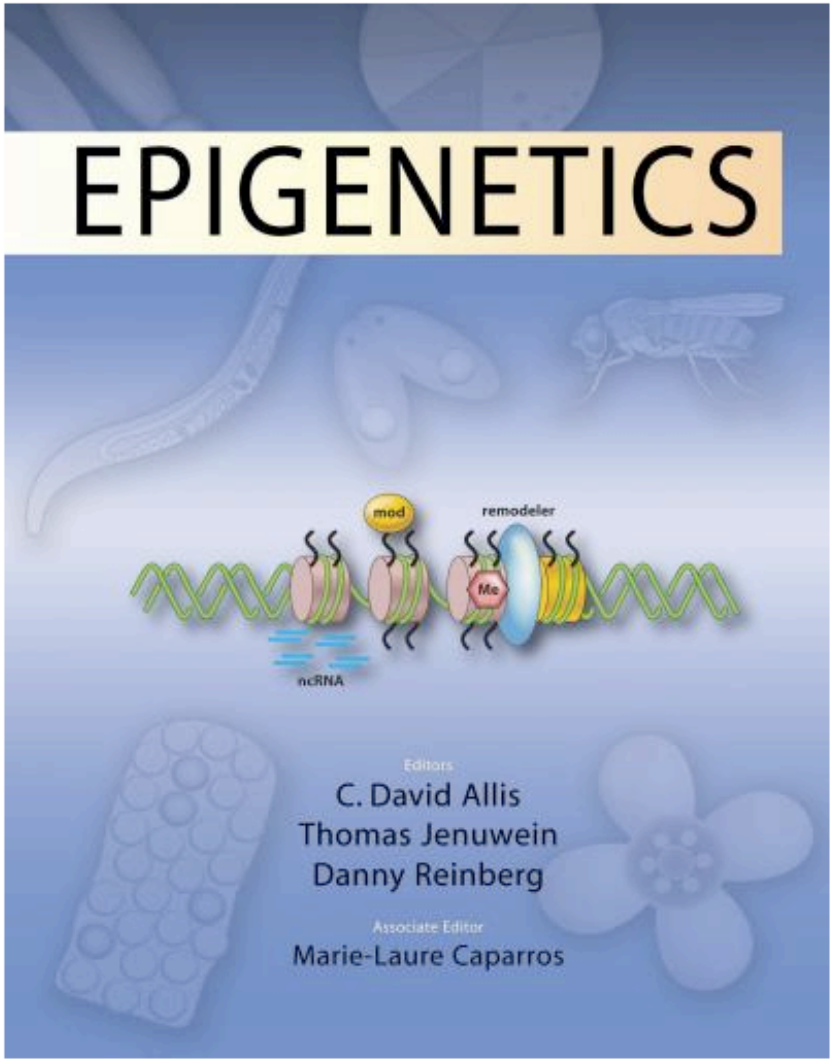
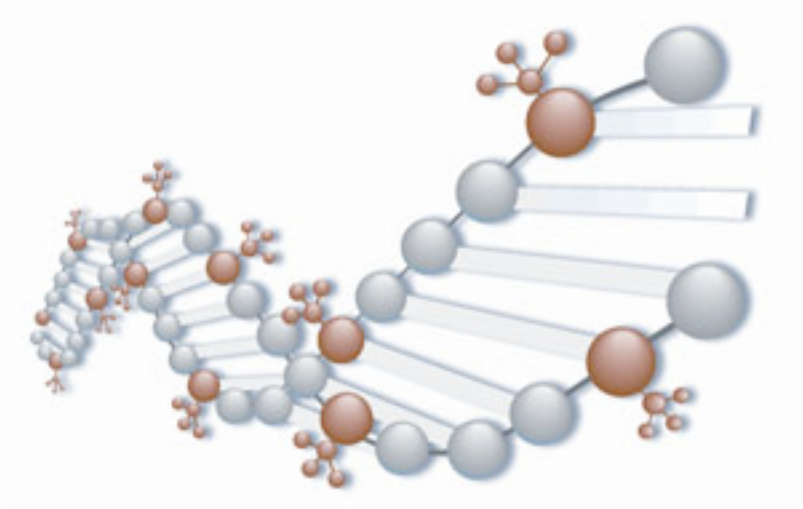
Gene Expression GE4055

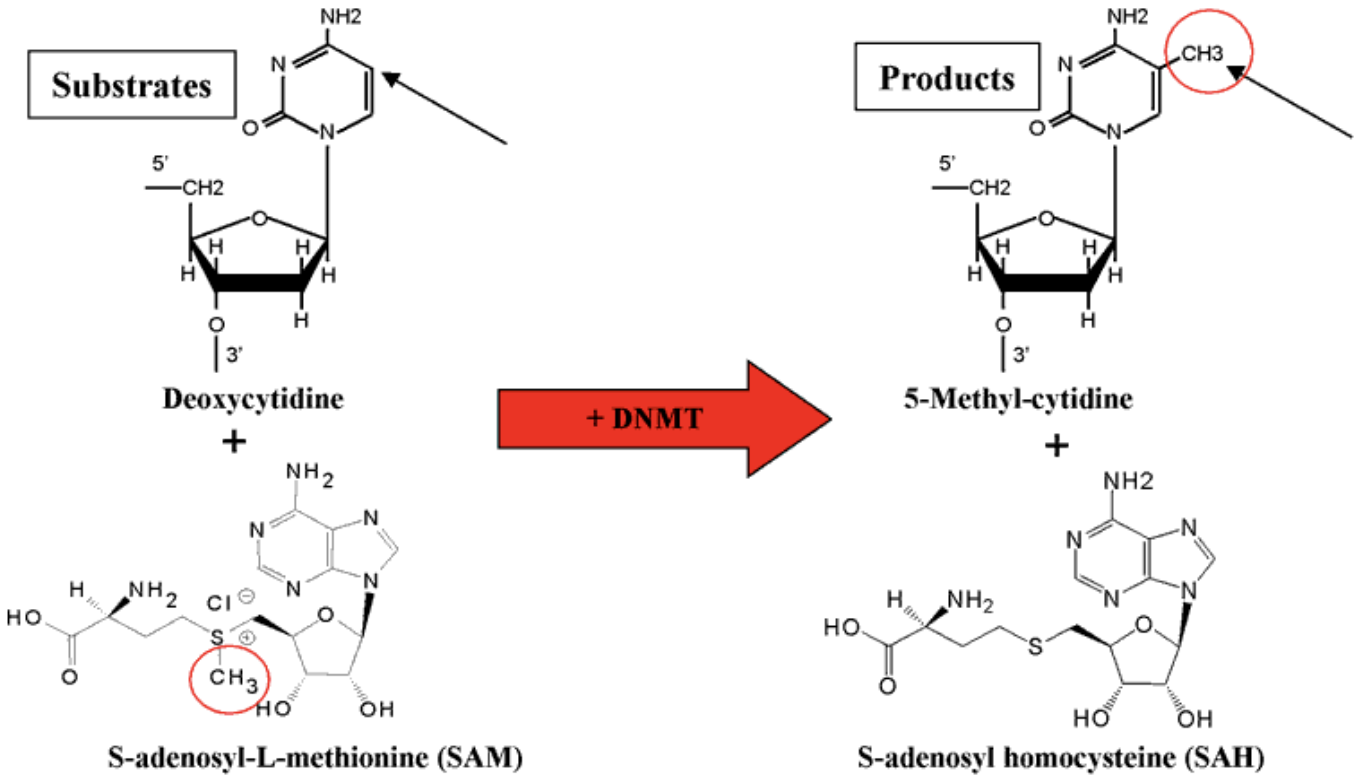
- Lecture 1: Introduction to Epigenetics
- Lecture 2: Histone modifications
- Lecture 3: Polycomb and Trithorax proteins
- Lecture 4: DNA Methylation
- Lecture 5: Non-Coding RNAs
- Lecture 6: Epigenetics in differentiation
- Lecture 7: Epigenetic reprogramming
- Lecture 8: Epigenetics in cancer and other disease



Week 4: DNA Methylation



DNA Methylation Reaction Catalyzed by DNA Methyltransferase (DNMT)

