HSTalks

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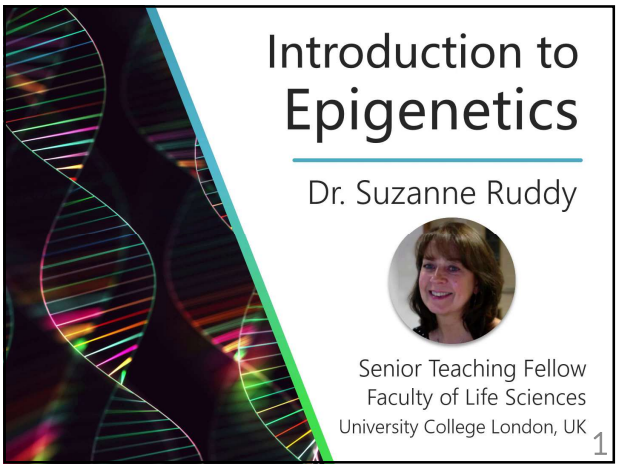

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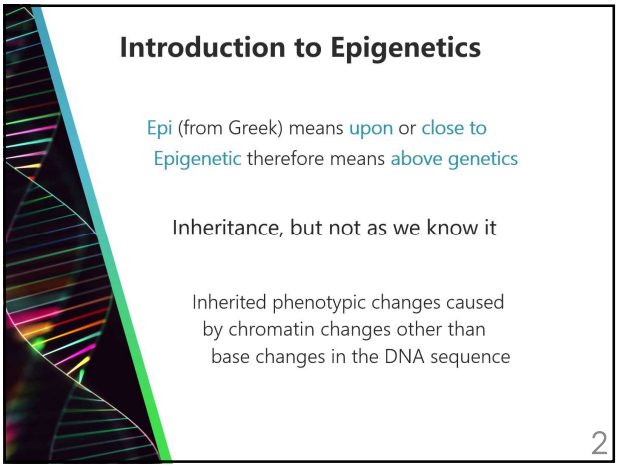

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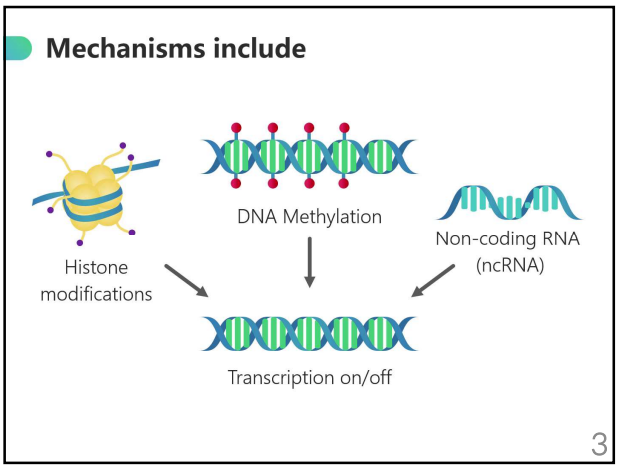

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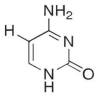
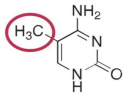
DNA methylation

- DNA methylation is commonly found in eukaryotes, including animals, plants, fungi and bacteria
- Addition of methyl groups (CH_3) at CpG sites on the DNA



Cytosine



Methylated cytosine

5′-CpG-3′

3′-GpC-5′

5′ GGAACCTCGACGGACTTG 3′

3′ CCTTGGAGCTGCCTGAAC 5′

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CpG site distribution is not random

- Clusters of CpG sites are known as CpG islands
- They are often found in the promoter regions of genes

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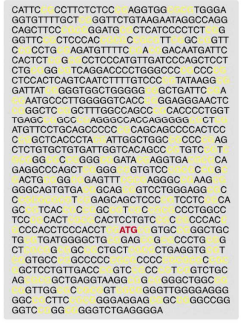
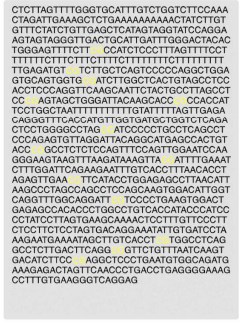
CpG site distribution is not random

Upstream promoter region



Main body of gene



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CpG site distribution is not random

- Clusters of CpG sites are known as CpG islands
- They are often found in the promoter regions of genes
- CpG sites in promoters are hypomethylated in active genes and hypermethylated in inactive genes
- Conversely, hypermethylated cytosines in the main body of the gene are often associated with active genes
- Regions of the genome that are switched off on a long-term basis, for example repetitive sequences which comprise 40% of the genome, are heavily methylated at CpG sites

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Three mechanisms for interaction

