

# **Eukaryotic Gene Expression: Basics & Benefits**

**P N RANGARAJAN**

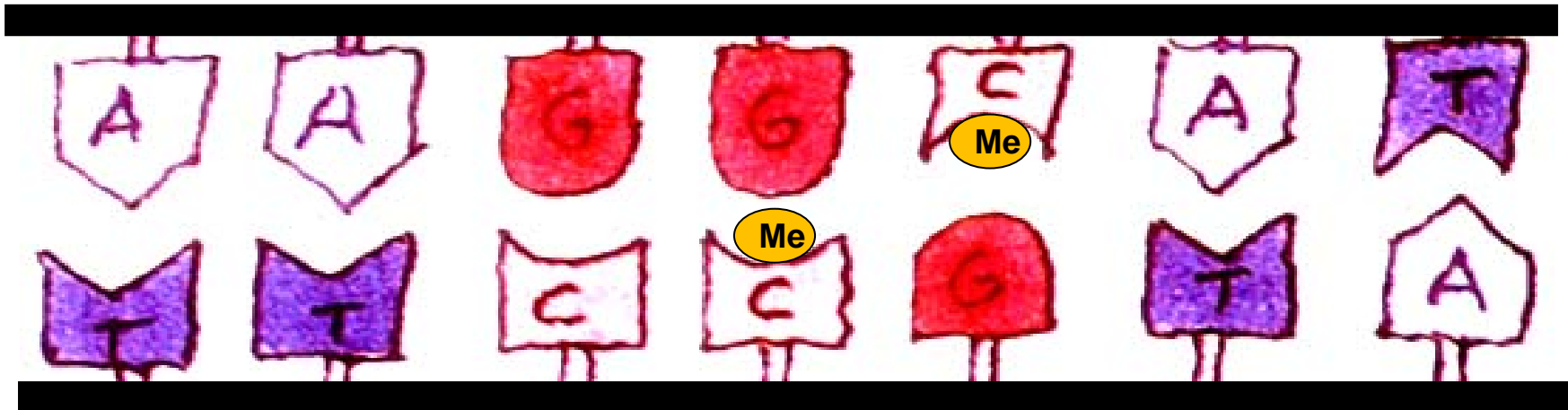
**Lecture 9**

**Eukaryotic gene regulation:  
DNA METHYLATION**

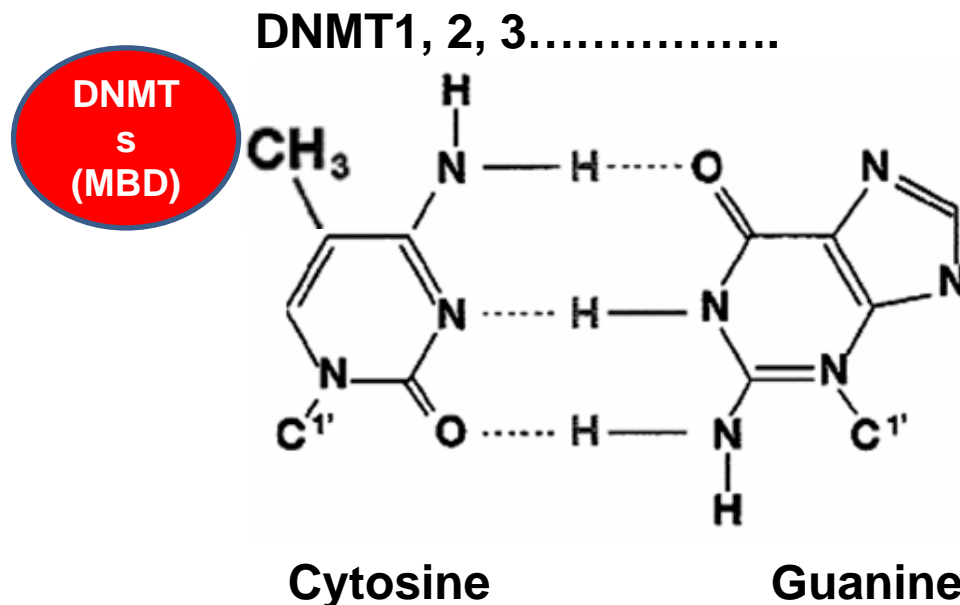
## **Recap.....**

- **Eukaryotic RNA polymerases**
- **Core promoter elements**
- **General transcription factors**
- **Enhancers and upstream activation sequences**
- **Transcriptional activators: DNA binding, transactivation**
- **Role of chromatin: Acetylation & deacetylation of histones**
- **Histone methylation, demethylation, phosphorylation etc.**
- **Histone code**

# DNA METHYLATION



Enzymes which methylate DNA are known as  
as  
DNA Methyl Transferases (DNMTs) which  
contain  
methyl-CpG-binding domains (MBDs)



In human DNA, methyl cytosine accounts for ~1% total DNA bases.