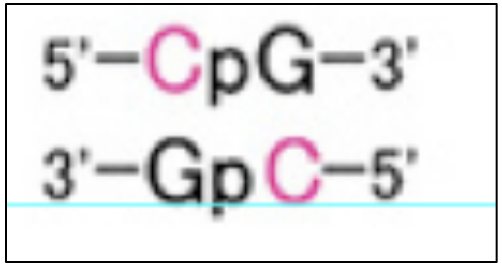


CpG – Cytosine phosphate Guanine

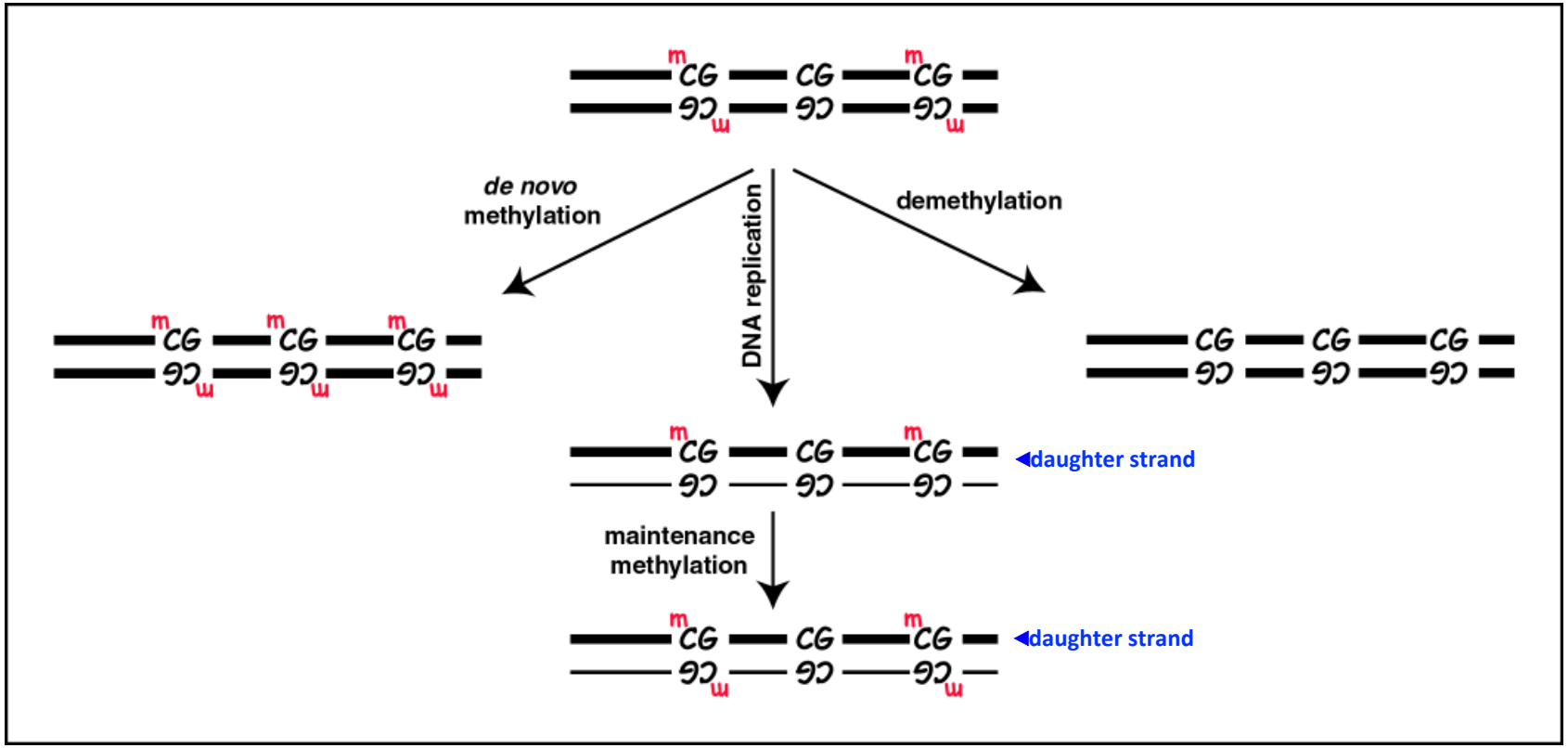
Methylation occurs at CpG dinucleotides in mammals

(No DNA methylation in flies. In plants CpG or CpNpG)

Note: the CpG is self complimentary

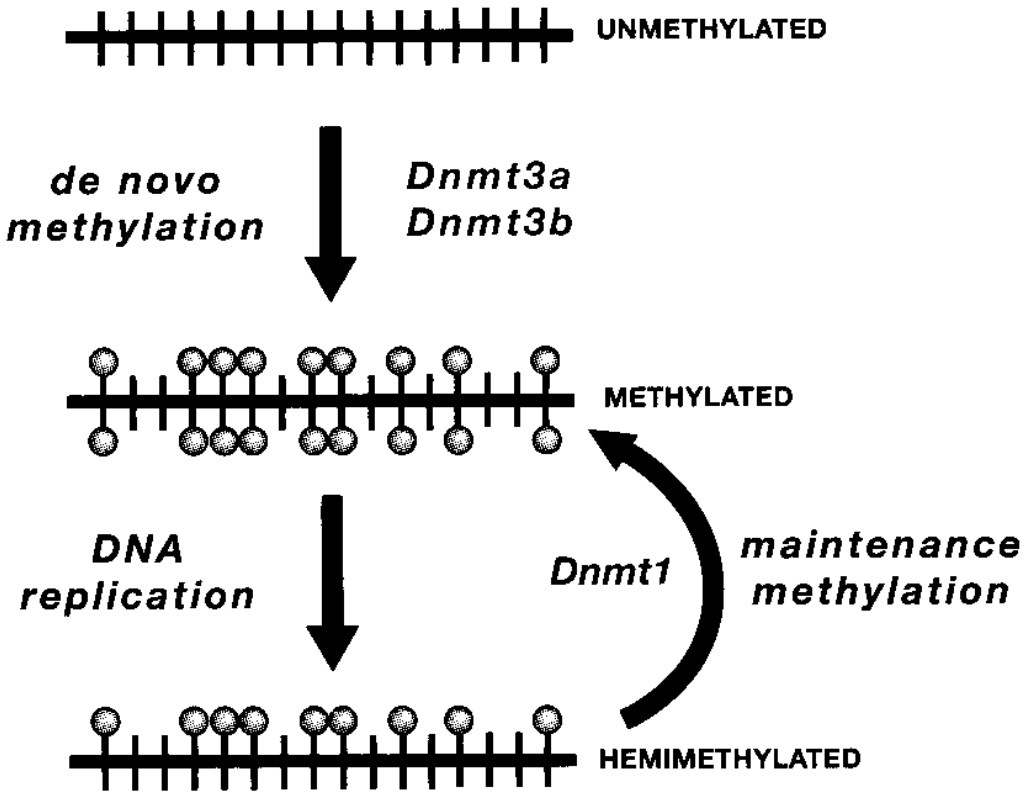


DNA methylation is stably maintained during DNA replication

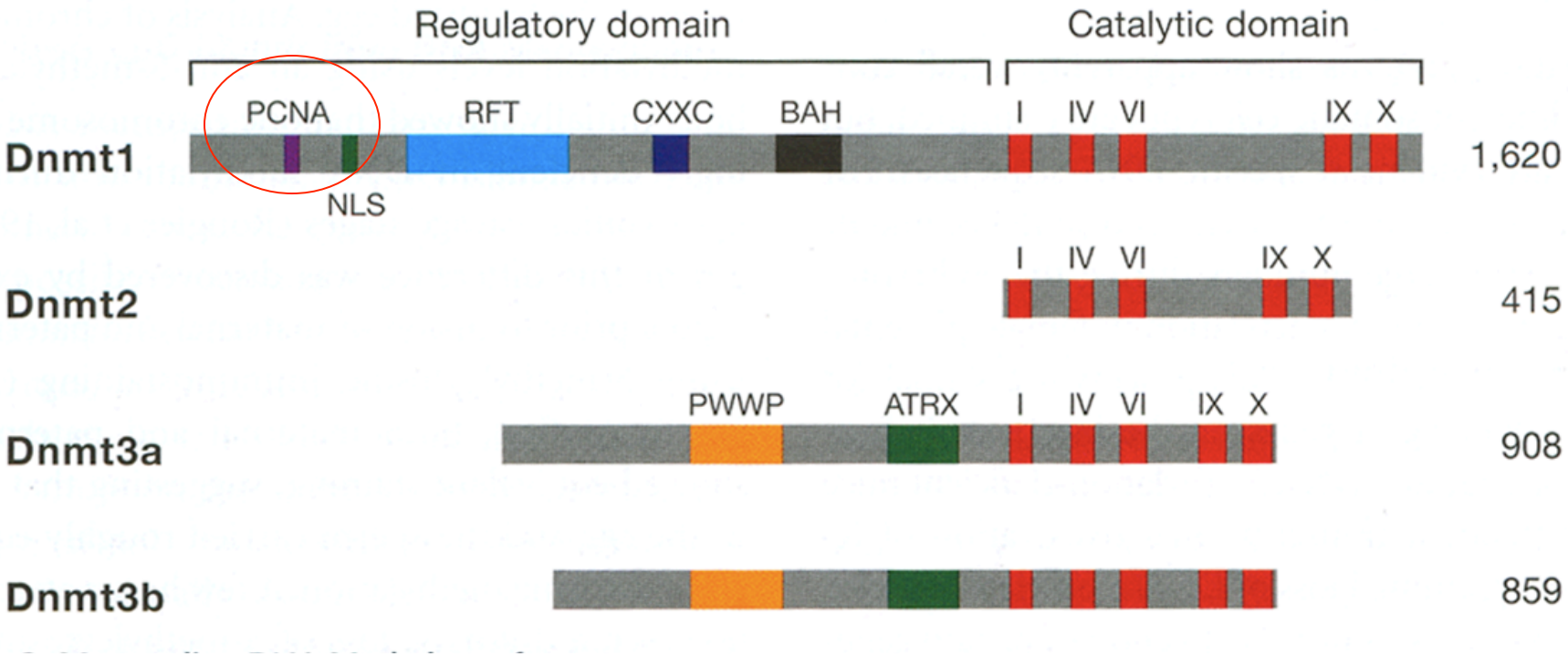


What are the enzymes involved?

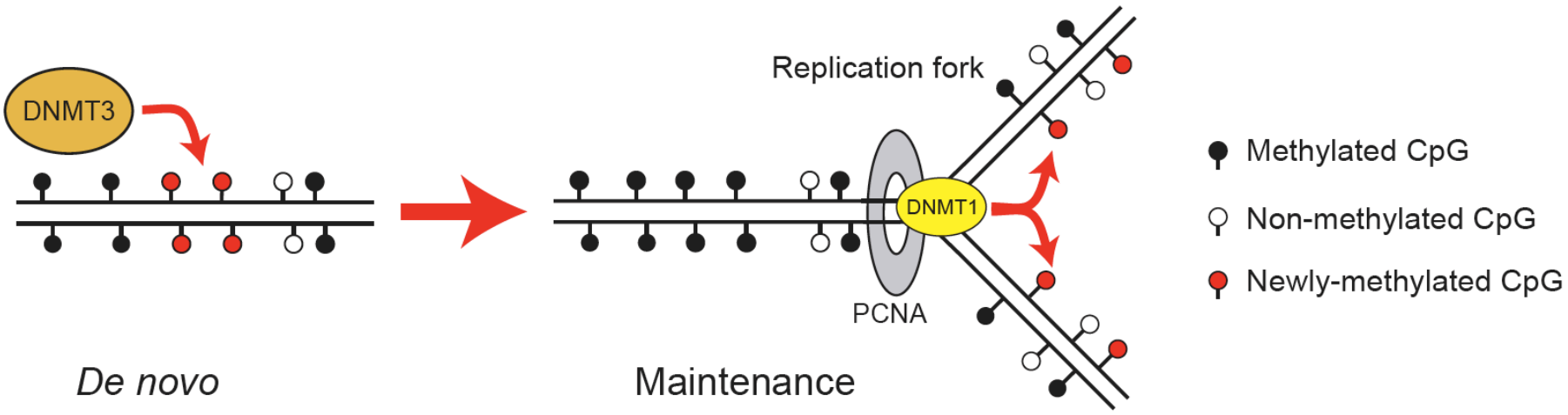
- CpG's are marked by vertical lines
- Unmethylated DNA becomes methylated (de novo) by Dnmt3a and Dnmt3b to give methylation on both strands at certain CpG pairs
- After Replication Dnmt1 functions to methylate the "daughter strand"



What makes Dnmt1 different?

