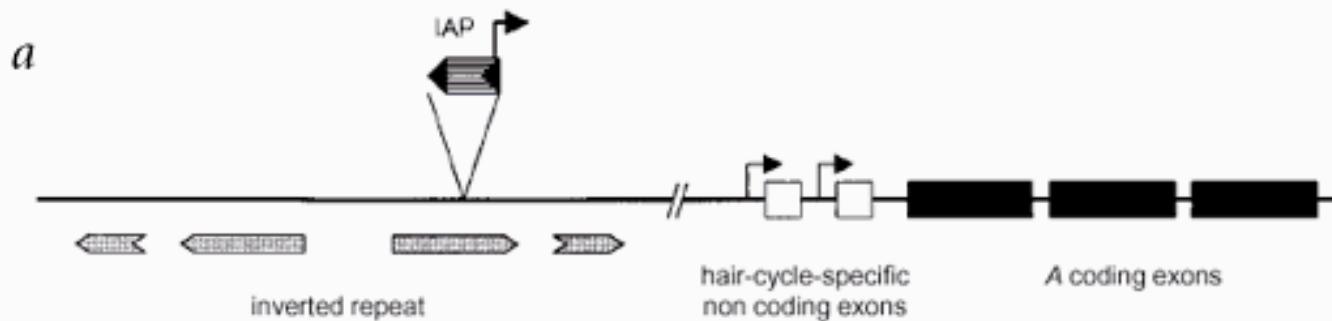
## Yellow mouse

- High risk of cancer, diabetes, obesity;
- Reduced lifespan

## Agouti mouse

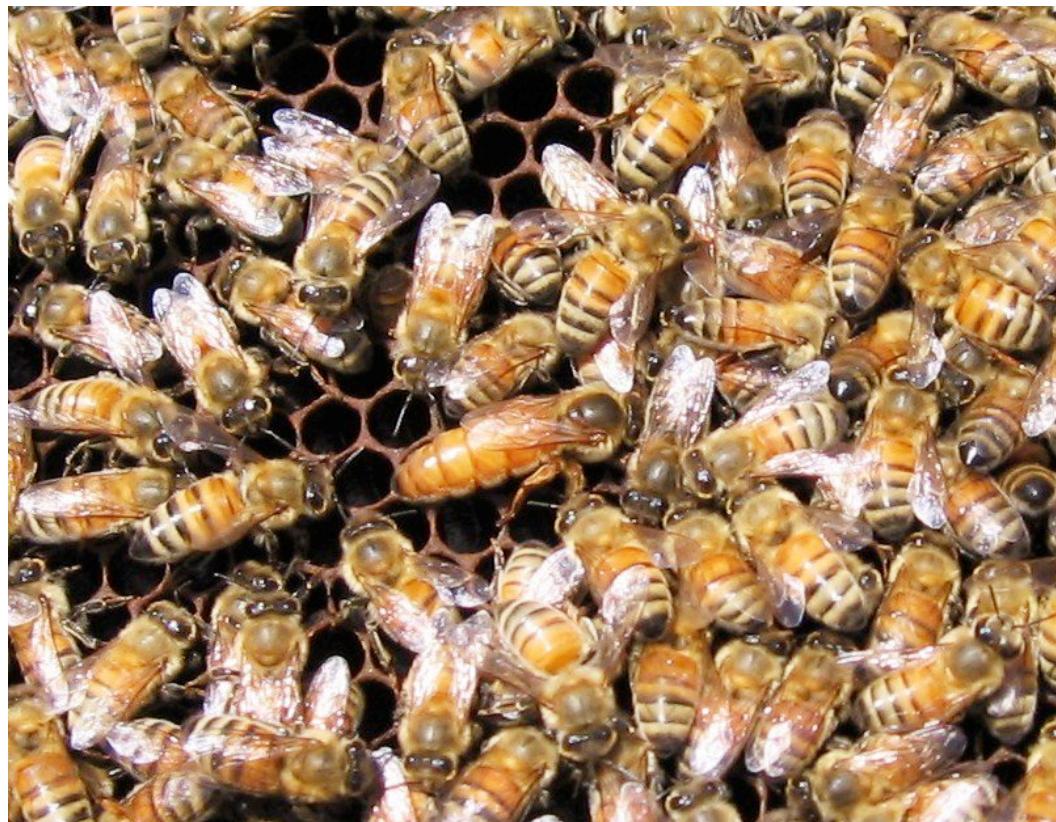
- Low risk of cancer, diabetes, obesity;
- Prolonged lifespan

Maternal supplements  
with Zinc, methionine,  
betaine, choline, folate,  
 $B_{12}$



Morgan, Whitelaw,  
1999  
Waterland, Jirtle,  
2004

# Where is the Queen?



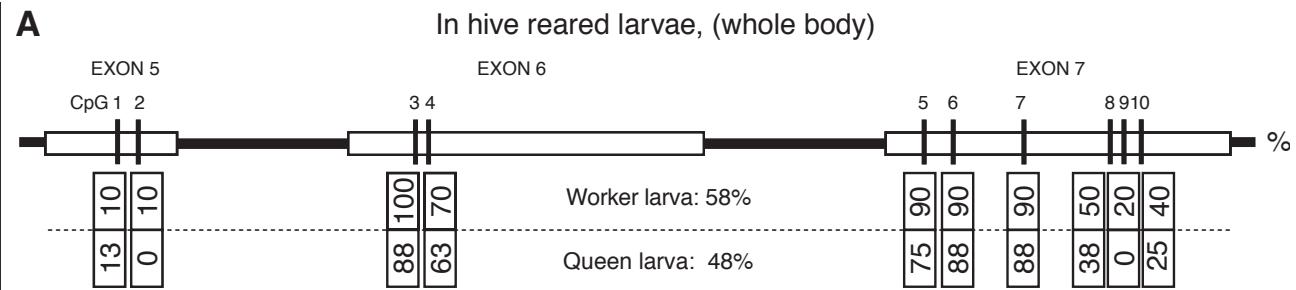
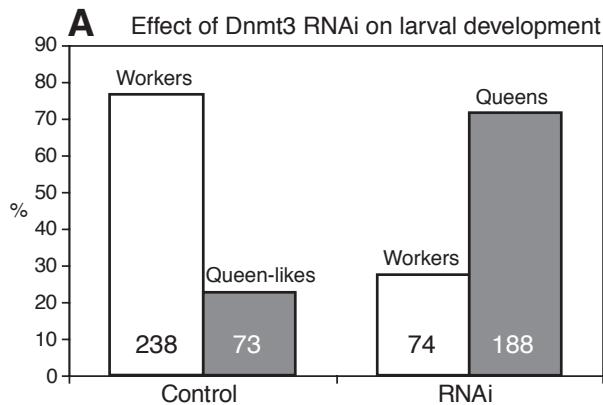
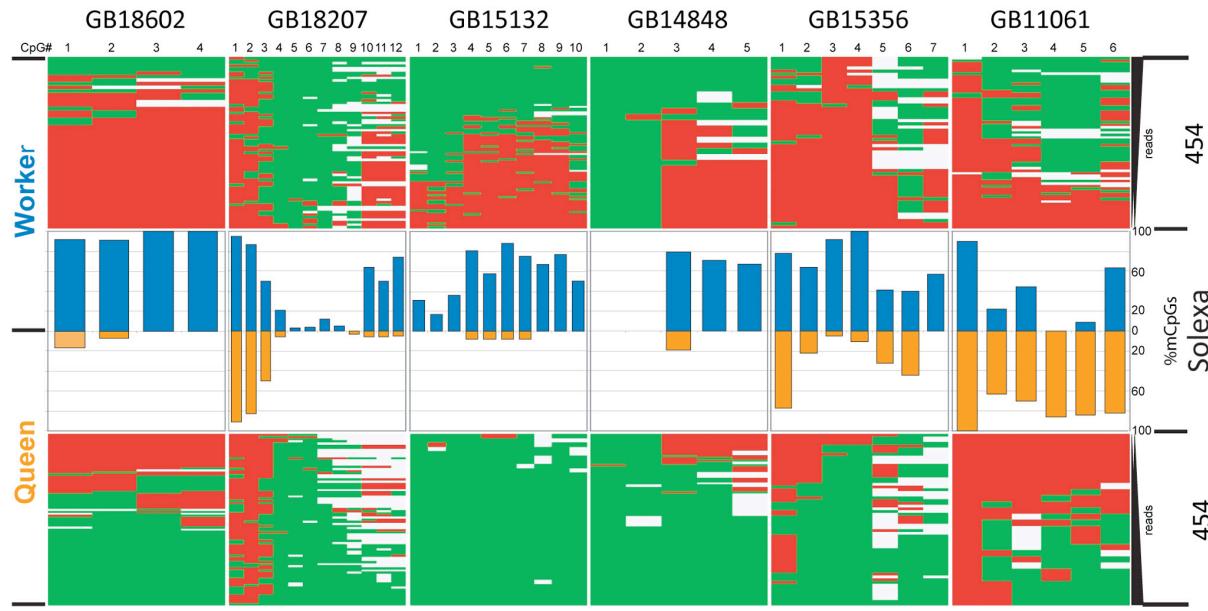
Larvae

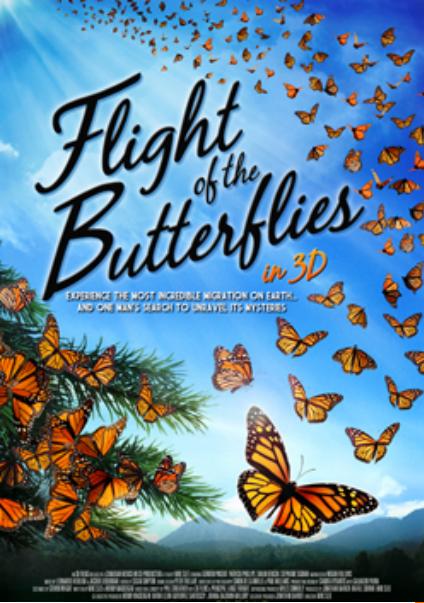


Queen

Worker

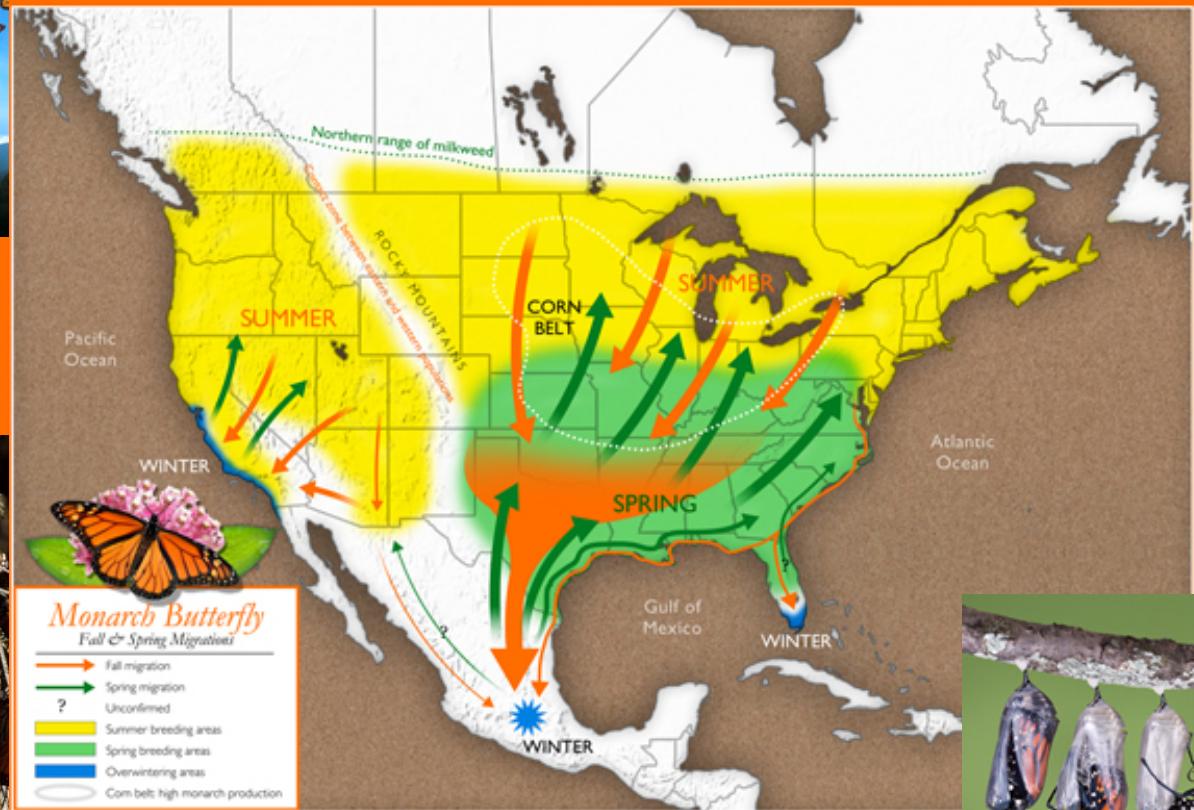
# Workers are more methylated Inhibition of Dnmt3 phenocopies Royal Jelly





# The King of Butterflies

## The Monarch



**Same Genome**

**Different Epigenome**

**Different Phenotype**

# What is Epigenetics/Epigenomics?

- A mitotically or meiotically heritable state of different gene activity and expression (phenotype) that is independent of differences in DNA sequence (genotype) – *based on Conrad Waddington, 1942*
- The sum of the alterations to the chromatin template that collectively establish and propagate different patterns of gene expression (transcription) and silencing from the same genome.
- Epigenetic changes influence the phenotype without altering the genotype.
- While epigenetics often refers to the study of single genes or sets of genes, epigenomics refers to more global analyses of epigenetic changes across the entire **genome**.