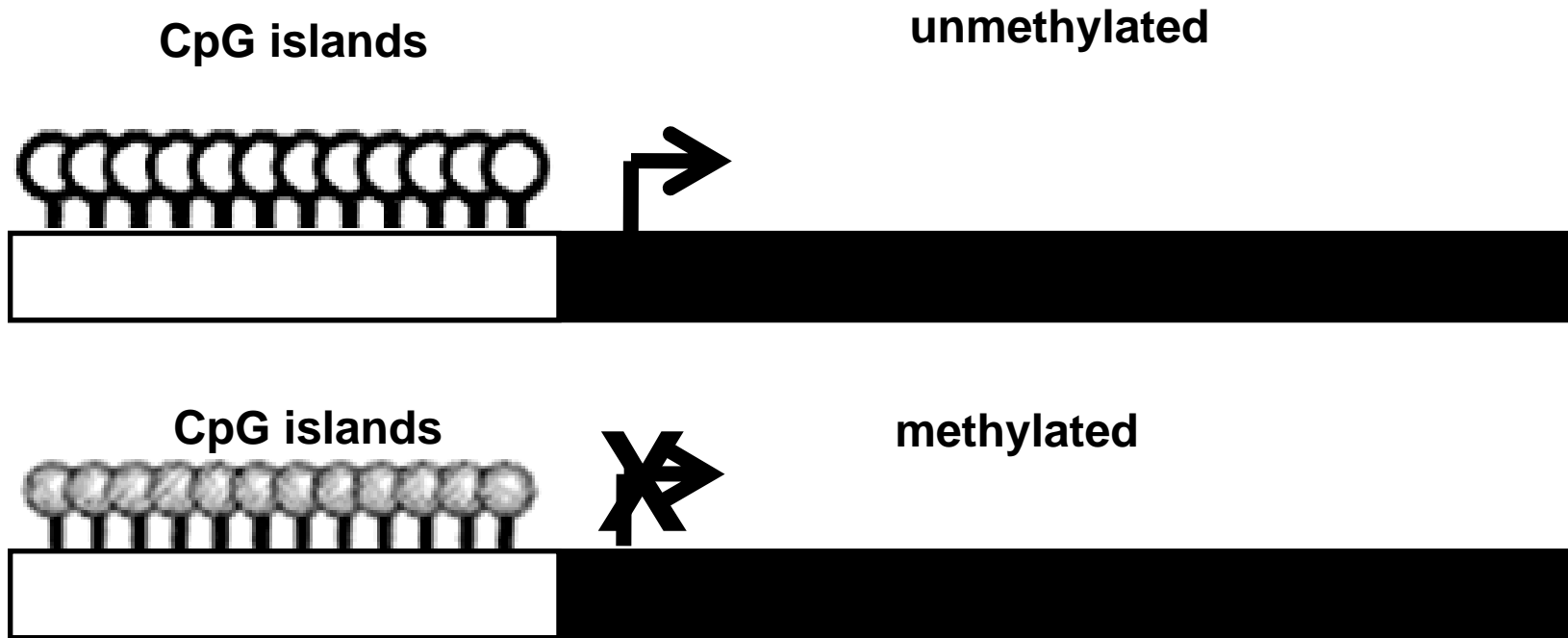
However, in several other eukaryotes such as *Drosophila* and *Caenorhabditis elegans*, very little DNA methylation is reported.

With reference to regulation of gene expression, methylation of a set of sequences known as CpG islands play a very important role.

The CpG sequences can often cover 1-2 kilobases at a stretch and A number of such CpG islands (~40,000, ~15,000) are reported to be present in the mammalian genomes.

Many genes which are constitutively expressed (house keeping genes), as well as those expressed in a tissue-specific manner contain CpG islands in their promoter regions.