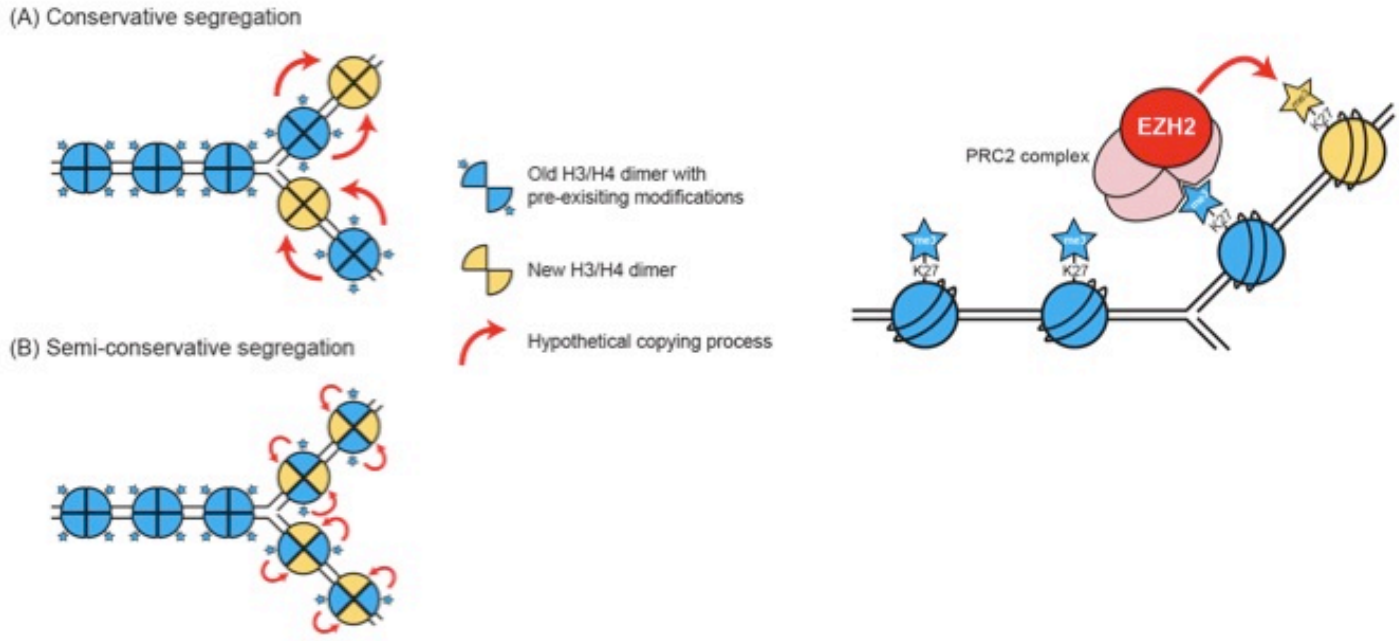


There are currently four members of the DNA methyltransferase family in mammalian cells. All of these proteins have their catalytic domain in the C-terminal region, and a regulatory domain in the N-terminal region (with the exception of DNMT2). Most of the protein-protein interactions are mediated by the N-terminal region


DNMT3
Replication fork
DNMT1
PCNA
Methylated CpG
Non-methylated CpG
Newly-methylated CpG
De novo
Maintenance

From lecture 3 – Polycombs….

A model for transmission of the H3K27me3 epigenetic mark through cell division

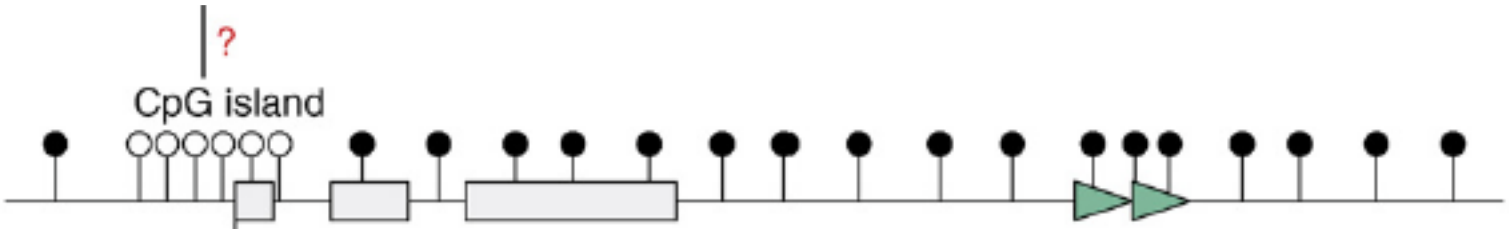


A model for transmission of the H3K27me3 epigenetic mark.
Hansen KH, Bracken AP, Pasini D, Dietrich N, Gehani SS, Monrad A, Rappsilber J, Lerdrup M, Helin K.
Nat Cell Biol. 2008 Nov;10(11):1291-300.

Role of the polycomb protein EED in the propagation of repressive histone marks.
Margueron R, Justin N, Ohno K, Sharpe ML, Son J, Drury WJ 3rd, Voigt P, Martin SR, Taylor WR, De Marco V, Pirrotta V, Reinberg D, Gamblin SJ.
Nature. 2009 Oct 8;461(7265):762-7

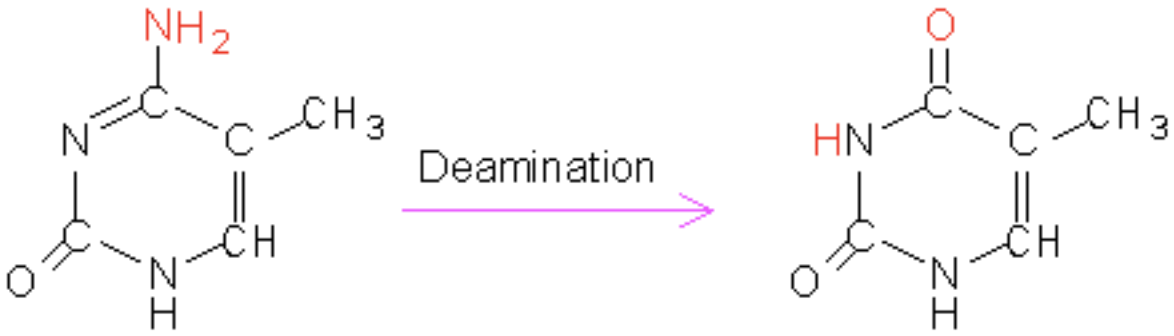
Where is DNA methylation in the Genome?

- Widespread DNA methylation in the entire genome e.g. non-coding regions e.g. introns and intragenic DNA, repetitive regions (transposons and retroviral derived sequences), centromeric heterochromatin and silent X-chromosome
- This is consistent with the idea that hypermethylation is the default epigenetic state and serves in maintaining genome integrity
- HOWEVER, there is limited DNA methylation at promoter regions



CpG Frequency in vertebrates

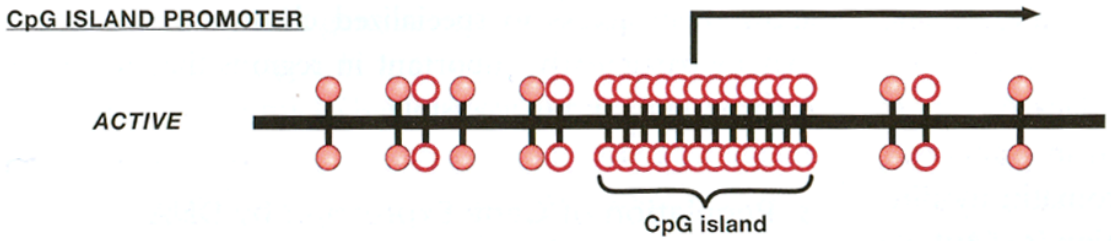
- CpG dinucleotides occur with a much lower frequency in the sequence of vertebrate genomes than would be expected due to random chance
- For example, the human genome has a 42% GC content. Therefore a pair of nucleotides consisting of cytosine followed by guanine would be expected to occur 0.21 x 0.21 = 4.41% of the time.
- BUT frequency of CpG dinucleotides in human genomes is only 1%.
- Scarano et al. proposed that the CpG deficiency is due to an increased vulnerability of methylcytosines to transition mutation in genomes with CpG cytosine methylation.



5-methylcytosine Thymine

An exception to the rule….