# The Epigenome

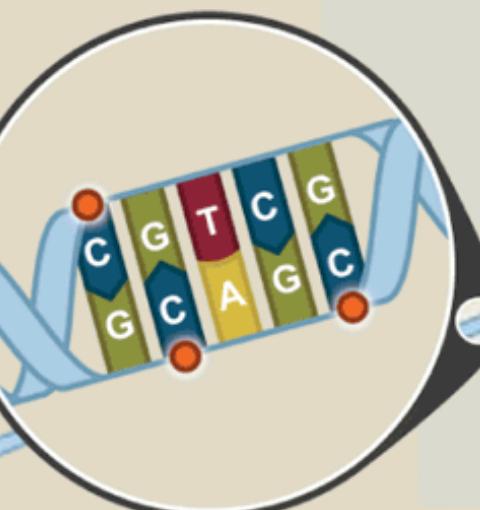
The complete number, location, and types of epigenetic modifications that occur in a given cell.

## The epigenome

DNA methylation



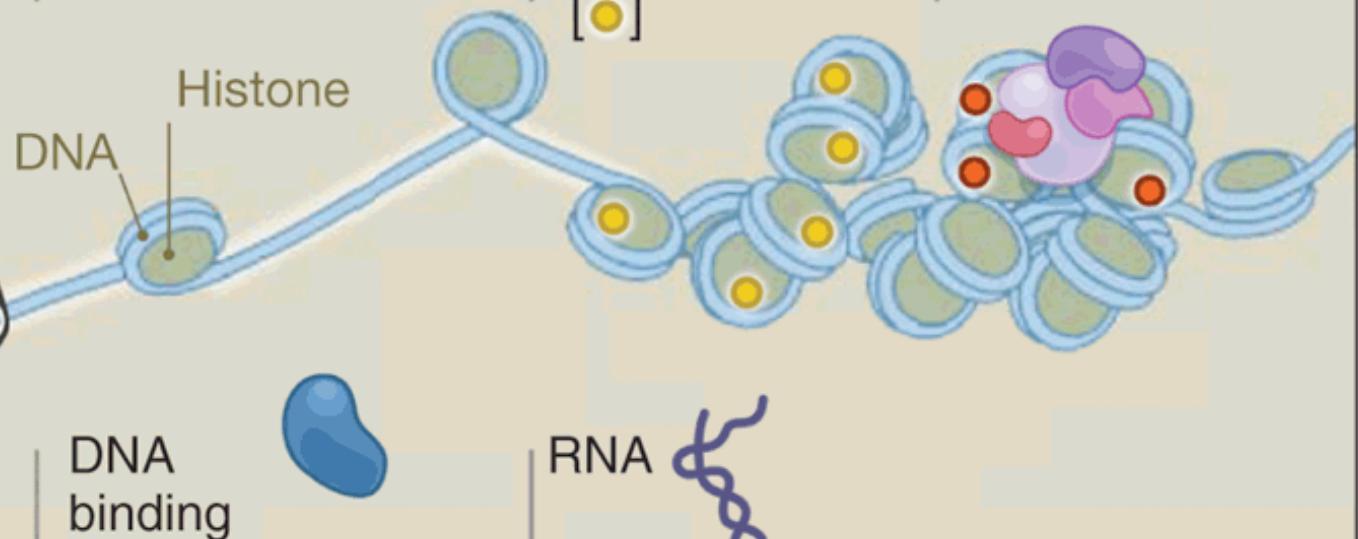
DNA accessibility



Histone modifications



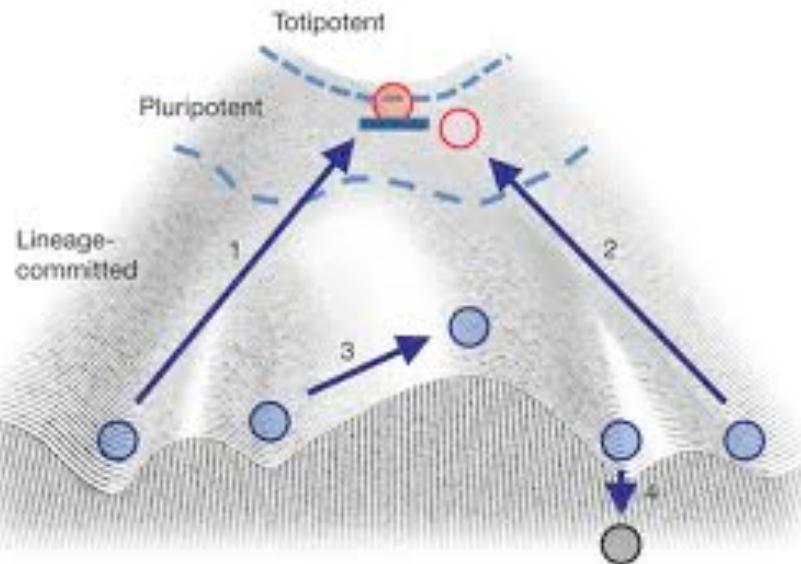
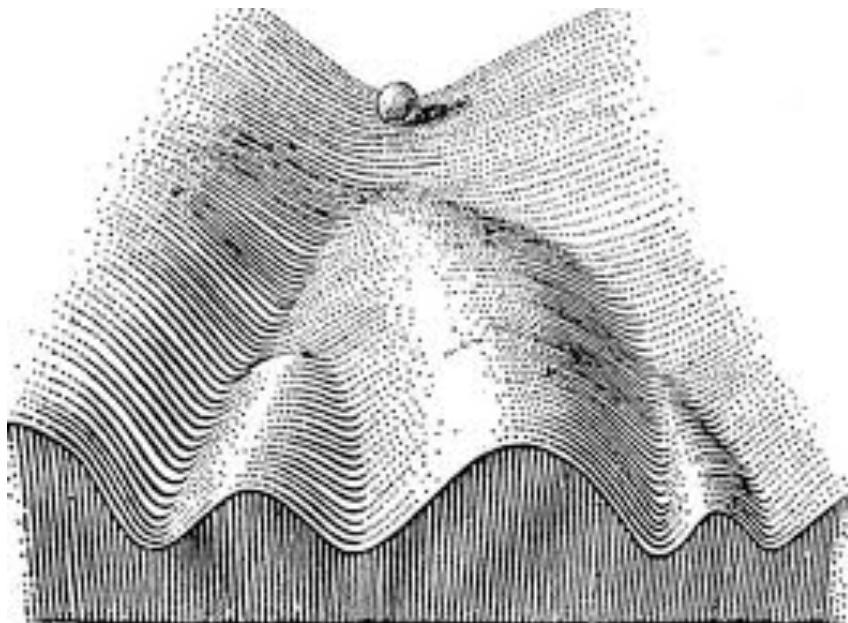
Polycomb complex