- Unmethylated DNA adopts an open conformation which is more accessible for non-histone proteins such as transcription factors
- Methyl groups can physically impede the binding of transcription factors
- Proteins called methyl-CpG-binding proteins preferentially bind methylated DNA through their methyl CpG binding domains (MBD)

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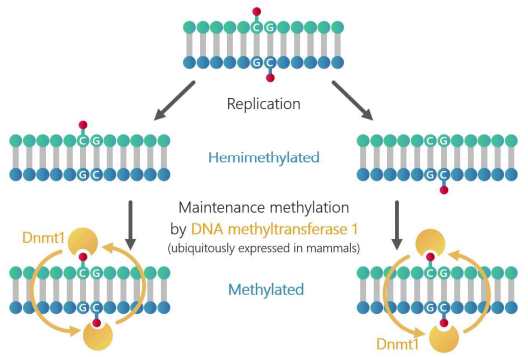
Ways to methylate DNA: maintenance methylation



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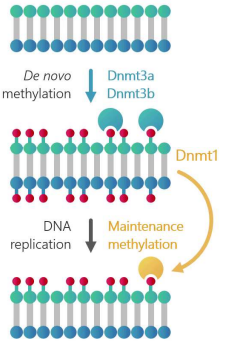
Ways to methylate DNA: *de novo* methylation

- Establishment of new methylation patterns by *de novo* methyltransferases Dnmt3a and Dnmt3b
- These are directed to DNA by sequence-specific DNA binding proteins and are developmentally regulated
- Occurs during early development, following the erasure of DNA methylation after fertilisation



Bird, *Science* (1999) **286**(5448) 2287-2288

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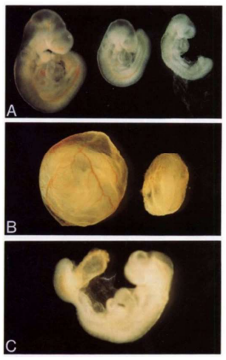
Phenotypic effects of Dnmt1 mutations



Severe effects of maintenance methylase (Dnmt1) mutations on mammalian development

Homozygous mutants of Dnmt1 die at 10.5 days gestation (recessive lethal phenotype)

Gross morphology of wild type and mutant embryos at day 10.5 gestation:

A. Whole view of wild type embryo (left) and two homozygous mutant littermates
B. Whole view of the yolk sac of a wild-type embryo (left) and that of a homozygous mutant littermate
C. Side view of a homozygous embryo showing an abnormal structure (arrow) near the tail

Li *et al.*, *Cell*, (1992) **69**(6) 915-926

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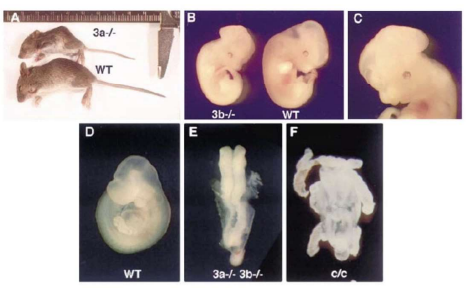
Dnmt3s are essential for development

In mammals, the de novo methylases (Dnmt3a and Dnmt3b) are essential for development



Adapted from Okano *et al.*, *Cell*, (1999) **99** 247-257

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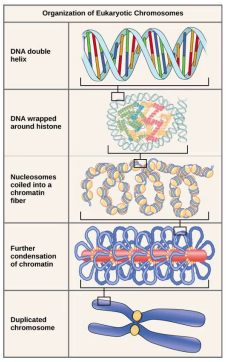
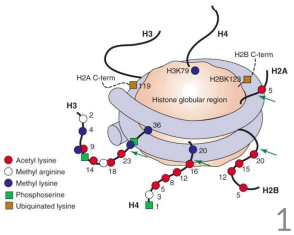
Histone modifications



- Importance of histone modifications was first discovered in yeast and tetrahymena model organisms
- Now known to be highly conserved across eukaryotes



Modified from Turner, *Cell*, (2002) 111(3), 285-91

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Different types of modifications make up the histone code

- Acetylation
  Addition of an acetyl group
- Methylation
  Addition of a methyl group
- Phosphorylation
  Addition of a phosphoryl group
- Ubiquitination
  Addition of a ubiquitin protein
- SUMOylation
  Small ubiquitin-like modifier proteins, H2A, H2B, probably transcriptionally repressive
- Biotinylation
  Addition of biotin, enriched in transcriptionally silent chromatin?
- ADP-Polyribosylation
  Addition of ADP-ribose polymers, H1, maintaining unmethylated CpG islands

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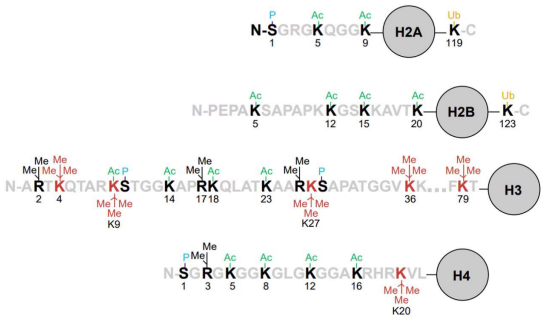
Histone modifications on the nucleosome