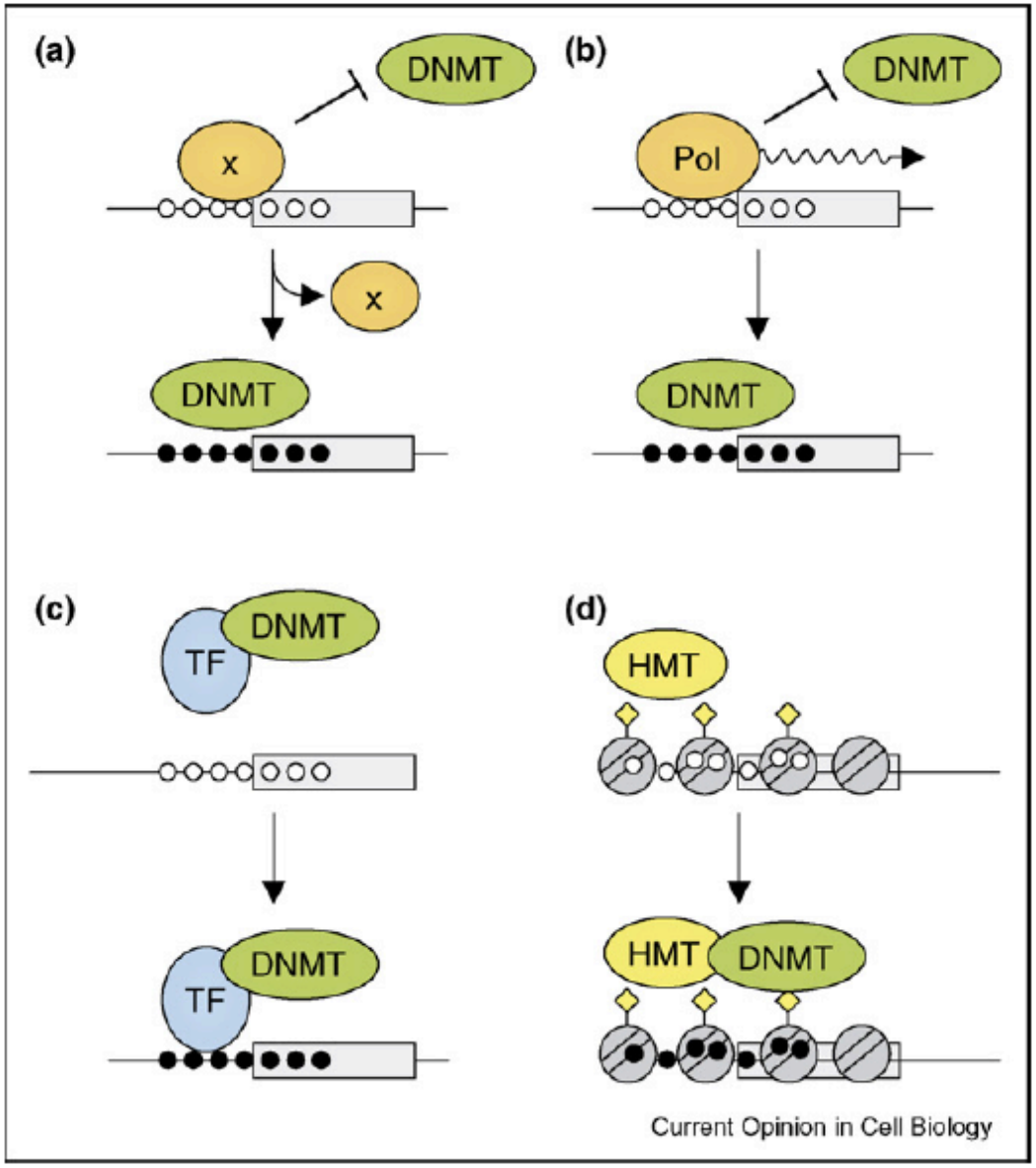
CpG Islands are ~1000bp stretches of DNA at the 5' end of 60-90% of all promoters in somatic cells



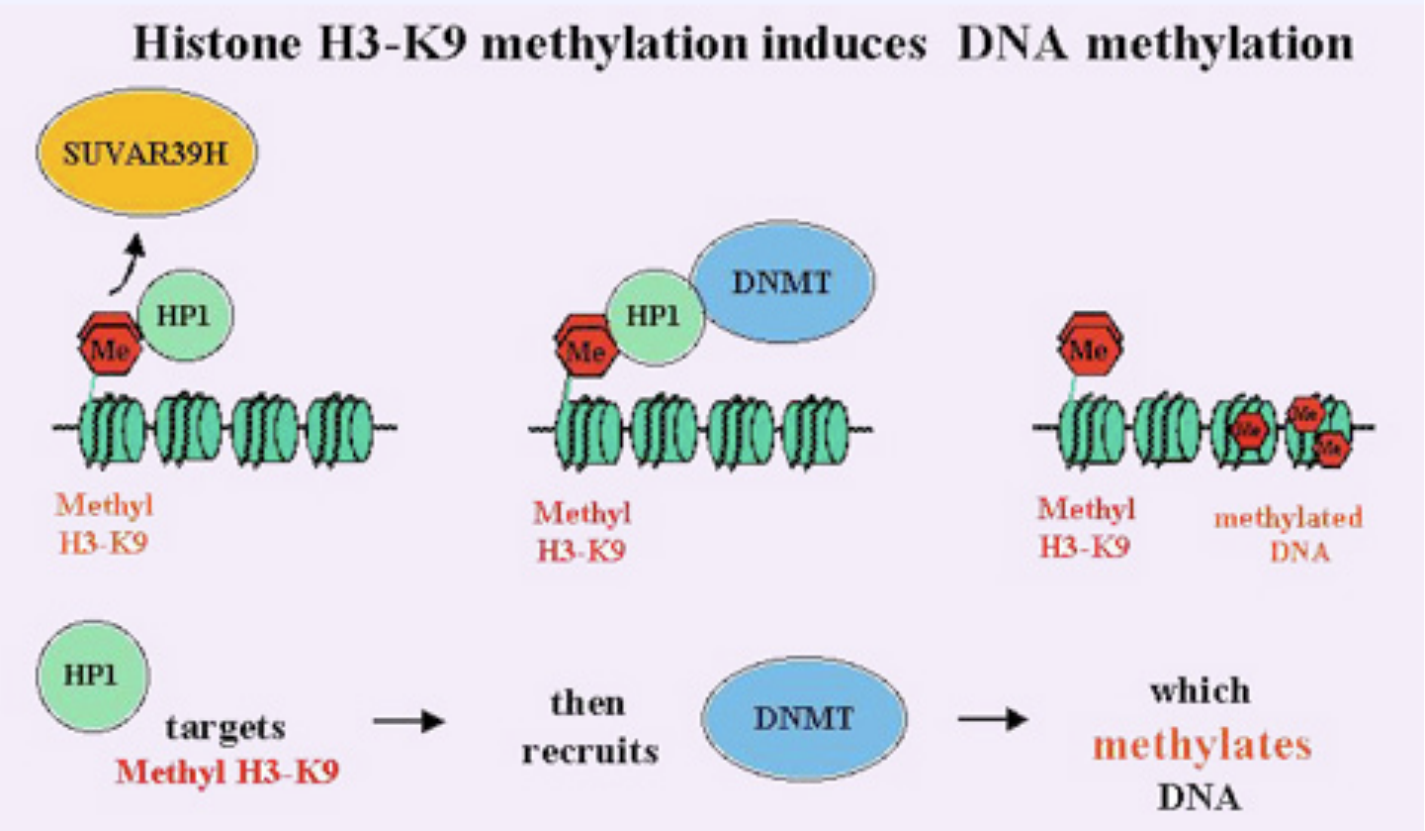
CpG islands are regions of high CpG density that lack CpG methylation found at promoters of most human genes. Long-term silencing of the gene can be ensured by methylation of the CpG island

- methylated Cytosine Spontaneously deaminates to form thymine
- Poorly recognised by DNA repair systems thus:
    - CG→TG mutation is propagated
    - CpG levels are less frequent than predicted 1/16
    - But they are enriched at gene promoters
    - Why?

How is DNA methylation recruited to DNA?



Histone H3-K9 tri-methylation potentially recruits DNA methylation…

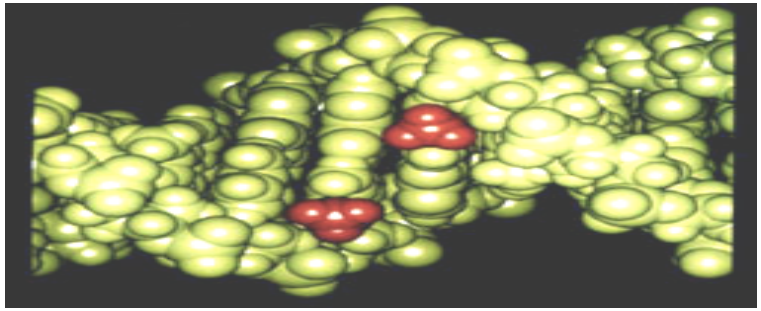


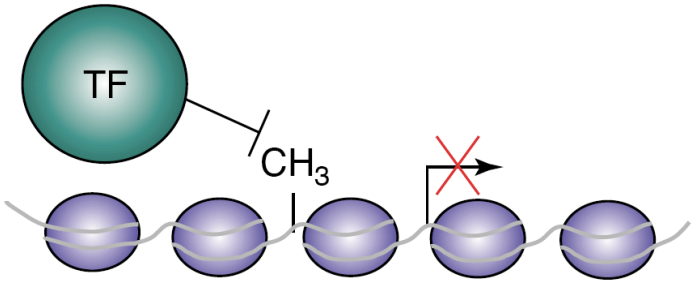
SUVAR39H is a methyltransferase which specifically methylates the Lysine 9 of histone H3.

This methylation creates a binding site for the Heterochromatin Protein HP1 which recruits a DNA methyltransferases

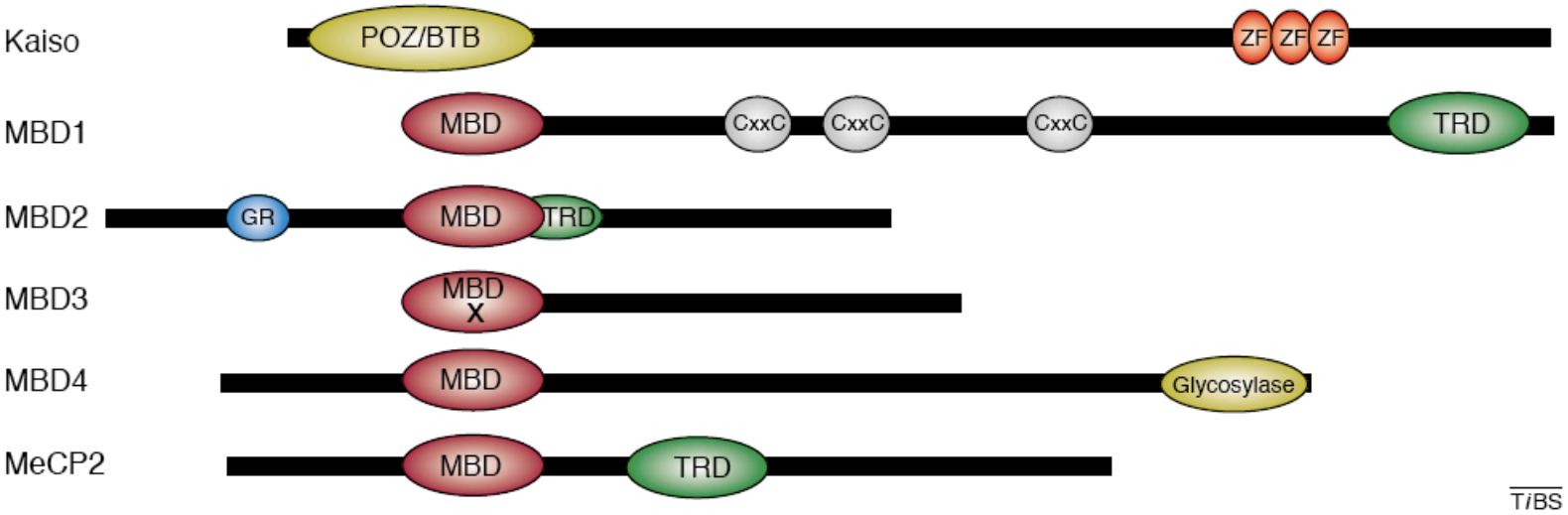
How does DNA methylation affect transcription?