In case of  $\gamma$ -globin gene, the promoter region in and around the transcription start site (-200 to +100) when methylated, leads to transcription repression



**DNA METHYLATION  
REPRESSES  
TRANSCRIPTION**

## How does DNA methylation results in transcriptional repression?

**MBD proteins  
(methyl-CpG-binding domain)**

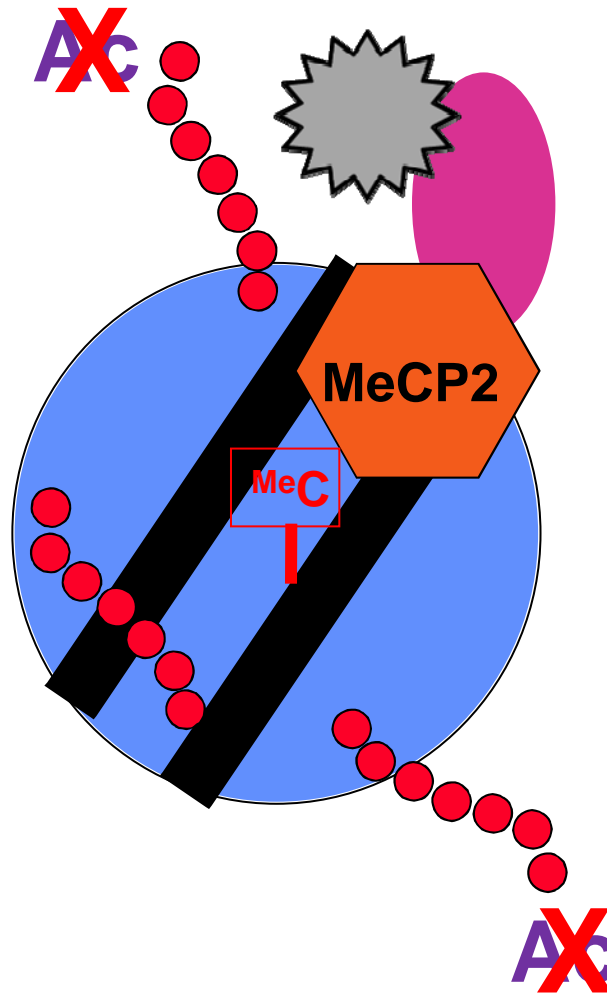
**MBD Proteins recognize methylated cytosines and bind to them**

**MeCP1 (many methylated CpGs)  
MeCP2 (single methylated CpG base pair)  
MBD1  
MBD2  
MBD3  
MBD4**

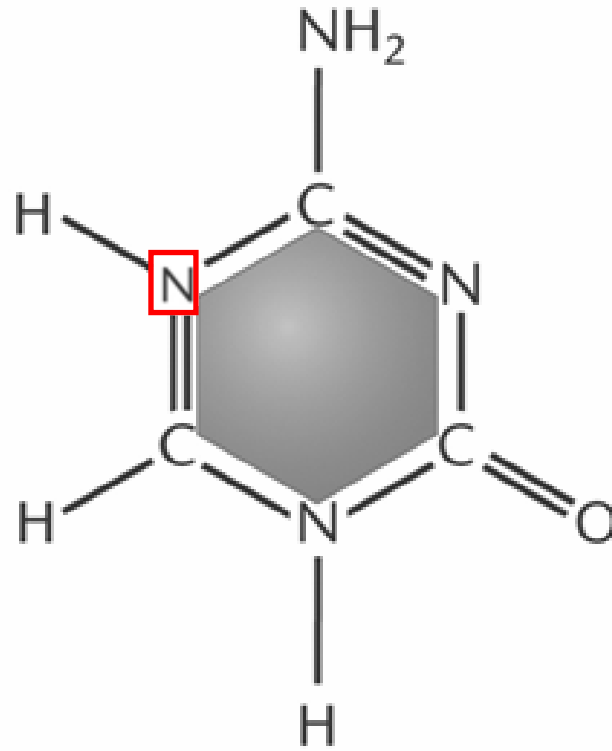
**These MBD proteins can then recruit repressors or co-repressors which often contain HDACs leading to histone deacetylation and repression of transcription**