DNA binding proteins

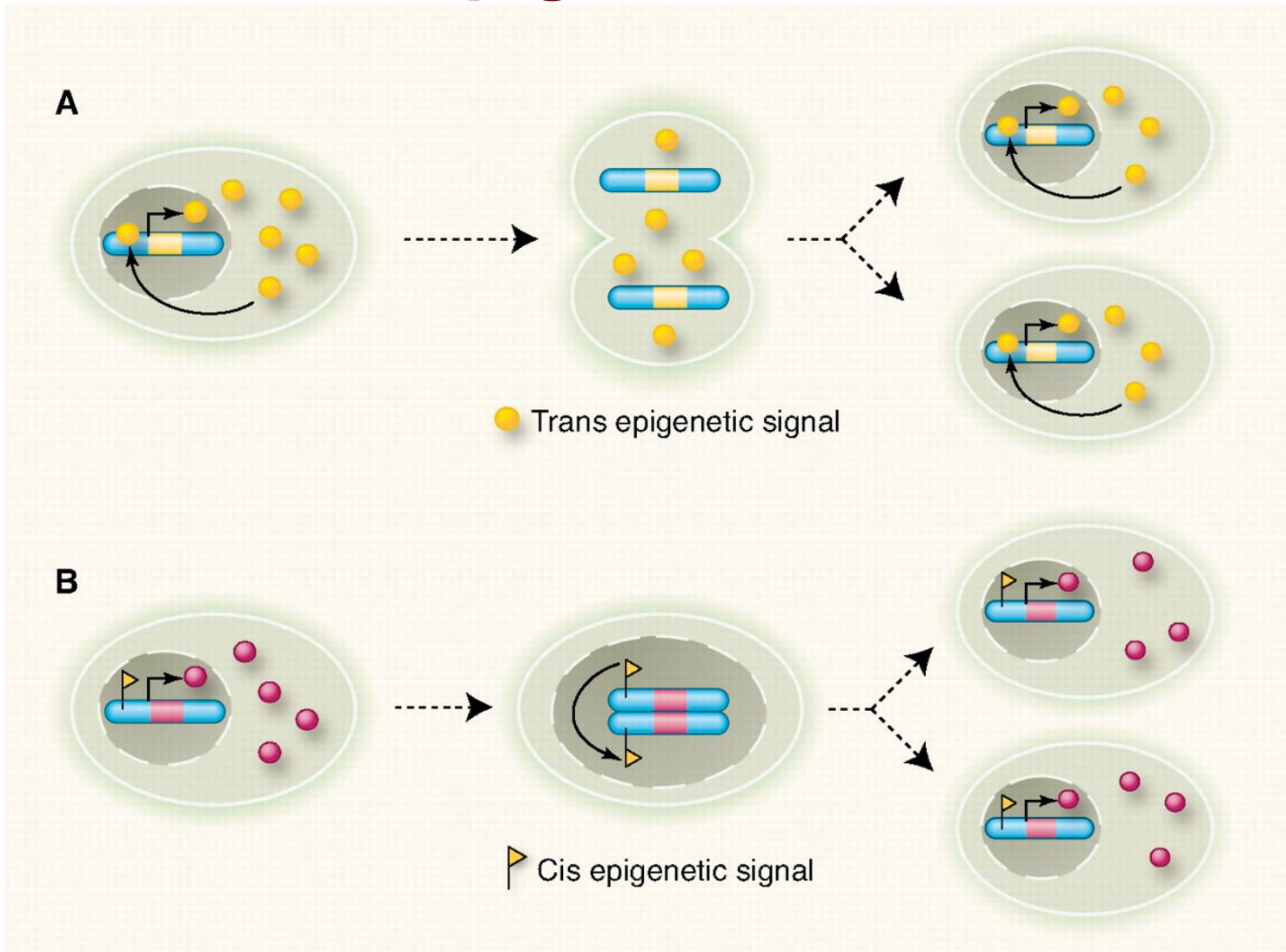
RNA

# Epigenetic Landscape

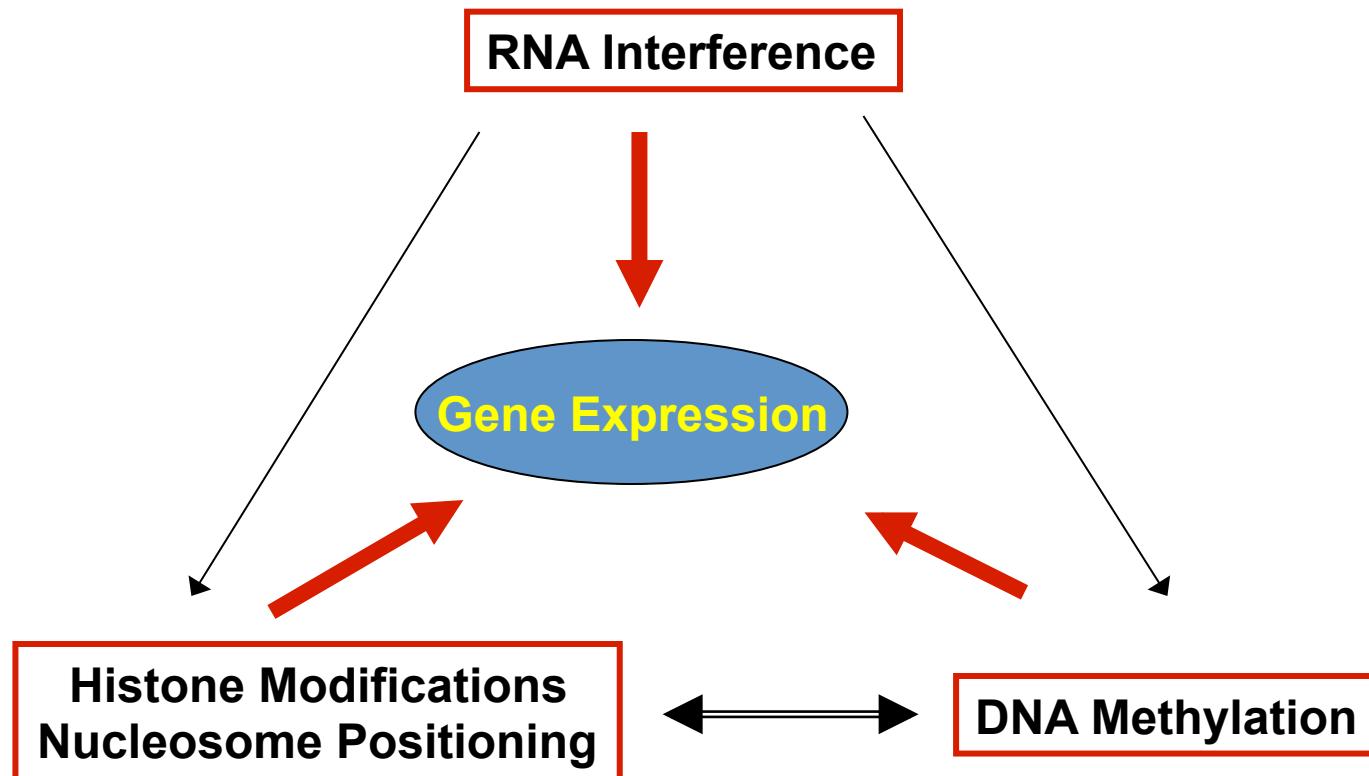


**Conrad Hal Waddington**  
(1905–1975)  
Developmental biologist  
Paleontologist  
Geneticist  
Embryologist  
Philosopher  
Founder for systems biology

# Inheritance, broad definition of epigenetics



# Epigenetics Mechanisms

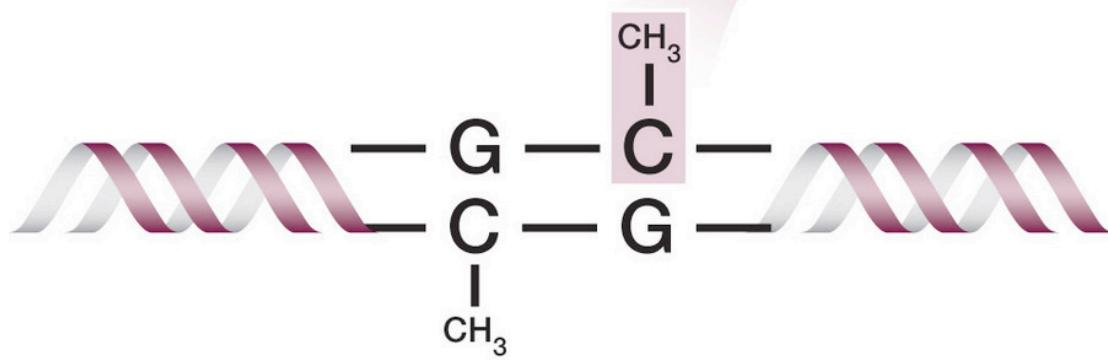
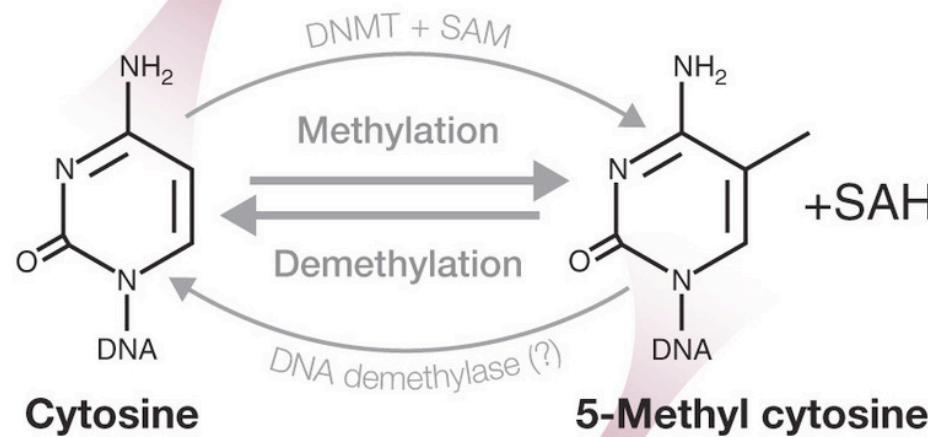
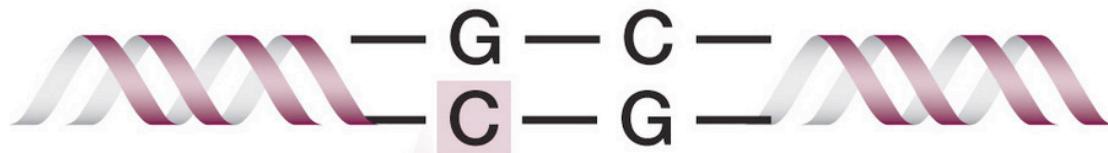


# Epigenetic mechanisms

- **DNA methylation**
  - Normal cells: role in gene expression and chromosome stability
  - Cancer cells: consequences of aberrant hypo- and hyper-methylation
- **Histone modification**
  - Normal cells - the histone code
  - Cancer cells - consequences of altered histone modifying enzymes
- **Interaction between DNA methylation, histone modifications and small RNAs**
- **Cell/tissue type specificity**
- **Gene/Environment interaction, disease susceptibility**

# **What is DNA Methylation?**

# The 5<sup>th</sup> base



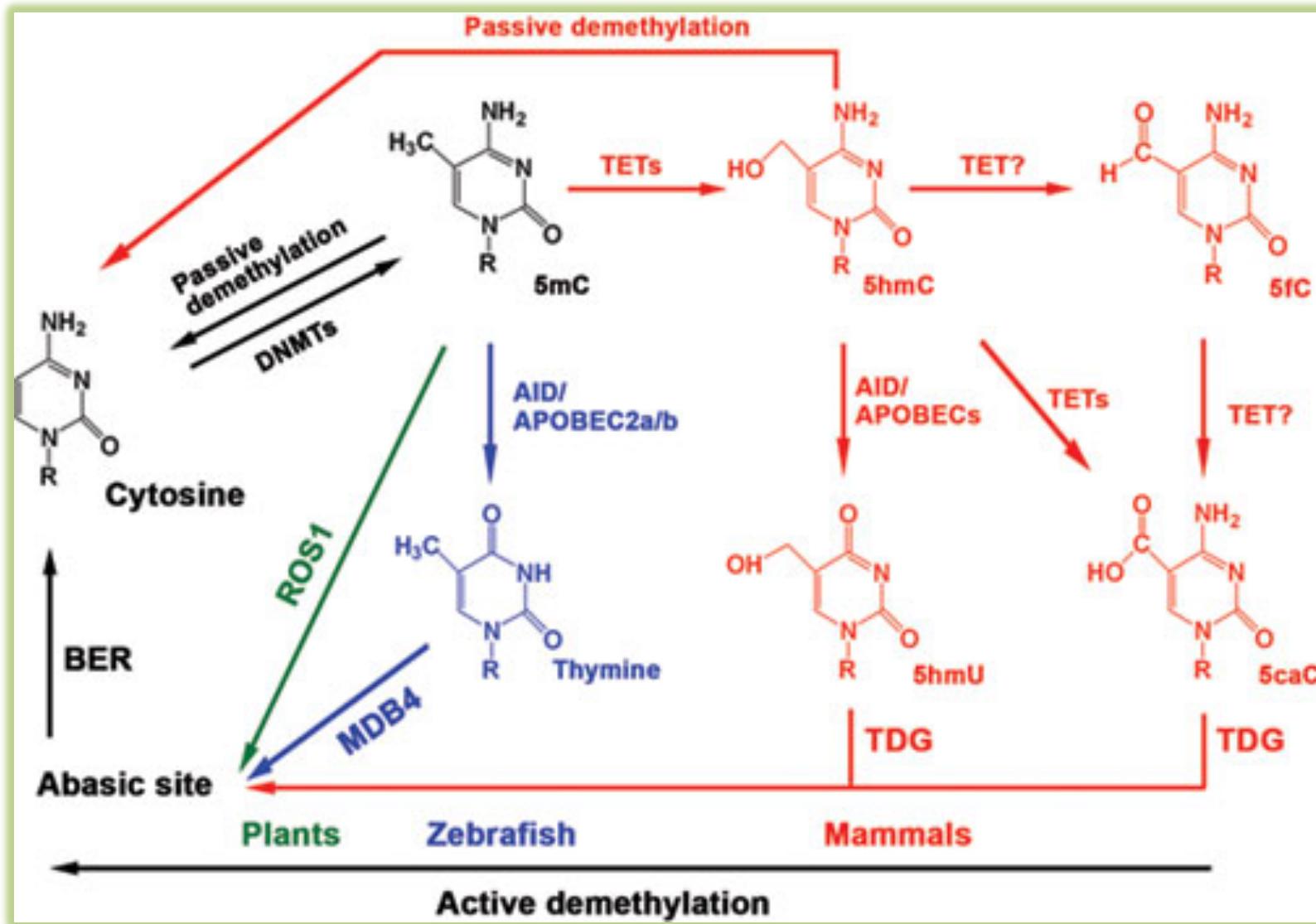
# History of DNA 5-mC

Year	Event	Scientists
1951	DNA 5-mC first reported	Wyatt
1968	Activity of a DNA 5-mC writer detected	Kalousek & Morris
1975	Model for maintaining 5-mC across cell divisions proposed by 2 independent groups	Riggs Holliday & Pugh
1980	DNA 5-mC is associated with gene repression using 5-azacytidine	Jones & Taylor
1982	<i>De novo</i> DNA methylation detected	Jahner et al.
1983	1 <sup>st</sup> DNA 5-mC writer, Dnmt1, purified	Bestor & Ingram
1987	DNA methylation of promoters associated with gene repression	Kovesdi et al.
1989	1 <sup>st</sup> DNA 5-mC reader, MeCP1, discovered	Meehan et al.
1993	DNA 5-mC is associated with gene repression using dnmt1 knockout mice	Li et al.
1998	Function of Dnmt3a and Dnmt3b ( <i>de novo</i> methylation of proviral DNA and repetitive sequences) determined	Okano et al.
1998	Additional DNA 5-mC readers, MeCP2, MBD1, MBD2 & MBD4, discovered	Hendrich & Bird
2002	Function of Dnmt3L ( <i>de novo</i> methylation of maternal imprinted genes) determined	Hata et al.
2007	DNA methylation of gene bodies associated with gene expression	Hellman & Chess
2009	DNA 5-mC erasers, TET1-3, discovered	Tahiliani et al. Kriaucionis & Heintz