Model 1: Methylated CpG's block the binding of transcriptional activators?





Model 2: Attraction of methyl-CpG-binding Proteins



From lecture 2 – Histones….

Effector proteins and domains

Effector

A protein domain that binds a particular histone modification to transduce a downstream function. Effector proteins themselves or other subunits of complexes in which they reside can be histone-modifying enzymes or chromatin remodelling factors, or they can be involved in stabilization of heterochromatin or have some other gene regulatory role.

Chromodomain

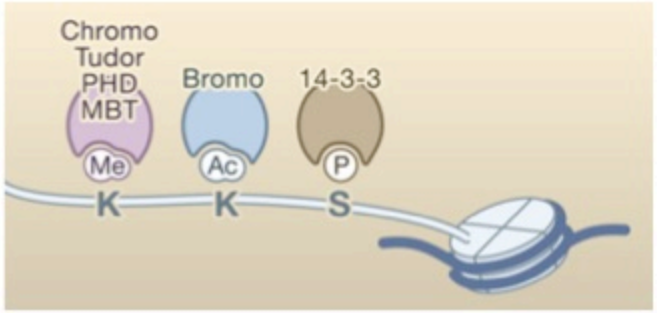
A domain with sequence conservation that is found in several transcriptional regulatory proteins and are known to bind methylated histones, HP1 and CBX4, CBX8

Bromodomain

A domain with sequence conservation that is found in several transcriptional regulatory proteins involved in gene activation, and that has acetyl-Lys-binding activity.

PHD finger domain

The plant homeodomain (PHD) zinc finger is found in many nuclear proteins that are thought to be involved in chromatin transactions.



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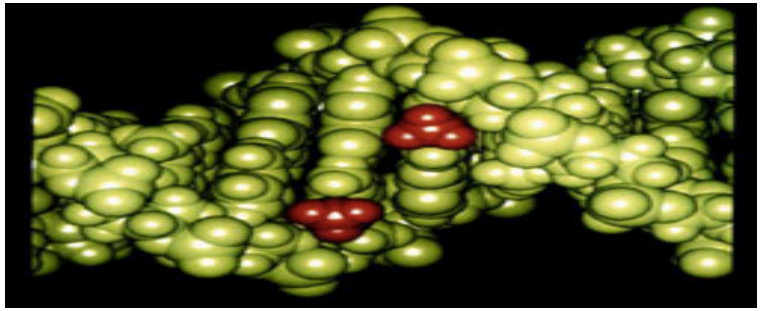

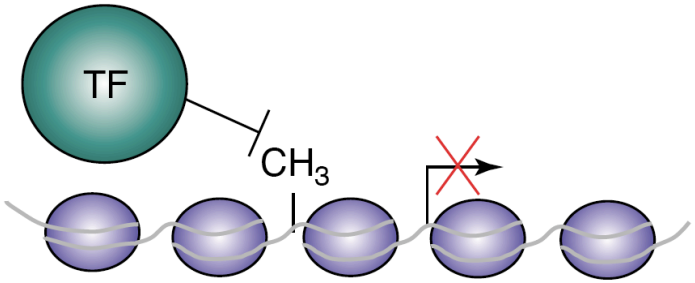

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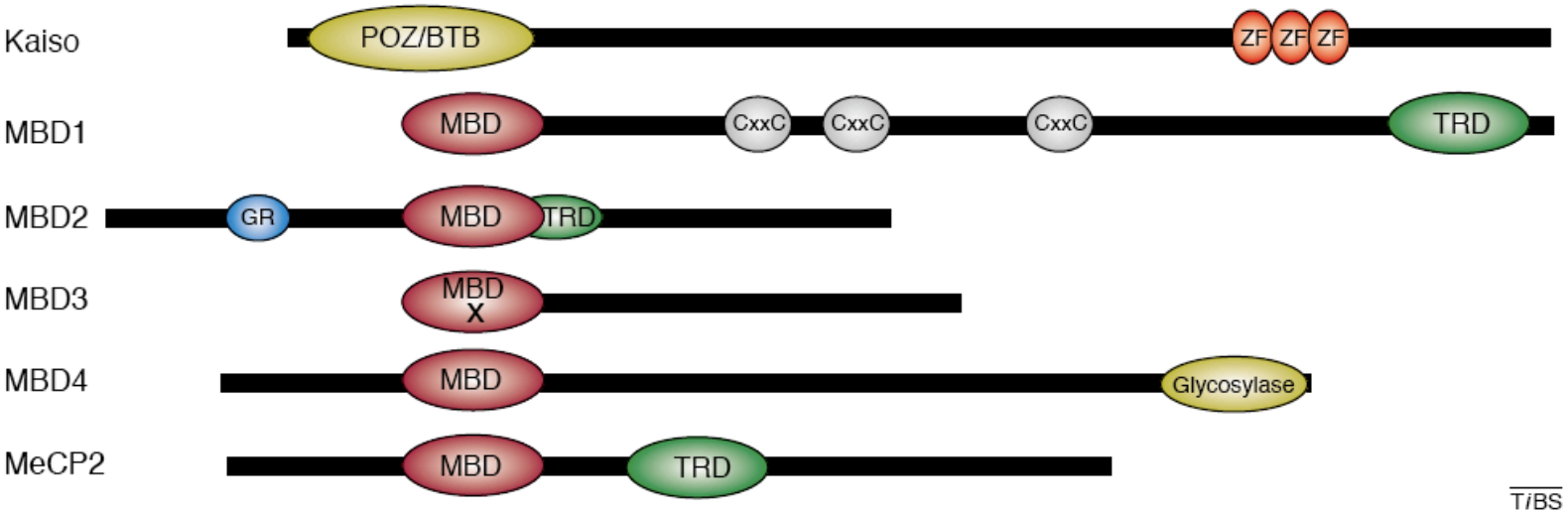
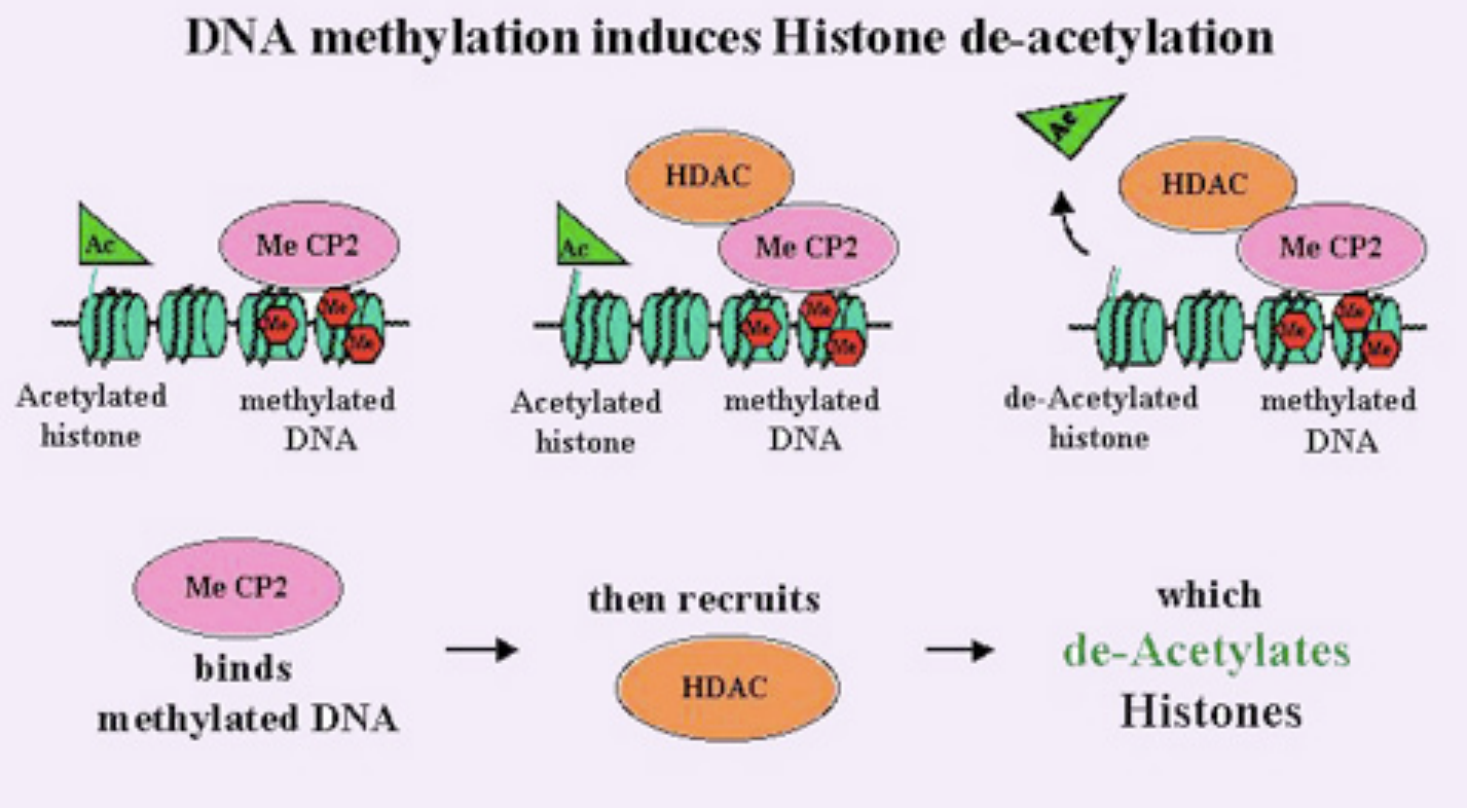
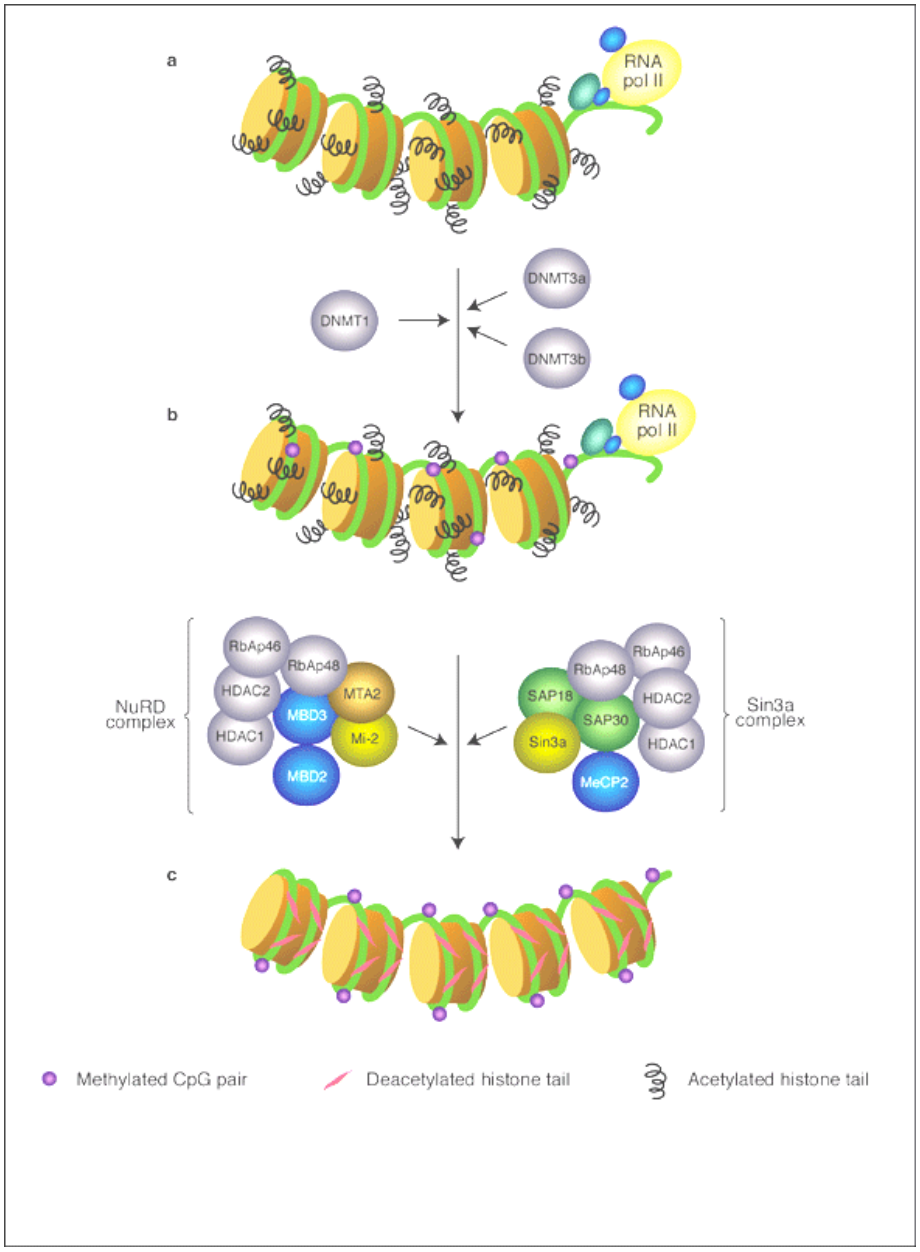