**For example, MBD proteins such as MeCP2 is a part of the Sin3 repressor complex which contains HDACs as well.**

## General mechanism of transcriptional repression by DNA methylation







5-Azacytosine

**Azacytosine when incorporated into DNA cannot be methylated**

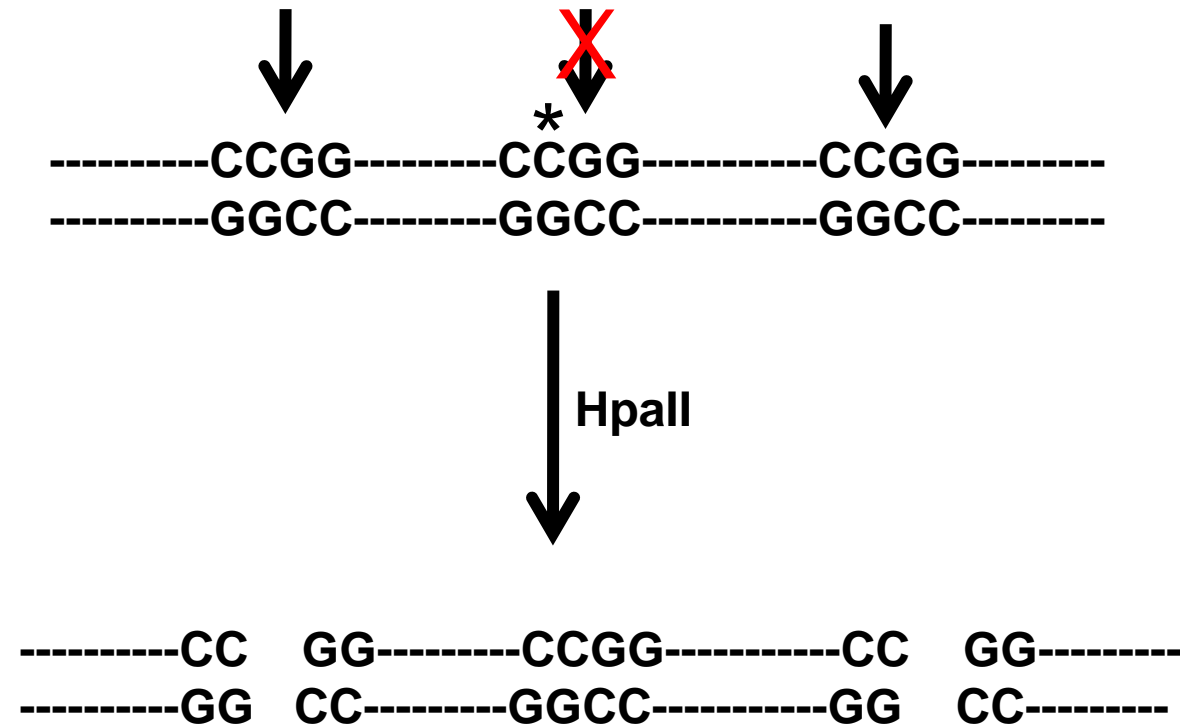
**5-AZACYTIDINE (AZT) IS OFTEN USED TO DEMONSTRATE OR STUDY THE EFFECT OF DNA METHYLATION ON GENE EXPRESSION**