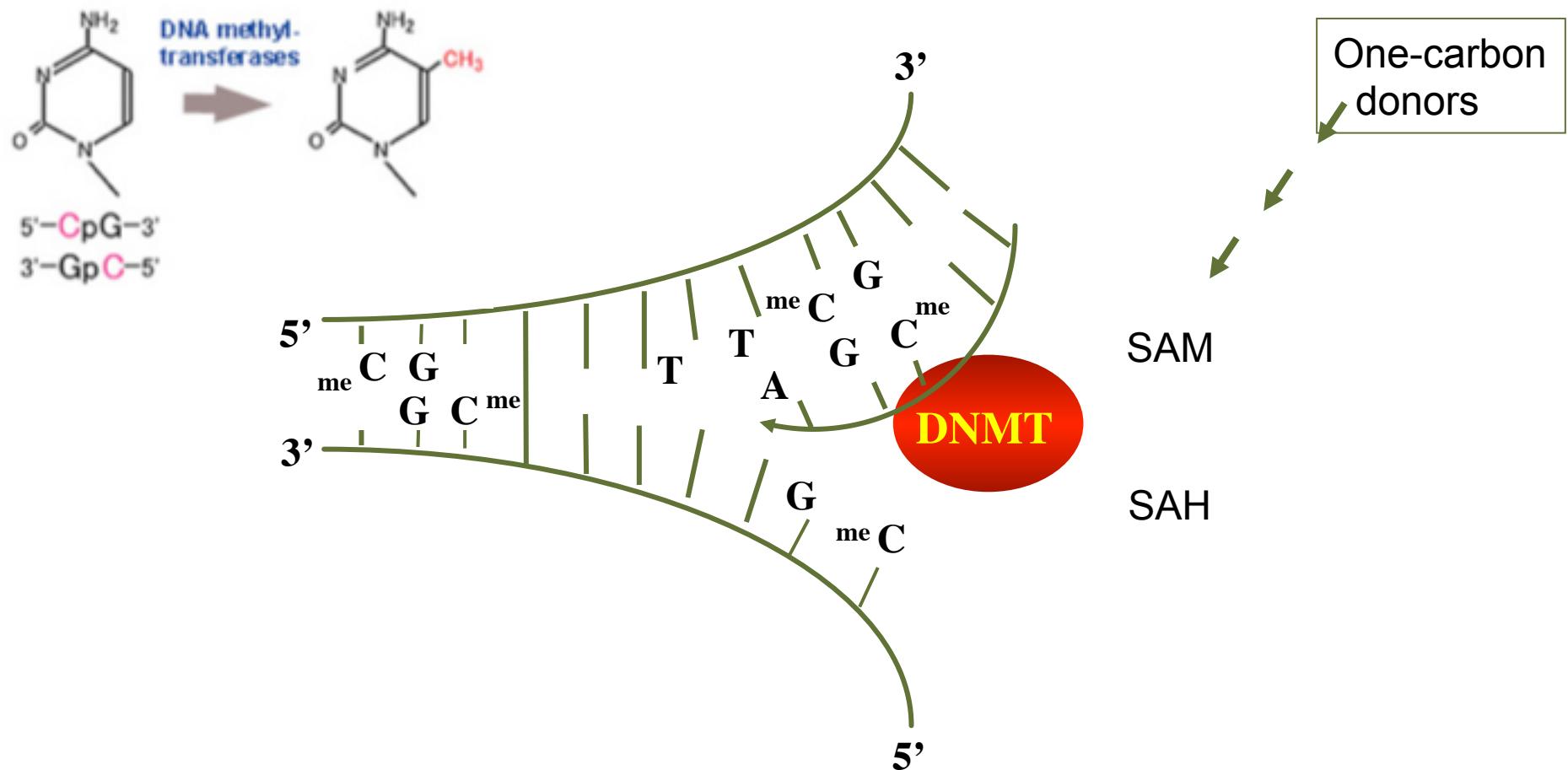
# DNA methylation is distributed nonuniformly in the genome

- CpGs comprise ~1% of the human genome
  - 5mCs are frequently mutated (spontaneous deamination) and thus become rare in genomes
- In human somatic cells, 60-80% of all CpGs are methylated
  - Most methylation is found in repetitive elements
- CpG islands
  - Are GC-rich regions that possess a high density of CpGs
  - Are usually hypomethylated
  - ~70% of gene promoters are embedded in CpG islands

# DNA methylation and demethylation



# DNA Methylation is Heritable

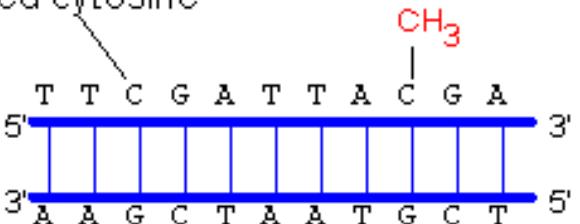


**DNMT:** DNA methyltransferase

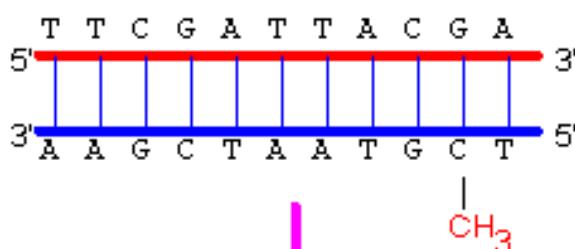
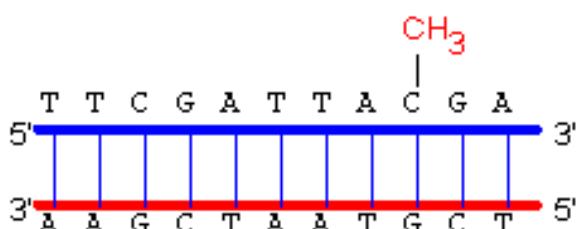
**SAM:** S-adenosyl-methionine

**SAH:** S-adenosyl-L-homocysteine

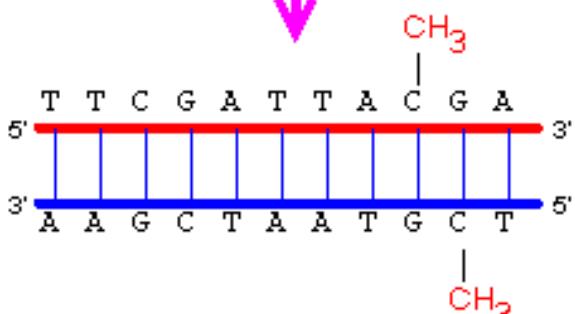
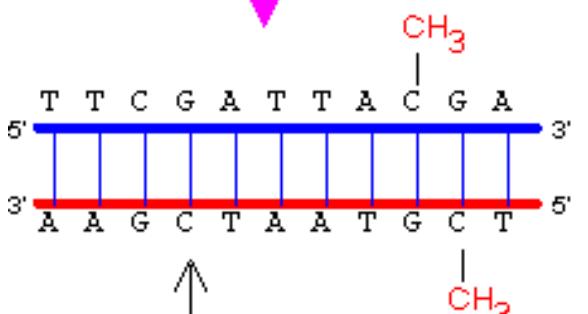
## Unmethylated cytosine



## DNA replication

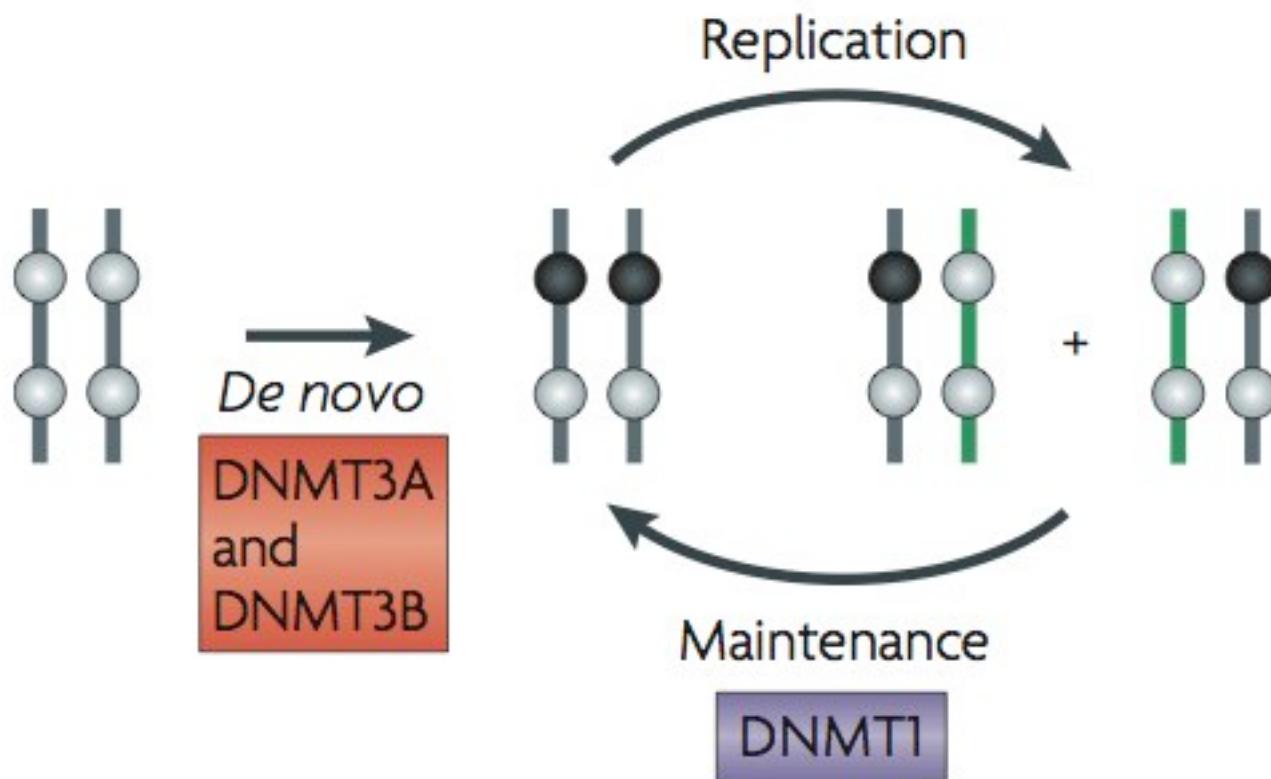


### Methylation



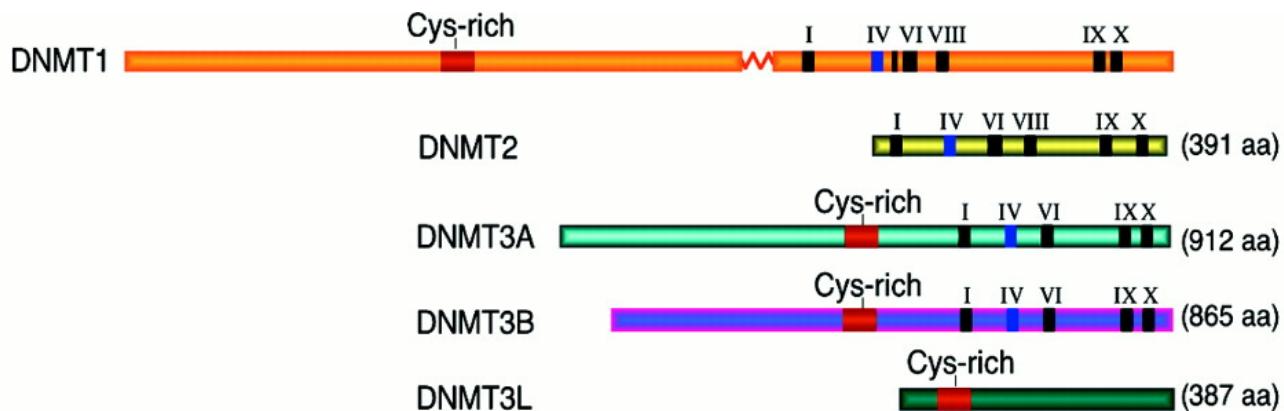
Not recognized by  
maintenance methylase