CpG–island methylation – how does it affect transcription?

- methylated-DNA binding proteins (e.g. *MECP2*, methyl CpG binding protein 2) bind to DNA
- this recruits a complex of histone deacetylases and *SIN3A*
- induces a closed chromatin structure → gene silencing
- in contrast to usual deacetylation-related silencing, when **DNA methylation** is involved, it's thought to be (almost) *irreversible*



Proposed mechanism by which DNA methylation leads to transcriptional repression.

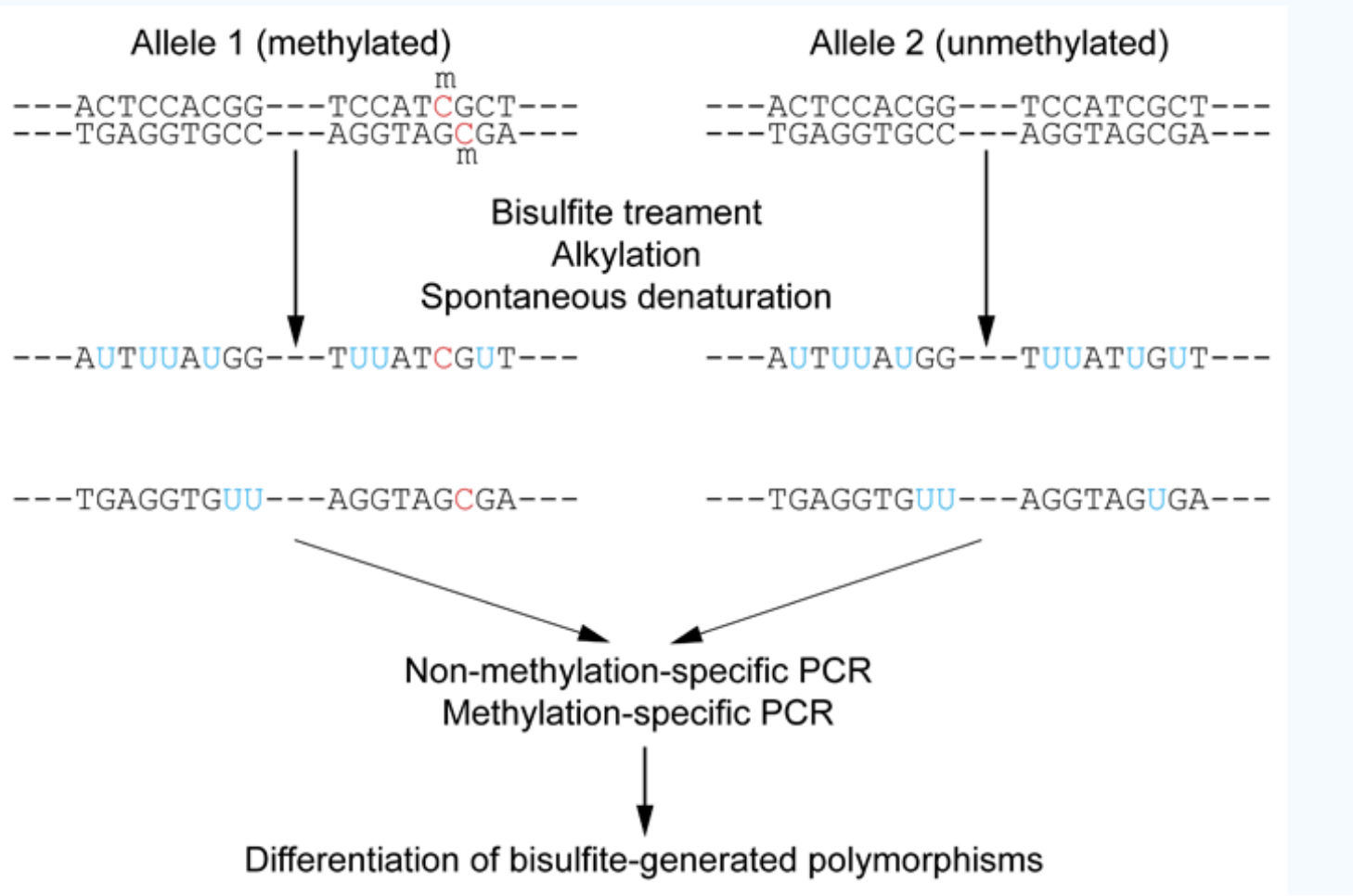


(a) Transcriptionally active chromatin is predominantly unmethylated and has high levels of acetylated histone tails (short black squiggles).

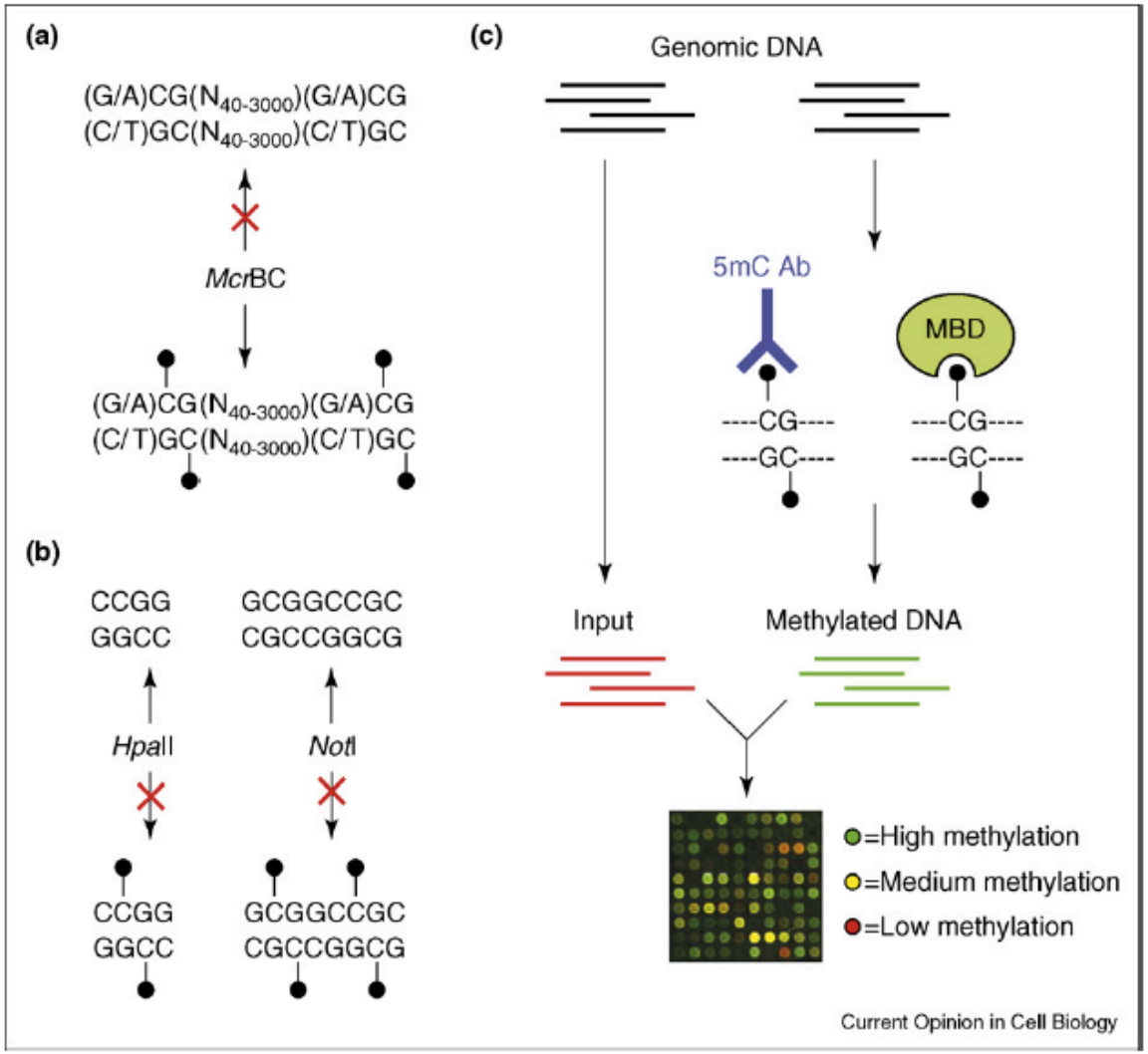
(b) Methylation at CpG dinucleotides is carried out by one of the three known human DNA methyltransferases (DNMT1, 3a and 3b), resulting in DNA with high levels of CpG methylation (purple circles), but still containing predominantly acetylated histone tails. DNA in this form would still be expected to be transcriptionally competent.