**WHEN INCORPORATED INTO DNA IN PLACE OF CYTOSINE, DEMETHYLATED SITES ARE CREATED.**

**WHEN CELLS ARE TREATED WITH AZT, IT RESULTS IN PROFOUND CHANGES IN GENE EXPRESSION PATTERNS**

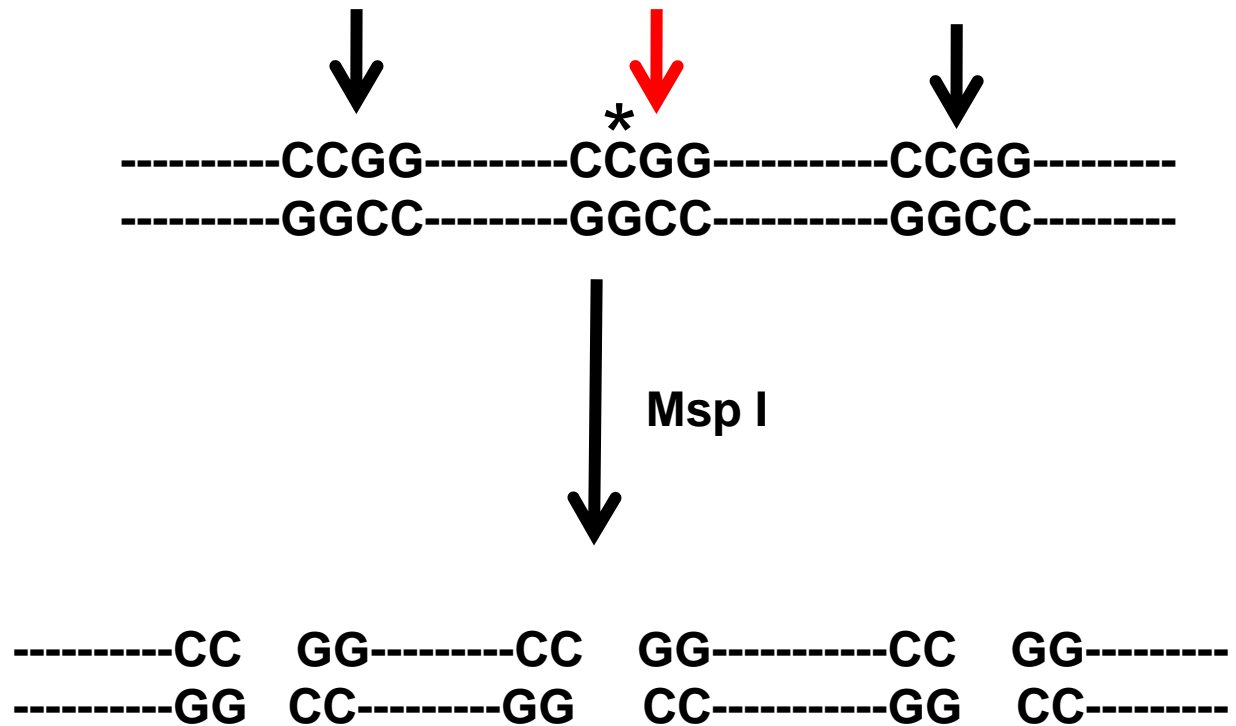
**CERTAIN GENES WHICH ARE NORMALLY NOT NORMALLY EXPRESSED ARE ACTIVATED.**

## Identification of CpG methylation in genomes by MspI, HpaII restriction digestion



HpaII action is blocked by methylation of internal C  
in the CCGG cutting sequence

## Identification of CpG methylation in genomes



Msp I action unaffected by methylation of internal C in the CCGG cutting sequence

## **Identification of CpG methylation in genomes**

**MspI CAN BE USED TO IDENTIFY ALL THE  
CCGG SEQUENCES IN THE GENOME**

**HpaII CAN BE USED TO DETERMINE  
HOW MANY OF THEM ARE METHYLATED**

**WHEN THE DNA IS NON METHYLATED, BOTH MspI AND HpaII  
WILL GENERATE IDENTICAL RESTRICTION DIGESTION PATTERNS**

**IF THE DNA IS METHYLATED, HpaII WILL GENERATE FEWER  
FRAGMENTS THAN MspI**

**Bisulfite sequencing** is used to identify the methylation pattern of DNA

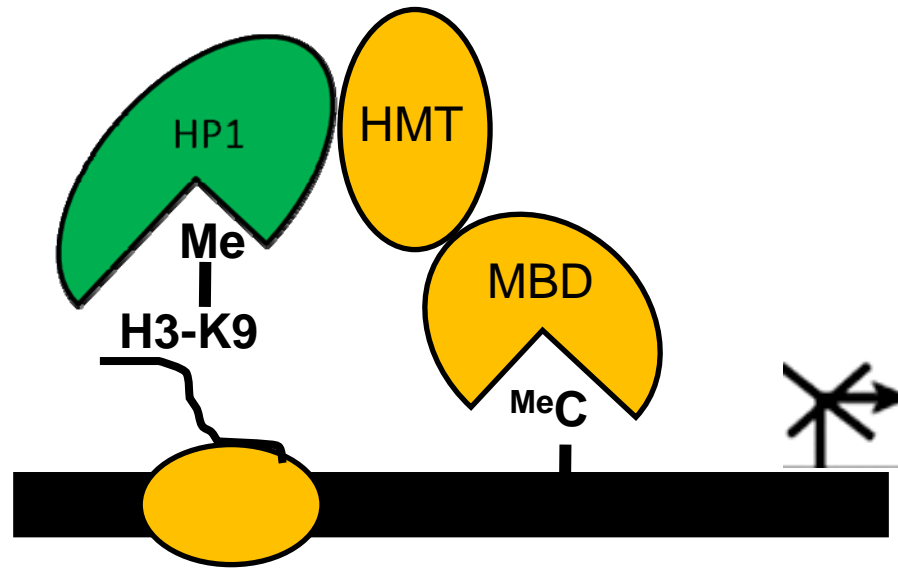
Treatment of DNA with bisulfite converts cytosine residues to uracil, but leaves 5-methylcytosine residues unaffected.

Thus, bisulfite treatment introduces specific changes in the DNA sequence that depend on the methylation status of individual cytosine residues.