# Two classes of DNA methyltransferases (DNMTs)



# Two classes of DNA methyltransferases (DNMTs)

	<u>function</u>	<u>knock-out</u>
DNMT1	maintenance	lethal
DNMT2	remains mystery, possibly a RNA methyl-transferase	
DNMT1o	maternal imprints	lethal*
DNMT3a	de novo	lethal
DNMT3b	de novo	lethal
DNMT3L	maternal imprints (indirect)	lethal *

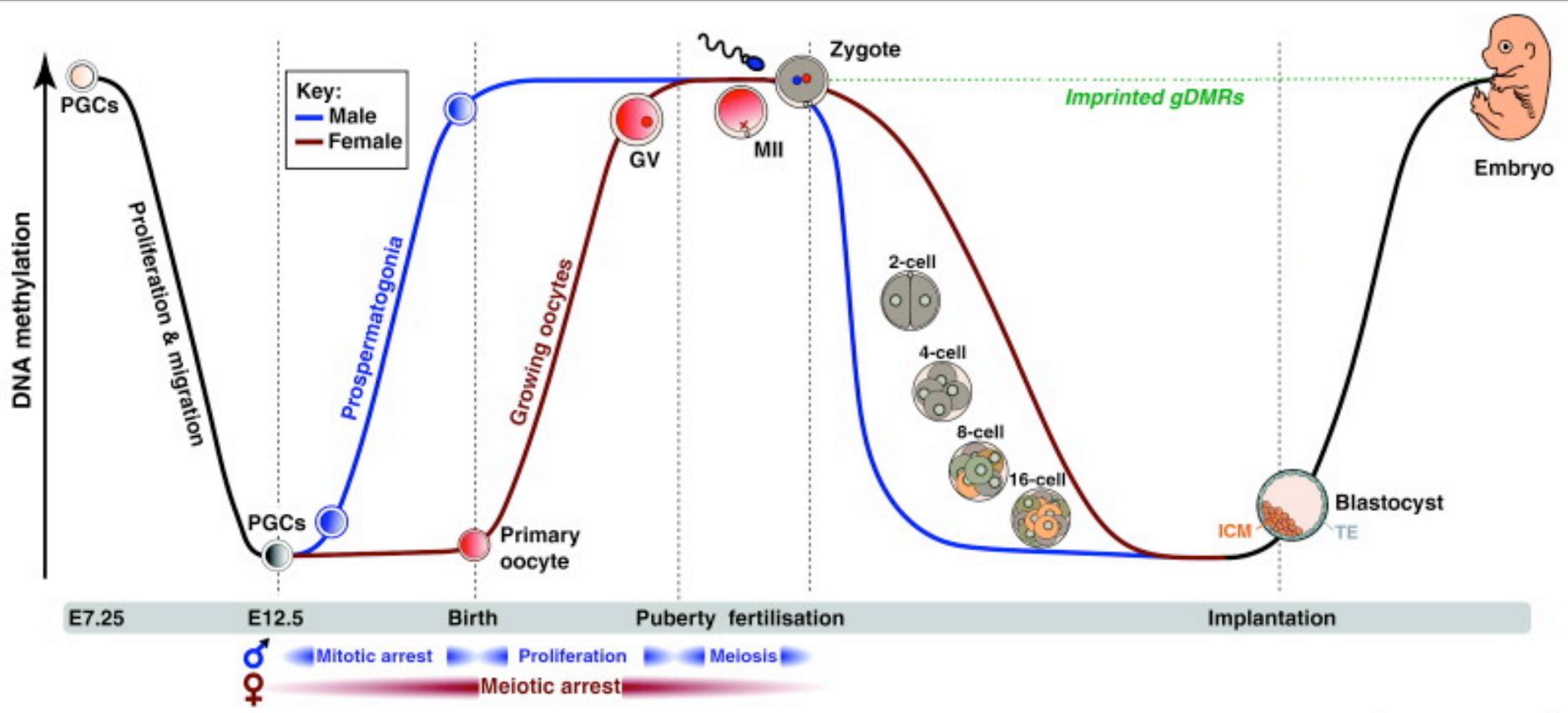


# **Normal pattern and function of DNA Methylation**

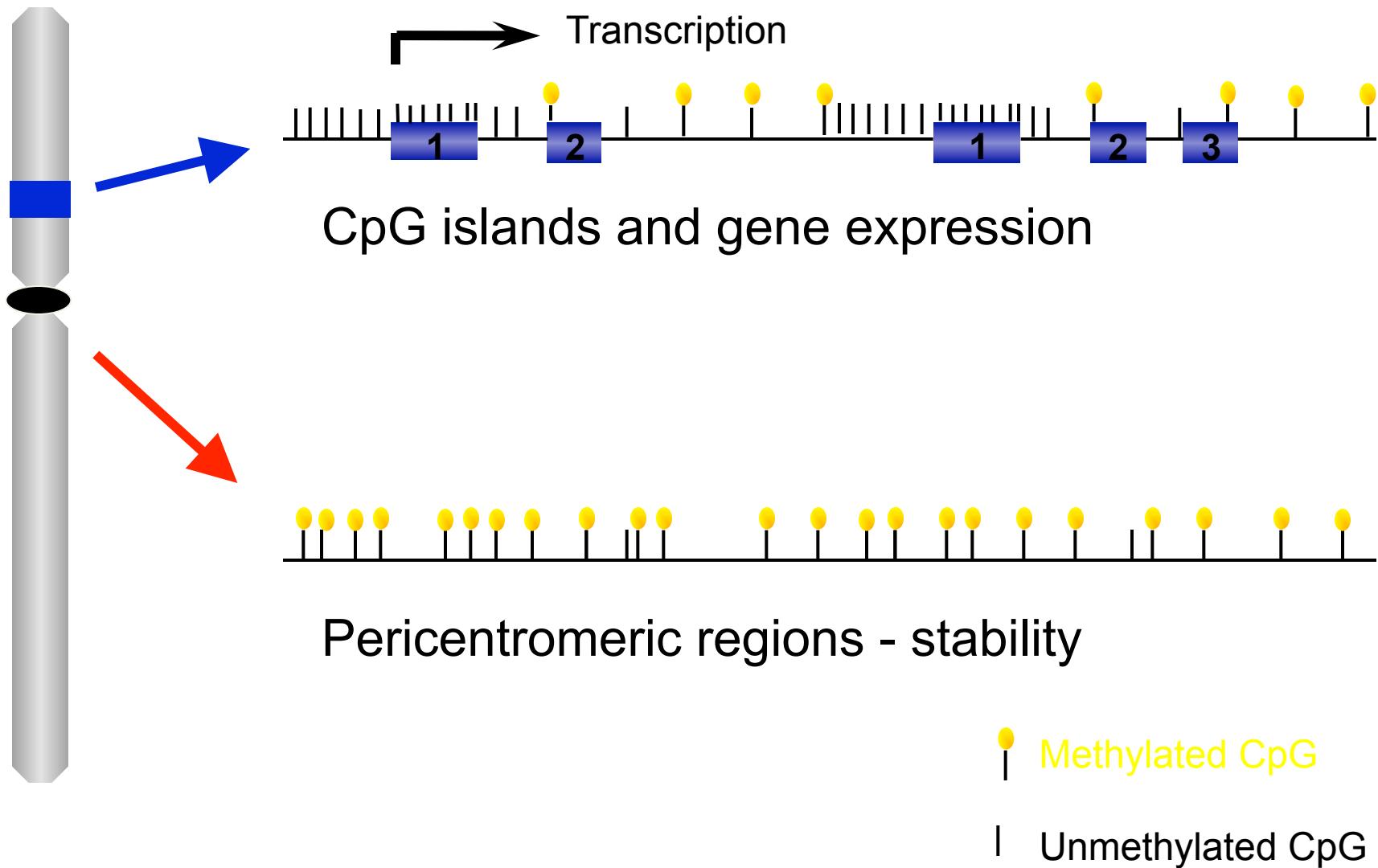
# Function of DNA methylation in mammals

- Host defense - endogenous parasitic sequence (repeats, etc.)
- Imprinting
- X chromosome inactivation
- Heterochromatin maintenance, chromosome stability, telomere length
- Gene expression controls

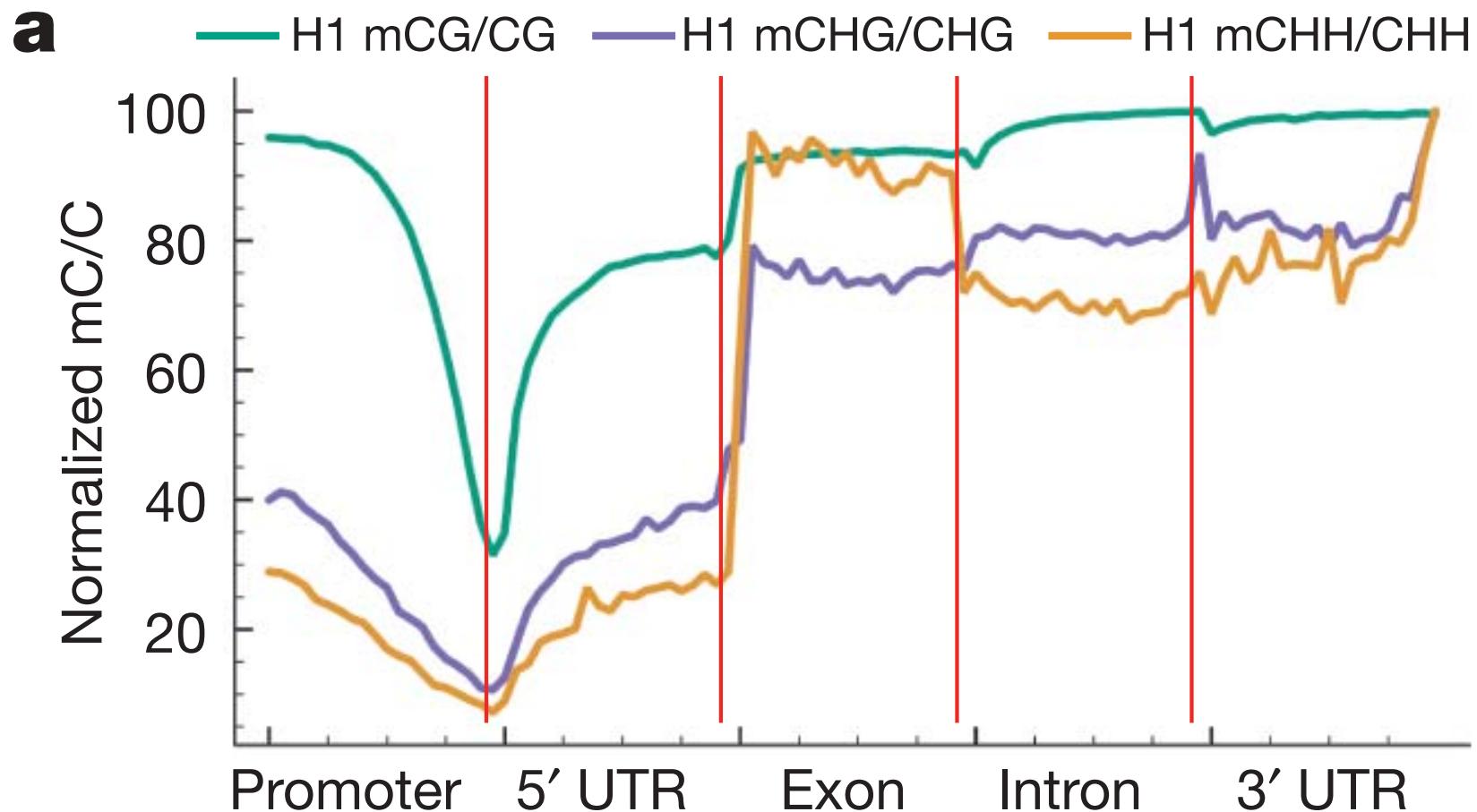
# DNA methylation changes during developmental epigenetic reprogramming