(c) Methylated DNA is targeted by methyl-binding domain (MBD) proteins such as MBD2 and MeCP2, which are found associated with large protein complexes such as the NuRD complex (MBD2) and the Sin3a complex (MeCP2). Histone deacetylase (HDAC1 and 2) and chromatin-remodelling activities (Mi-2 and Sin3a) within these complexes result in alterations in chromatin structure, producing chromatin that is refractory to transcriptional activation (pink streaks represent deacetylated histone tails). The functional roles of other components in these complexes are not yet known. Abbreviations: MTA2, metastasis-associated protein 2; RbAp46/48, retinoblastoma-associated protein 46/48; RNA pol II, RNA polymerase II; SAP18/30, Sin3-associated polypeptides 18/30

How do you check if a stretch of DNA is methylated?



Mapping DNA methylation in the (epi)genome

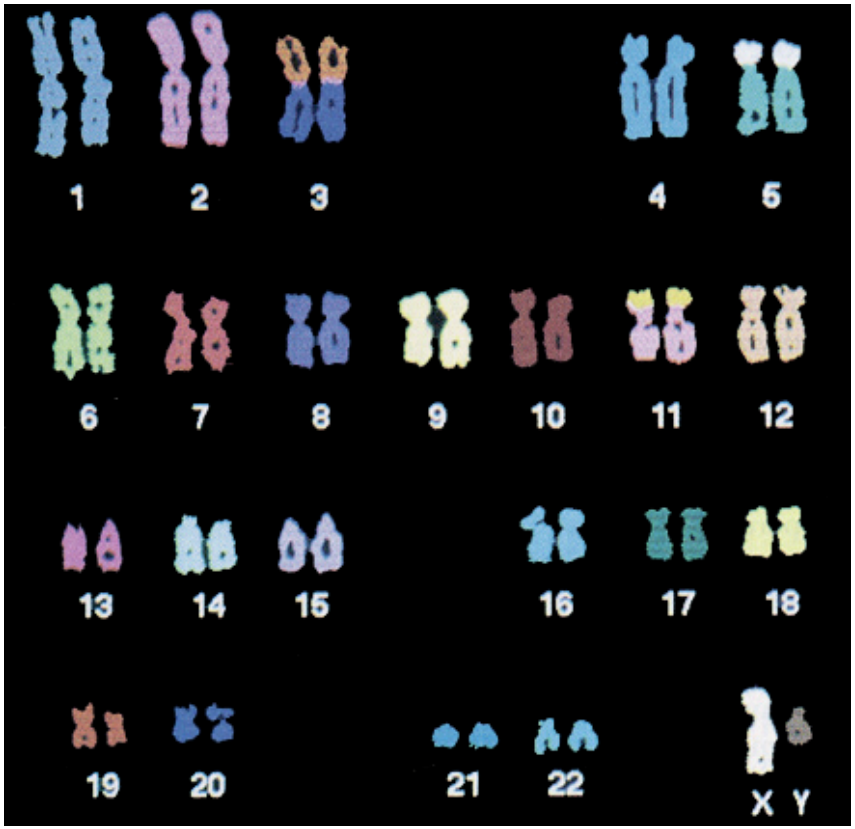


Technologies to map DNA methylation genome-wide. Classical approaches to study DNA methylation use restriction enzymes that cut only (**a**) methylated (*Mcr*BC) or (**b**) unmethylated (*Hpa*II, *Not*I) DNA; however, these methods limit the analysis to particular sequence motifs. (**c**) Alternative methods use isolation of methylated DNA with antibodies or MBD proteins. The methylated DNA (labeled in green) can be used for cohybridization with input DNA (labeled in red) on any existing microarray. Lollipop shapes denote methyl groups.

A break from enzymes and mechanisms!

….a story about female cat development….

What makes boys different to girls??



Chromosomal dosage and compensation

- Women are XX, men are XY
- How are levels of all essential X-encoded gene products similar between men and women if women have twice the number of alleles?

Mary Lyon – 1961

In cells with multiple X chromosomes, all but one is inactivated during mammalian embryogenesis – the "*Lyon effect*"

X-inactivation; which X? Usually random

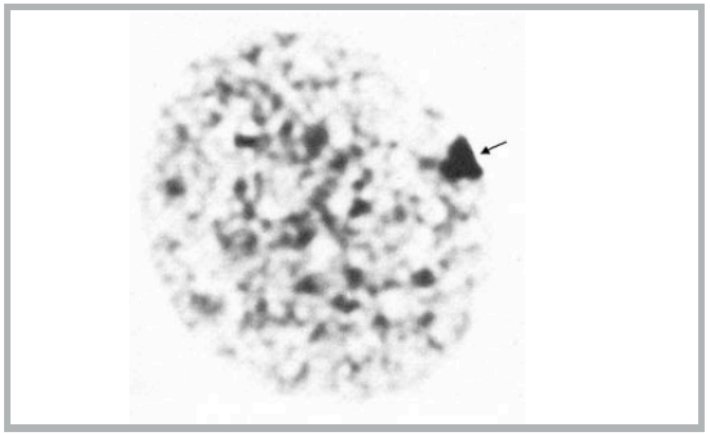


Figure 14.3: *A female cell showing a darkly stained 'Barr Body' which is the inactivated X chromosome (source: Gardner RJM & Sutherland GR (1996):*

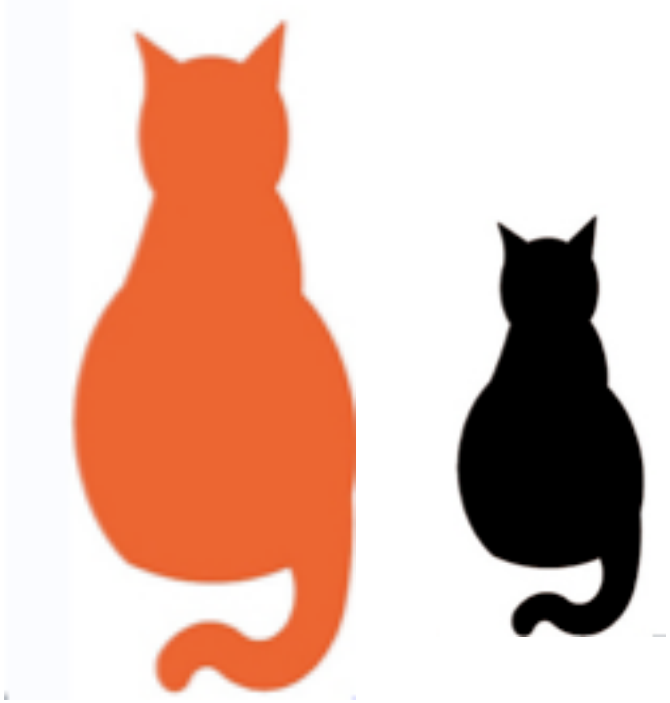
Cats: One X-linked Gene with Two Alleles for Coat Color

In cats, one of several genes controlling fur color is located on the X chromosome.

The gene has two alleles.

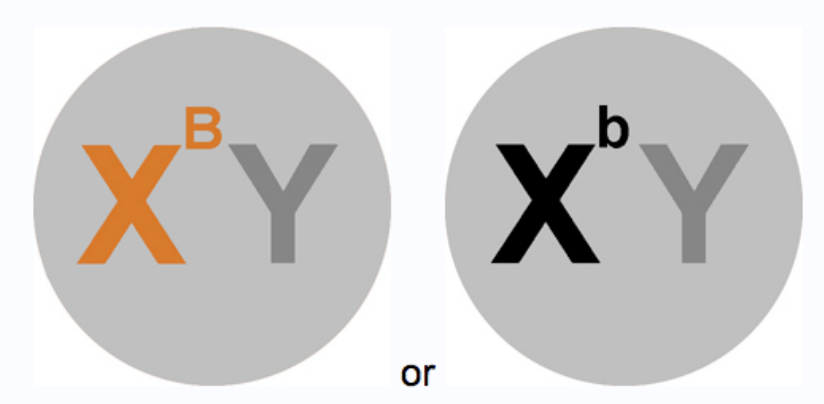
One form of the gene codes for orange fur (XB) and the other form codes for black fur (Xb).

The orange allele is dominant to the black allele.