[http://en.wikipedia.org/wiki/Bisulfite\\_sequencing](http://en.wikipedia.org/wiki/Bisulfite_sequencing)

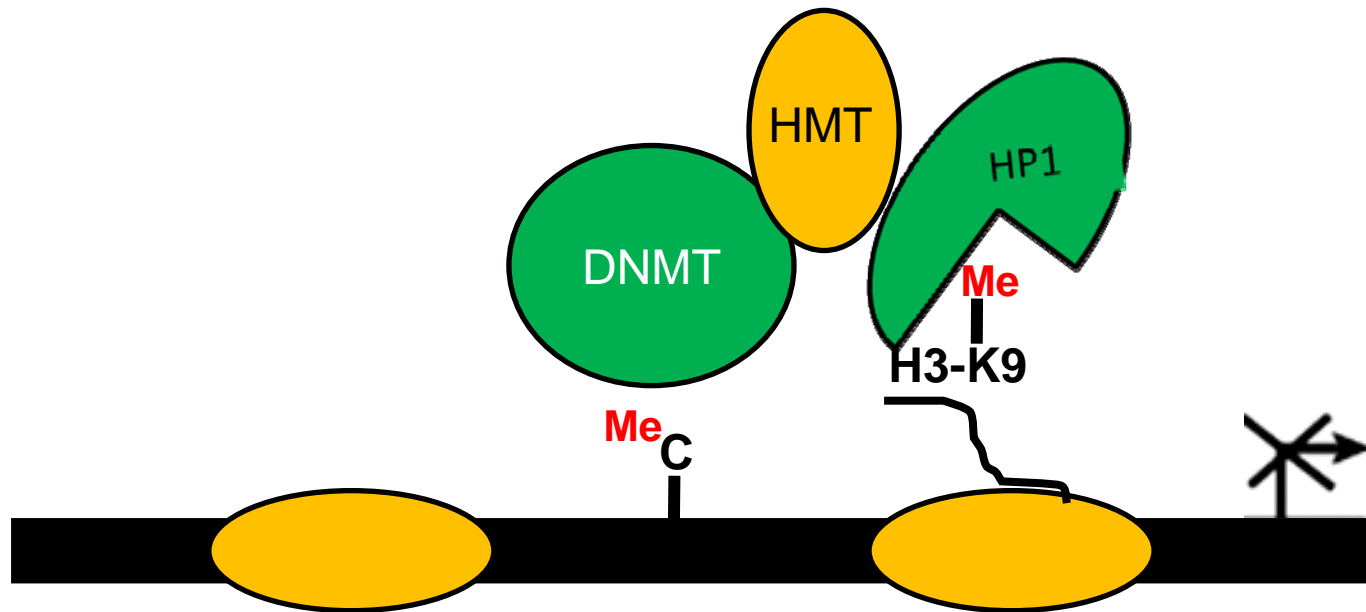
**DNA METHYLATION  
HISTONE METHYLATION  
AND  
HISTONE DEACETYLATION  
ARE ALL INTERCONNECTED**