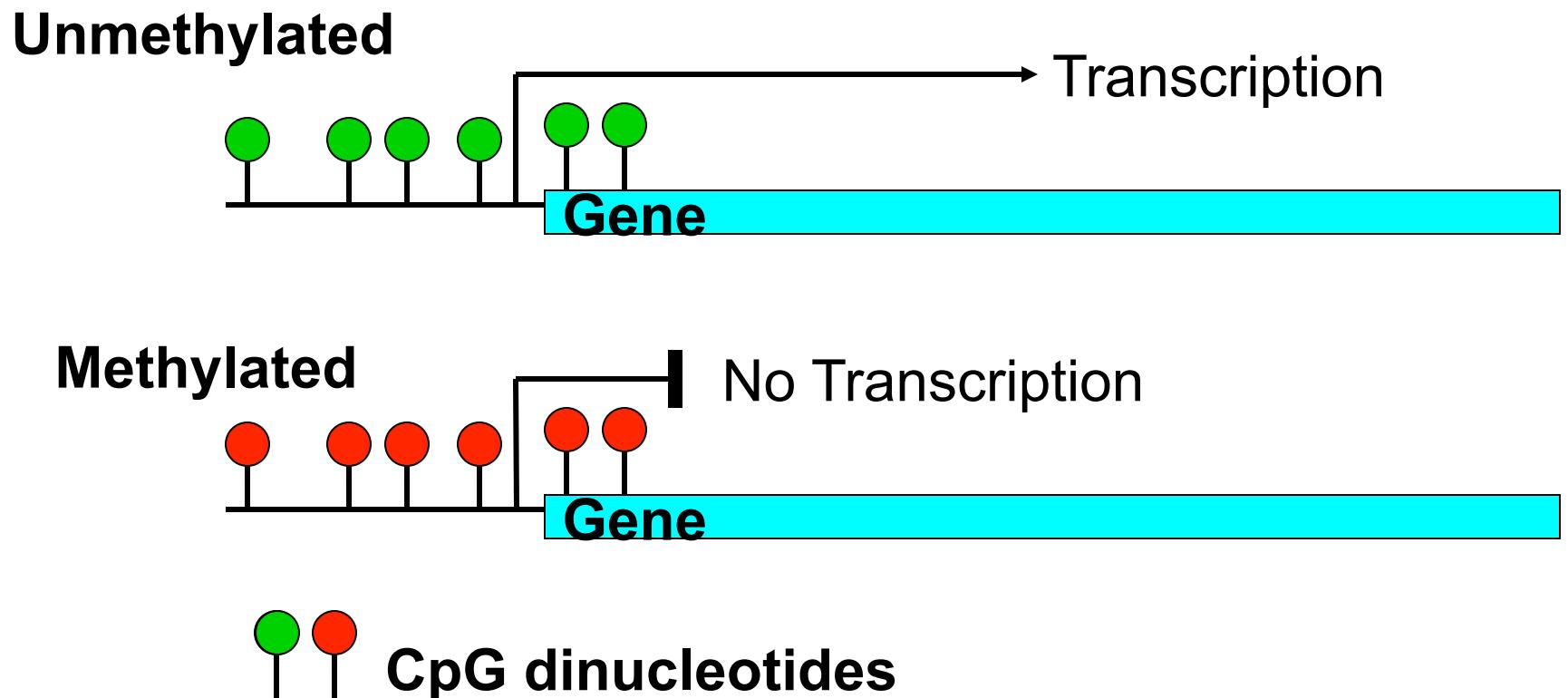
# Normal Patterns of DNA Methylation



# DNA methylation pattern across a gene structure



# Methylation and Gene Expression



# **Mechanisms of gene silencing by methylation**

**Direct mechanism:**

**Inhibition of transcription factor binding (eg.CTCF, UBF)**

Not a universal mechanism since not all transcription factor binding sites contain CG dinucleotides

**Indirect mechanism:**

**Inhibition mediated by methyl-CpG binding proteins MeCP1/ MeCP2**

Recruitment of corepressor complexes including histone deacetylases (HDAC)

Change in chromatin conformation

# Methylated DNA binding proteins

## Protein

MeCP2

MBD1

MBD2

MBD3

MBD4

## Mutant phenotype

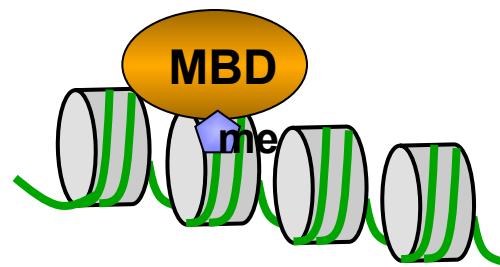
Rett syndrome

behaviour abnormalities

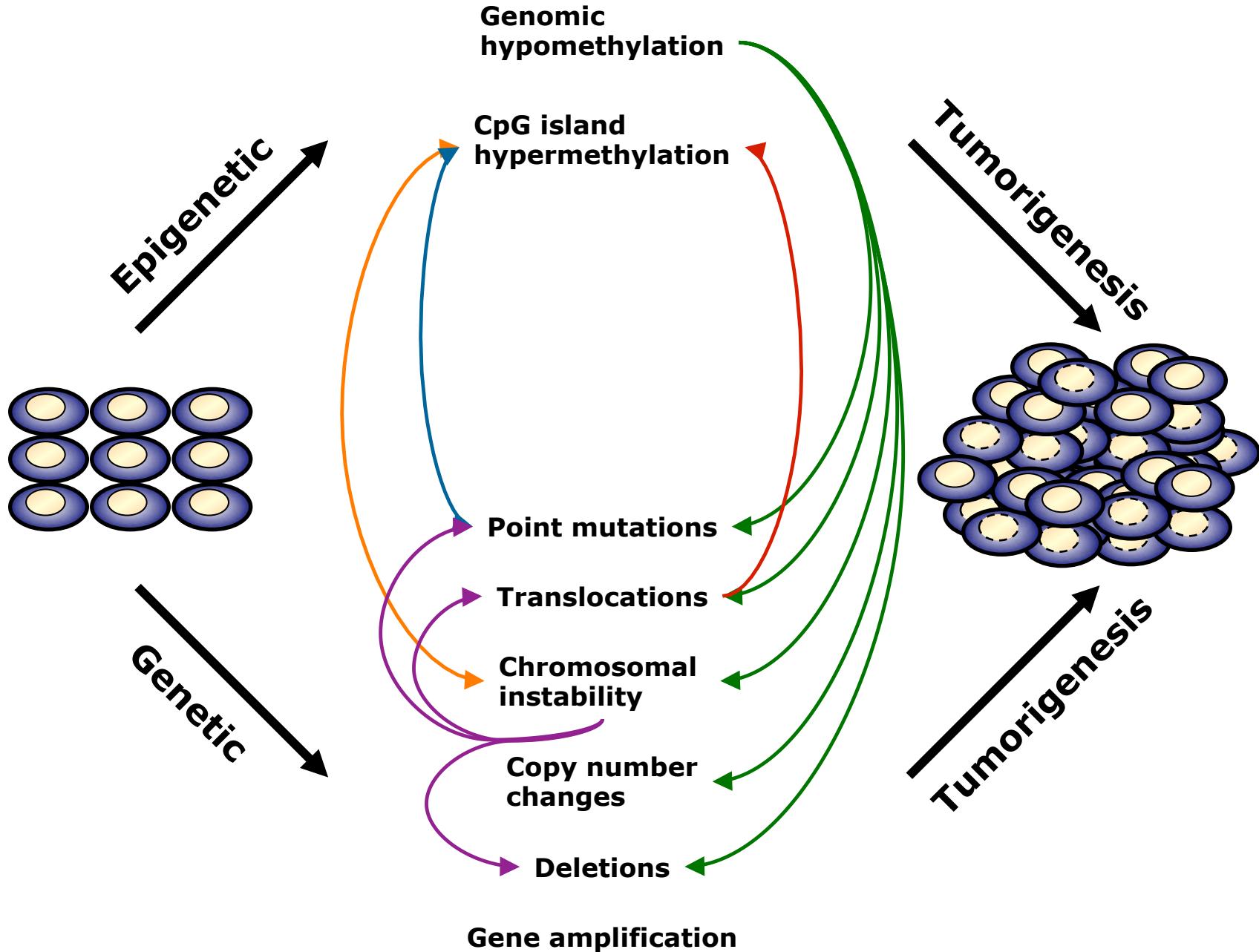
lethal

increased mutation frequency

(a glycosylase for mismatch repair)

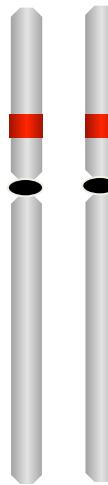


# DNA methylation in cancer

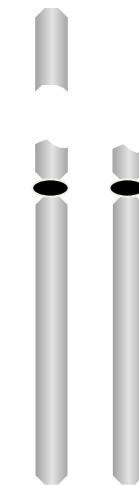
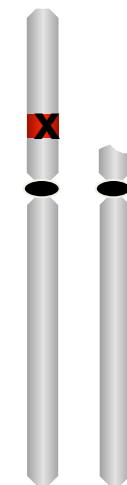