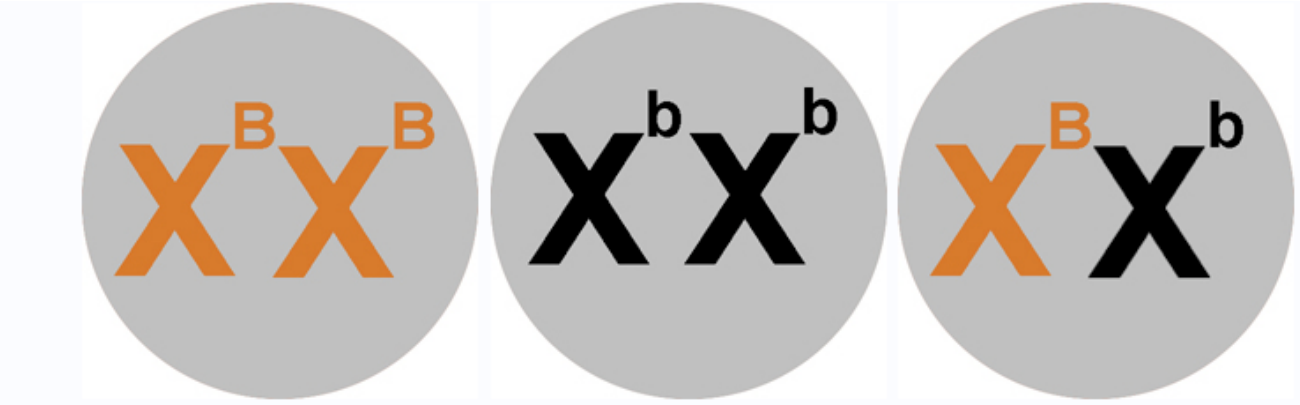


Cats: One X-linked Gene with Two Alleles for Coat Color

Male Genotypes
There are two possible (normal) male genotypes:

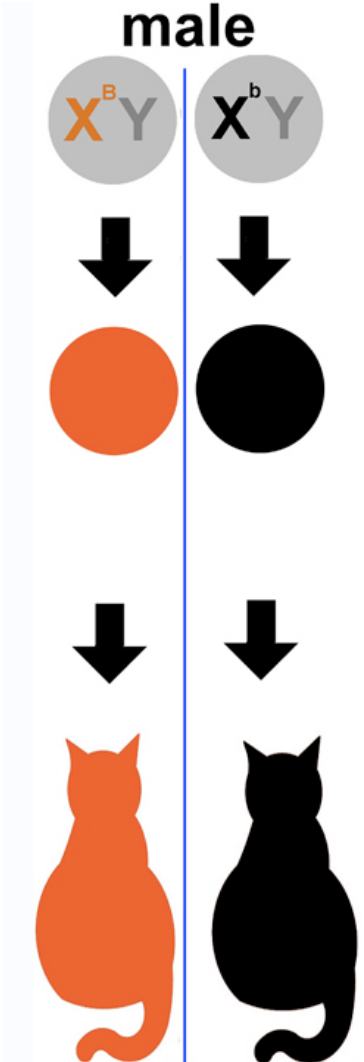


Female Genotypes
There are three possible female genotypes for this locus:



Expression of Coat Color in Males

Male color expression is straightforward. Because only one allele exists for the gene in each cell, it will be expressed. And all males have only B or only b. There are no heterozygotes, because the Y does not carry the allele.



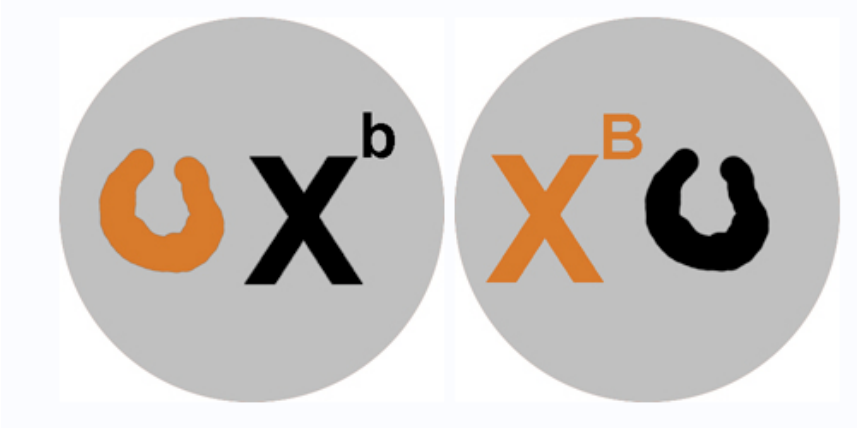
Expression of Coat Color in Females

In females, the situation is more complicated because of an interesting process that prevents the female from expressing double the amount of X-linked gene products as the male, who has only one copy of each X-linked gene.

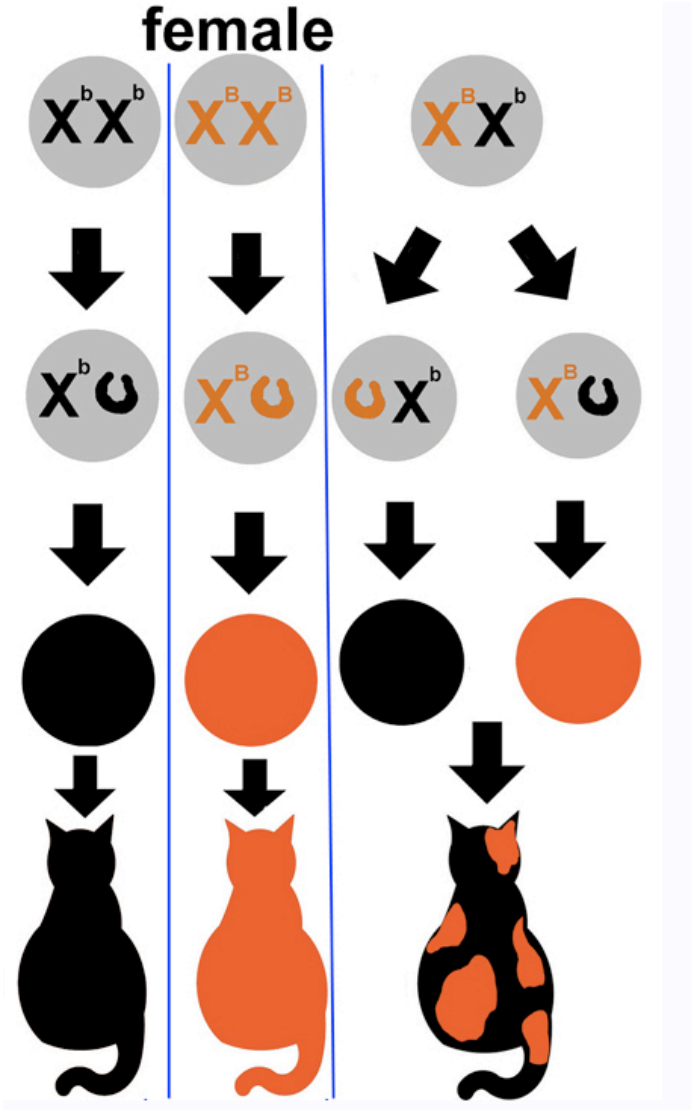
At a certain point in the embryonic development of every female mammal (including cats), one of the two X chromosomes in each cell is “inactivated”.

This irreversible process leaves only ONE active X chromosome in each cell of the female embryo. Only the alleles on the active X chromosome are expressed.

This is a random event in each cell: there's no way to predict which of the two X chromosomes will become inactivated. Hence, any given cell of a heterozygous female could end up as either of the following:



Expression of Coat Color in Females



Phoebe the Calcico cat has an X chromosome that has the orange color and an X chromosome that has the black color.

What happens if you “Clone” a Calcio cat??

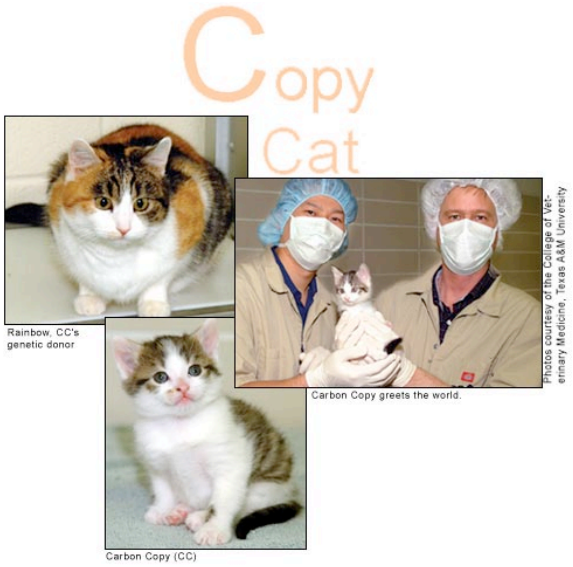
The first cloned cat, Carbon Copy, was created in Texas from a calico cat.

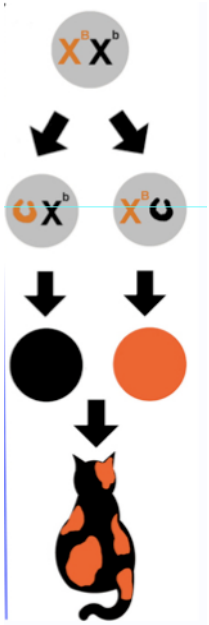
But the kitty didn’t turn out to be a ‘carbon copy’ despite having identical DNA to her genetic ‘mother’ Rainbow.

The process of inactivation is random, which is why cloning a calico cat will never produce the same pattern.

In the case of Carbon Copy, she was created from an egg cell that had its nucleus replaced with one from Rainbow.

Although the cell from which Carbon Copy was cloned had one inactive X, the developmental programme reactivates both X-chromosomes, and the process of inactivation recurs in a random manner. This results in an entirely different coat pattern even when two individuals are genetically identical.





Recommended reading material

- Epigenetics
  by C. David Allis, Thomas Jenuwein, Danny Reinberg
  CHAPTER 18 DNA methylation and CHAPTER 17 Dosage Compensation

Extra Reading:

- **Genomic patterns of DNA methylation: targets and function of an epigenetic mark**
  Michael Weber and Dirk Schübeler
  Current Opinion in Cell Biology 2007, **19**:273–280

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