# DNA METHYLATION – HISTONE METHYLATION

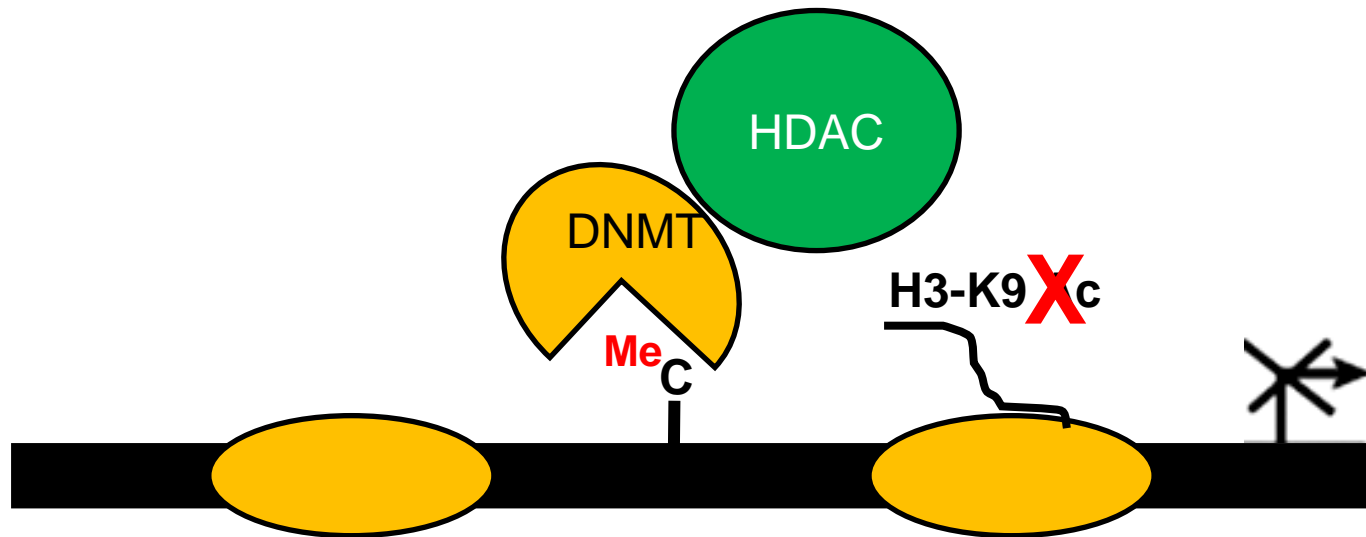




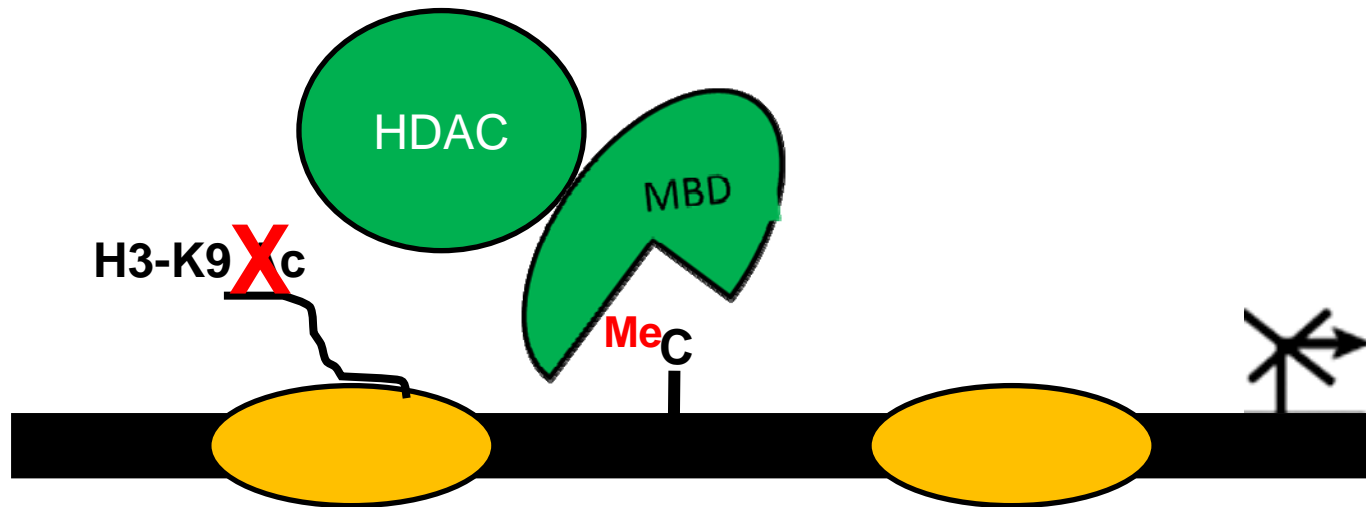
# DNA METHYLATION – HISTONE METHYLATION



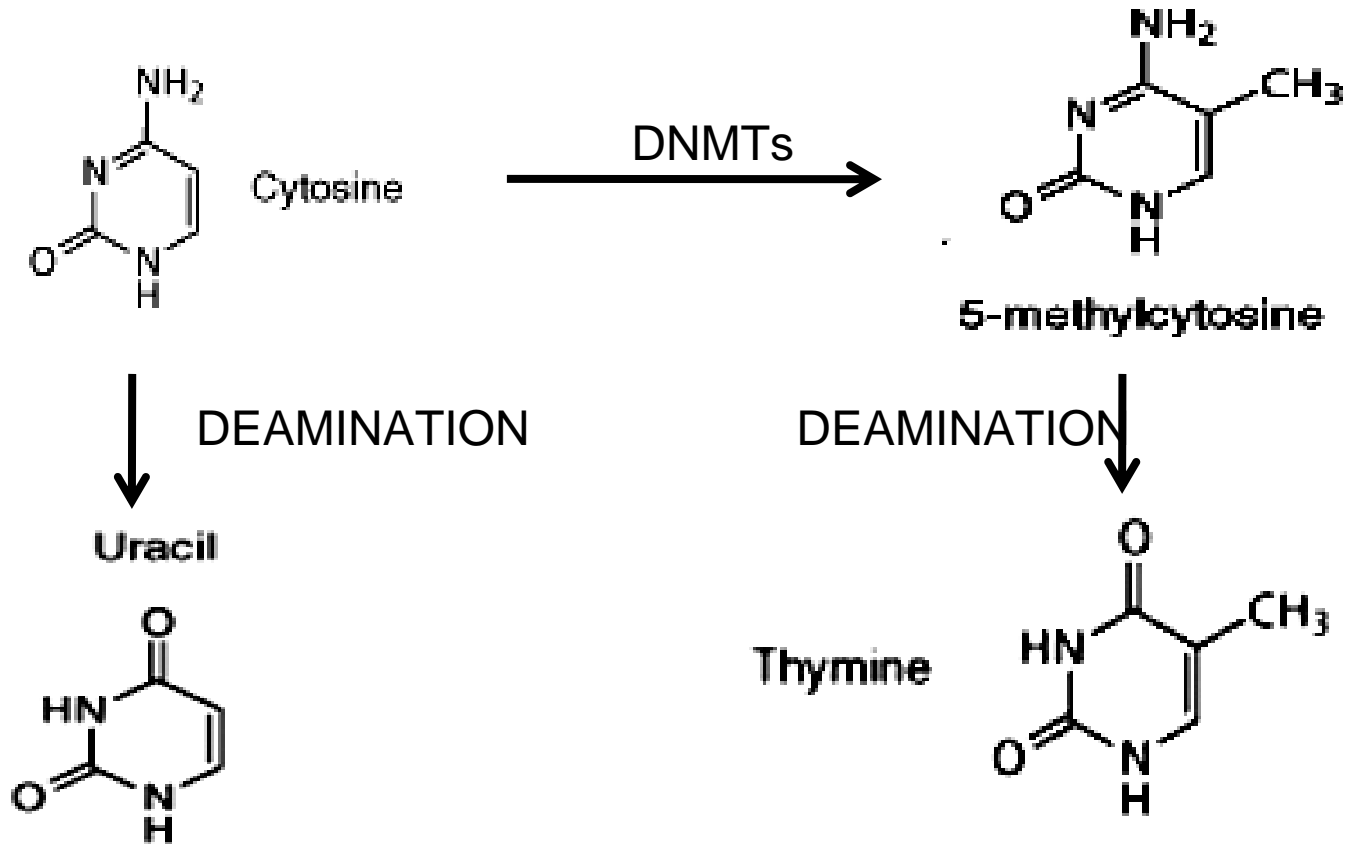
# DNA METHYLATION – HISTONE DEACETYLATION



# DNA METHYLATION – HISTONE DEACETYLATION



## DNA methylation and Mutations



# **DNA methylation and cancer**

**Methylation of CpGs in the promoters of tumor suppressor genes**

**Demethylation of CpGs in the promoters of oncogenes**

## **DNA methylation**

Genomic imprinting,  
X chromosome inactivation,  
Gene silencing,  
Regulation of tissue-specific gene expression  
Maintenance of heterochromatin

**The generation of genomic methylation patterns is a dynamic process that requires demethylation and *de novo* methylation by the action of the two *de novo* methyltransferases:**

**Dnmt3a  
Dnmt3b**

**during gametogenesis and early embryonic development.**

**Once the methylation patterns are created, they are perpetuated by the maintenance methyltransferase Dnmt1, leading to somatic inheritance**

## **Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene**

**Nature** (1999) 402: 187-191

The recessive autosomal disorder known as ICF syndrome (for immunodeficiency, centromere instability and facial anomalies) is characterized by variable reductions in serum immunoglobulin levels which cause most ICF patients to succumb to infectious diseases before adulthood.