# Tumor Suppressor Gene Inactivation in Human Cancer

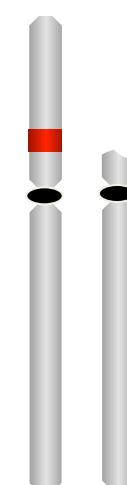
Normal



Tumor



$\text{CH}_3$



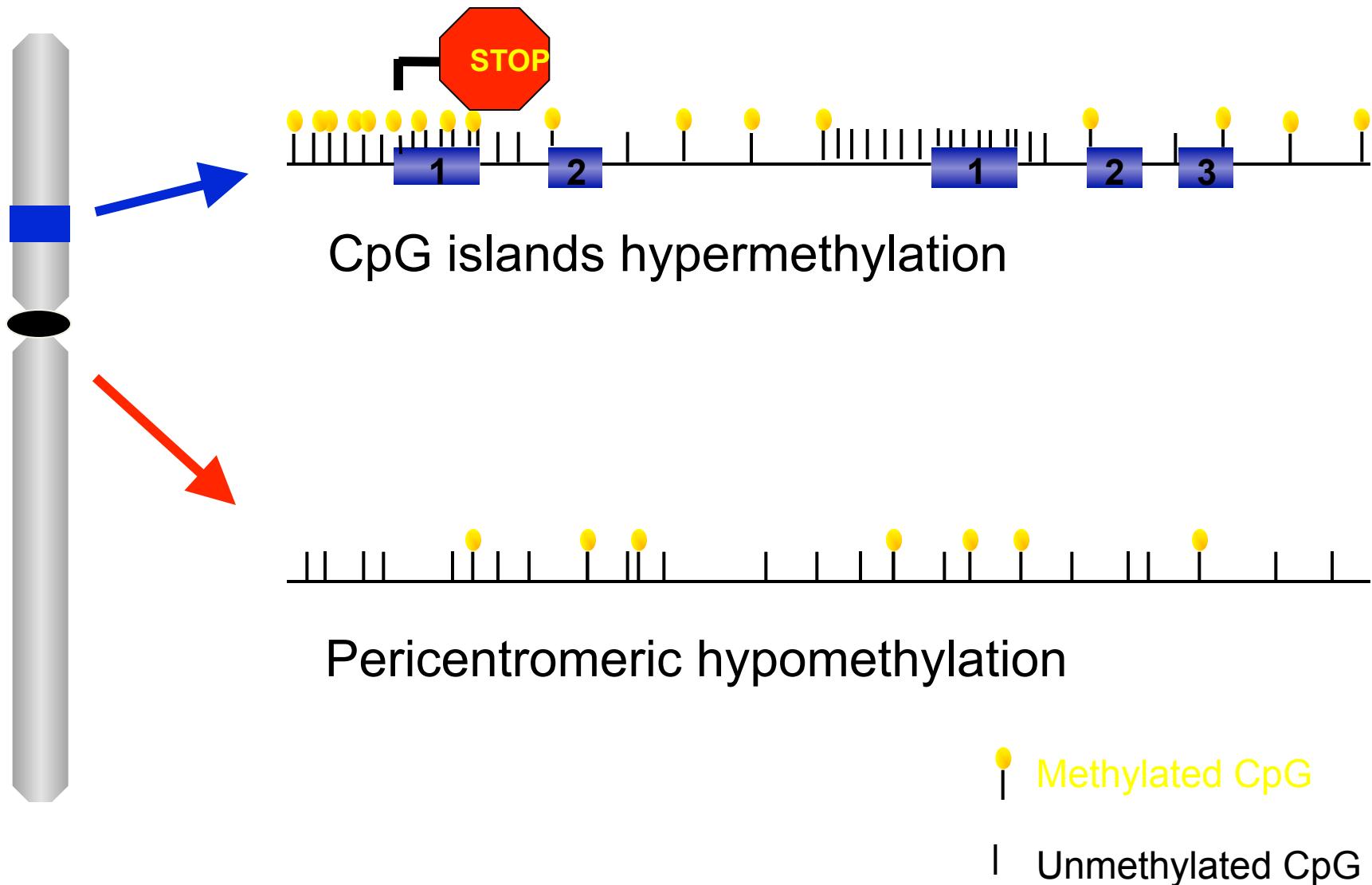
Mutation  
And  
Deletion

Homozygous  
Deletion

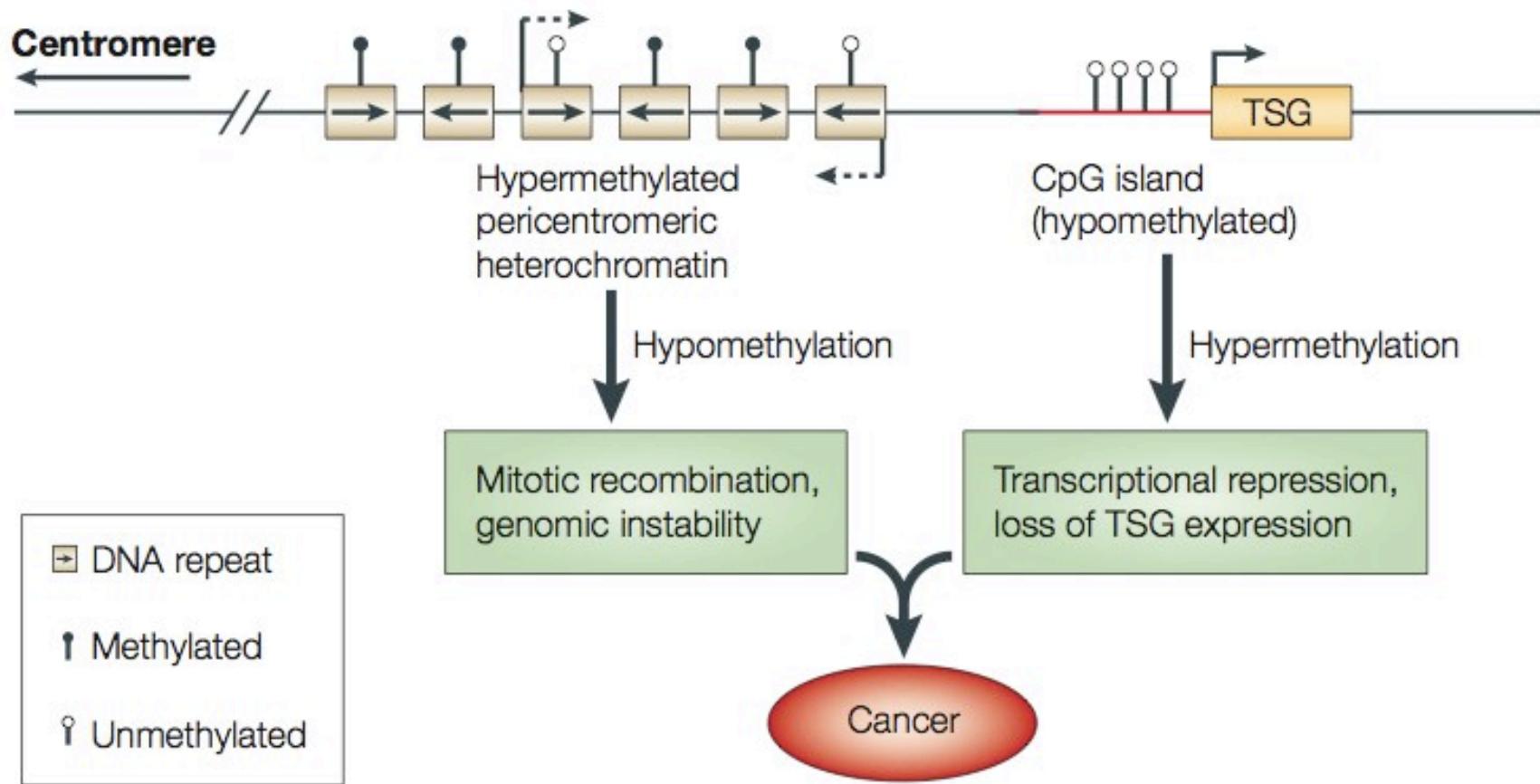
Methylation

Knudsen's 2-hit hypothesis, 1971

# Aberrant DNA Methylation in Cancer



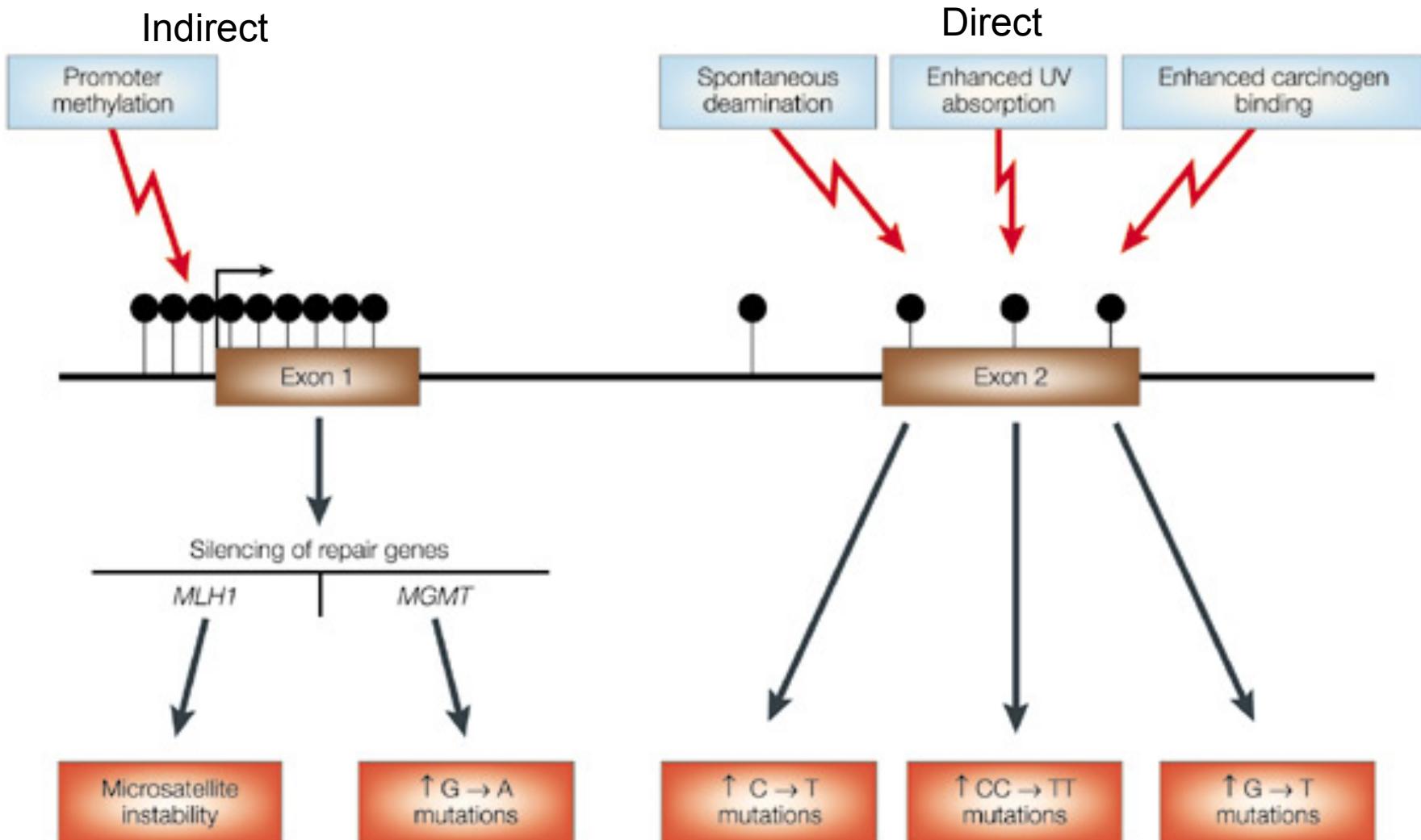
# Different regions of the genome are hypermethylated or hypomethylated in cancer



# Hypomethylation and Cancer

- The overall level of 5-methylcytosine is decreased in a proportion of human tumors. (Gama-Sosa et al. 1983)
- Hypomethylation of c-Ha-ras and c-Ki-ras, two cellular oncogenes, was identified in primary colon tumors. (Feinberg and Vogelstein 1983)
- Loss of IGF2 imprinting increases colorectal cancer risk (Cui et al. 2003)
- Consequences of Hypomethylation?
  - Chromosome instability (ICF syndrome)
  - Transcriptional activation of retrotransposons
  - Transcriptional activation of oncogenes

# Methylation promotes mutation



# **DNA methylation and other diseases**

## -- **Imprinting Disorder:**

- Beckwith-Wiedemann syndrom (BWS)
- Prader-Willi syndrome (PWS)
- Transient neonatal diabetes mellitus (TNDM)

## -- **Repeat-instability diseases**

- Fragile X syndrome (FRAXA)
- Facioscapulohumeral muscular dystroph

## -- **Defects of the methylation machinery**

- Systemic lupus erythemtosus (SLE)
- Immunodeficiency, centromeric instability and facial anomalies (ICF) syndrome

# Imprinting Diseases: Angelman and Prader-Willi Syndromes

## Angelman Syndrome

- “Happy puppet”
- Severe mental retardation
- Absence of speech
- Happy disposition
- Excessive laughing
- Hyperactive, with jerky repetitive motions
- Red cheeks, large jaw and mouth

## Prader Willi Syndrome

- Small hands and feet
- Underactive gonads, tiny external genitals
- Short stature
- Mentally retarded
- Slow-moving
- Compulsive overaters
- Obese

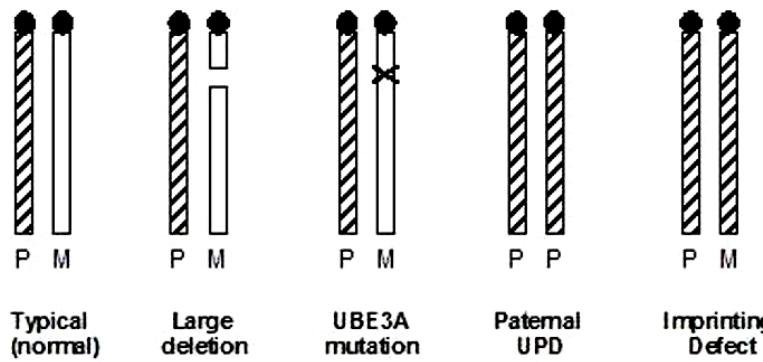
# Imprinting Diseases: Angelman and Prader-Willi Syndromes



Chr15 deletion, ~4Mb

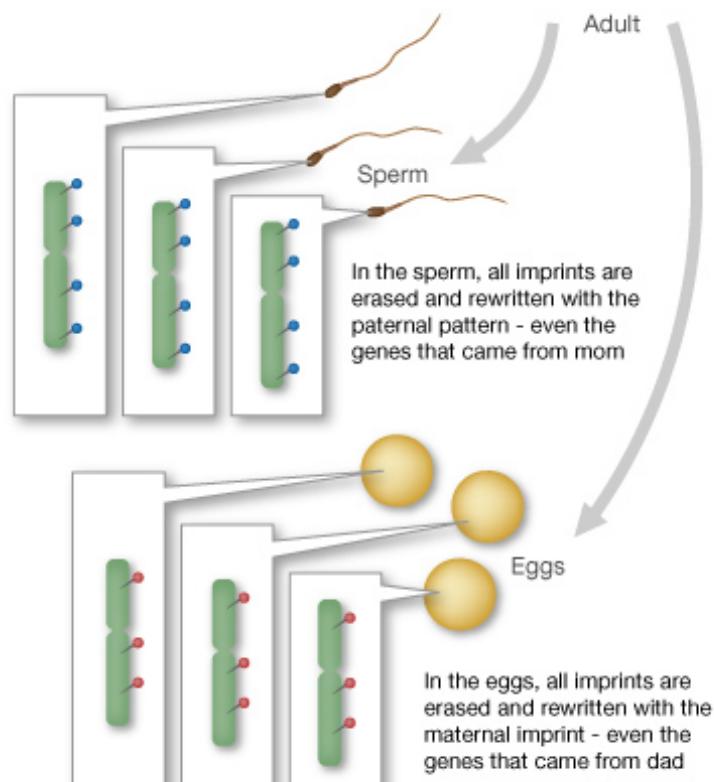
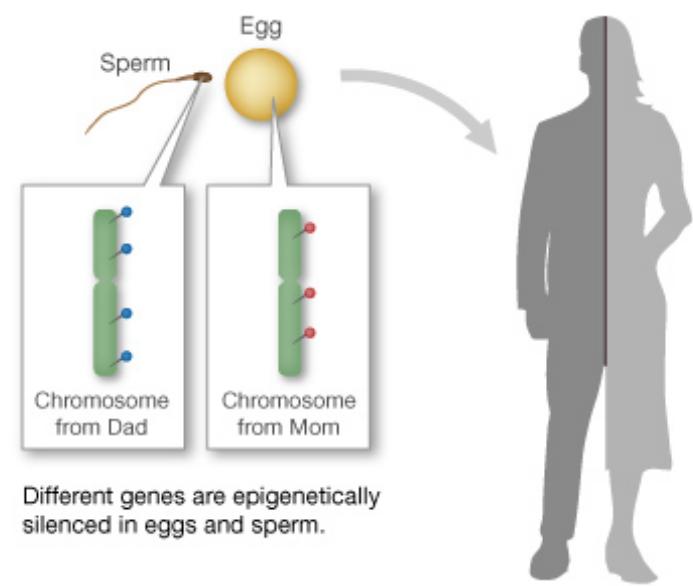
Loss of maternal contribution  
**UBE3A**  
**Angelman Syndrome**

Loss of paternal contribution  
**SNRPN**  
**Prader-Willi Syndrome**



# Genomic imprinting

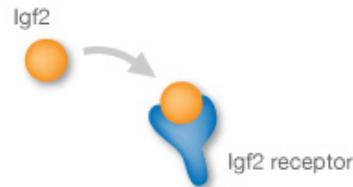
- Imprinting is unique to mammals and flowering plants. In mammals, about 1% of genes are imprinted.
- For imprinted genes, one allele is expressed and the other is silent.
- This is typically controlled epigenetically. The expressed alleles are unmethylated and associated with loosely packed chromatin.
- Imprinted genes bypass epigenetic reprogramming.
- Imprinting is required for normal development



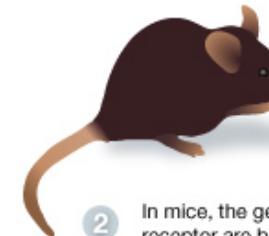
# Why imprinting?

- The Genetic Conflict Hypothesis
  - Many imprinted genes are involved in growth and metabolism.
  - Paternal imprinting favors the production of larger offspring, and maternal imprinting favors smaller offspring
- Imprinted genes are under greater selective pressure.
  - No back up!
  - Any variation in single gene is expressed
  - Closely related species have different imprinting patterns
    - Liger and Tigon
- Imprinted genes are sensitive to environmental signals.

## AN EXAMPLE OF IMPRINTING



1 In mammals, the growth factor Igf2 interacts with the Igf2 receptor.



2 In mice, the genes for Igf2 and the Igf2 receptor are both imprinted.

Deleting the mother's Igf2 receptor gene produces overly large offspring.



Deleting the father's Igf2 gene produces dwarf offspring.



Deleting the mother's Igf2 receptor gene AND the father's Igf2 gene produces normally sized offspring.

3 The imprints on the Igf2 and Igf2 receptor genes normally cancel each other out. Changing the imprint on one copy of the gene has a dramatic effect on the size of the offspring. This result supports the genetic conflict hypothesis



Genes from mom:  
Igf2 receptor - ON  
Igf2 - OFF

Genes from dad:  
Igf2 receptor - OFF  
Igf2 - ON

# What we don't know about imprinting

- What targets a gene for imprinting?
  - Why are some genes expressed from both alleles and other expressed from only one allele?
- How are the imprints imposed?
  - Do males and females have different mechanisms for imprinting genes?

# Rett Syndrome

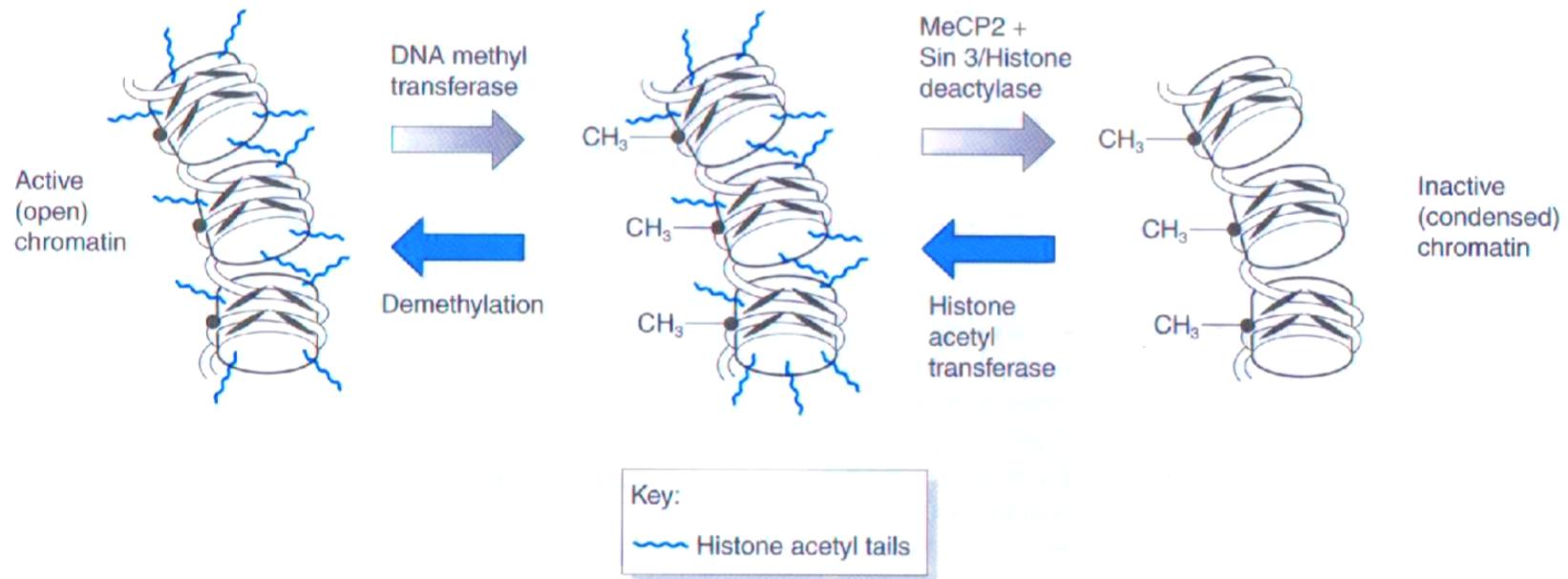


Nature Genetics (1999) 23:127-128

- X-linked trait
- Mainly girls affected
- Normal at birth
- At 6-18 months, begin losing purposeful movement
- Persistent wringing of hands
- Loss of speech, gait
- Mental retardation ensues

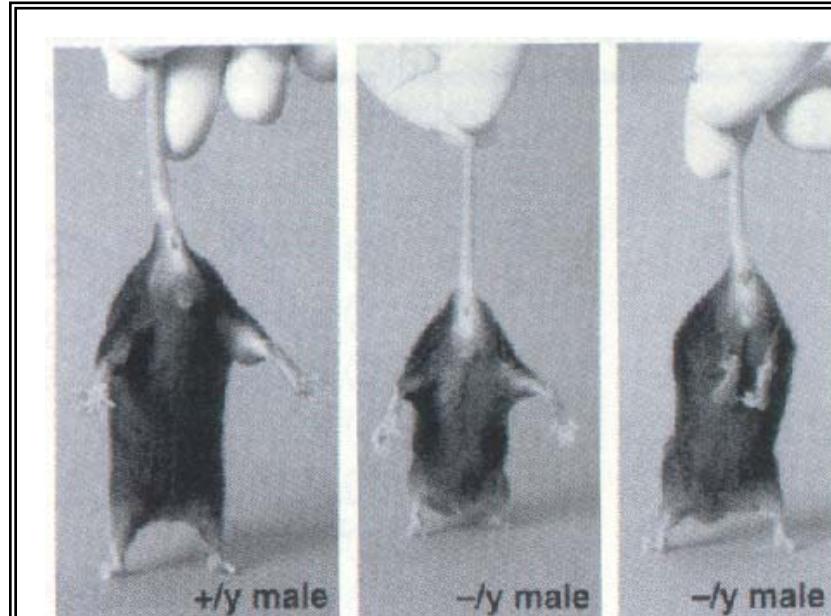
# Rett's is due to defect in MeCP2

- Methyl-cytosine binding protein 2 (MeCP2) binds methylated DNA and recruits binding of a histone deacetylase
- Normal role is tightening chromatin packing, leading to gene silencing



# Mouse model for Rett's

- Mice have a gene that is homologous to MeCP2
- Knocking out the gene in mouse gives a phenotype similar to human Rett's
- This model offers good experimental system for studying the human disease



Nature Genetics (2001) 27:332-336

Male mice with MeCP2 knockout develop normally for a while (middle), but at 6 weeks of age, they begin to develop neurological symptoms, such as hindlimb clasping (right).

# Why is the phenotype neurological?

- The phenotype suggests that the targets are genes in the brain
- Normal neurological differentiation requires silencing of MeCP2 gene target(s)
- The target(s) of MeCP2 are not known

## LETTER

doi:10.1038/nature09544

### L1 retrotransposition in neurons is modulated by MeCP2

Alysson R. Muotri<sup>1\*</sup>, Maria C. N. Marchetto<sup>2\*</sup>, Nicole G. Coufal<sup>2</sup>, Ruth Oefner<sup>2</sup>, Gene Yeo<sup>3</sup>, Kinichi Nakashima<sup>4</sup> & Fred H. Gage<sup>2</sup>