Five unrelated ICF patients have mutations in both alleles of the gene that encodes DNA methyltransferase 3B



**DNA Methylation is globally erased  
during gametogenesis  
and  
embryogenesis  
and then re-established**

## **Genomic imprinting**

**Some genes are expressed only from the maternal genome and some only from the paternal genome**

**It is estimated that about 40 genes are imprinted and they can be found on several different chromosomes**

**DNA methylation plays a key role in genome imprinting**

**THERE ARE FOUR BASES IN DNA**

**ADENINE  
GUANINE  
CYTOSINE  
THYMINE**

**THERE ARE **FIVE** BASES IN DNA**

**ADENINE  
GUANINE  
CYTOSINE  
THYMINE**

**METHYL CYTOSINE**

**(epigenetic regulation of gene expression)**

## HISTONE MODIFICATIONS - HISTONE CODE

### DNA METHYLATION

**HISTONE MODIFICATIONS + DNA METHYLATION = EPIGENETIC CODE**

The epigenetic code is a defining code in every eukaryotic cell consisting of the specific epigenetic modification in each cell.

It consists of histone modifications defined by the histone code and additional epigenetic modifications such as DNA methylation.

The basis for the epigenetic code is a system above the genetic code of a single cell.

While in one individual the genetic code in each cell is the same, the epigenetic code is tissue and cell specific.

**The term EPIGENETICS is often used to study heritable traits that do not involve changes to the underlying DNA sequence.**