Sims *et al., Trends in Genetics*, (2003) 19(11), 629-639

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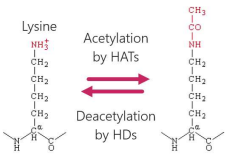
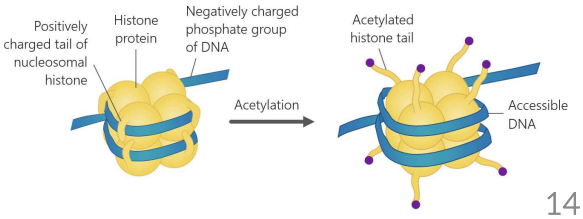
Histone acetylation

- Addition of acetyl groups (CH_3CO) from acetyl CoA to specific histones
- This usually stimulates transcription





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Flowering control in *Arabidopsis thaliana*

Arabidopsis thaliana

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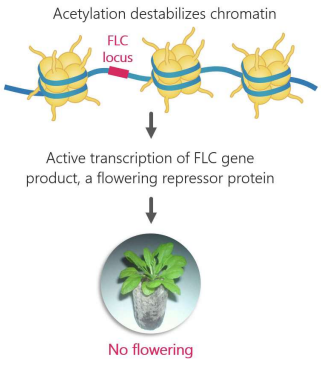
Flowering control in *Arabidopsis thaliana*



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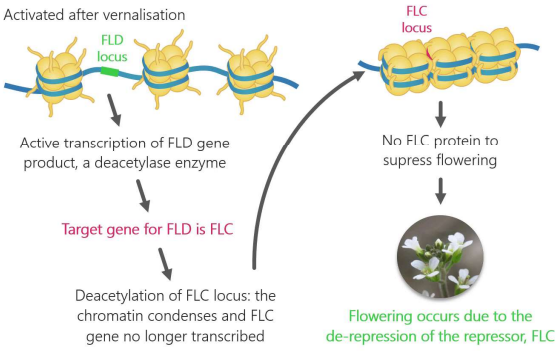
Flowering control in *Arabidopsis thaliana*



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Histone methylation

- Addition of methyl (CH3) groups to histone tails
- Can be mono-, di- or tri-methylated (1, 2 or 3 methyl groups attached respectively)
- Specific lysine or arginine residues are commonly modified on H3 and H4
- Can indicate active or inactive chromatin

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How does histone methylation work?

- Recruits effector proteins to chromatin which have enzymatic activities and can lead to chromatin remodelling
- Histone methylation can have an activating or repressing effect on transcription
- It does not change the overall charge of histones

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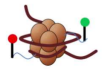
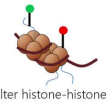
Modifications can act directly or indirectly

Direct modifications can alter nucleosome stability:

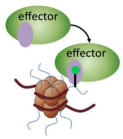


Alter DNA-histone interactions

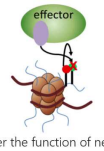


Alter histone-histone interactions

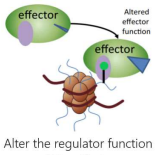
Indirect modifications can:



Recruit effector factors



Alter the function of nearby histone modifications



Alter the regulator function of the effector

Blakey and Litt, *Epigenetic Gene Expression and Regulation*, (2015) 21-42

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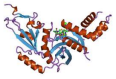
Enzymes

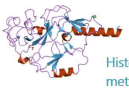


HAT1

- Histone acetyltransferases (HAT)
  Transfer the acetyl groups to the histone residues and are usually involved in activation of transcription

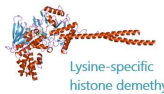
HDAC8

- Histone Deacetylases (HDAC)
  Remove acetyl groups and are usually involved in repression of transcription



Histone lysine methyltransferase

- Histone methyltransferase (HMT)
  Add methyl groups to histones



Lysine-specific histone demethylase 1, LSD1

- Histone demethylases
  Remove methyl groups from histone

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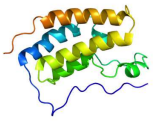
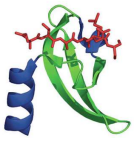
Binding domains

- Regulatory proteins bind to acetylated lysines through bromodomains



Bromodomain of human BRD4 protein

- Effector proteins bind to methylated lysines via different conserved domains, called chromodomains



Chromodomain of HP1 bound to methylated histone H3-K9

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Model for action of histone code

The histone code can be thought as an orchestra of different instruments playing together to create a unanimous sound

Histone modifications interact in infinitely complex ways to regulate transcription

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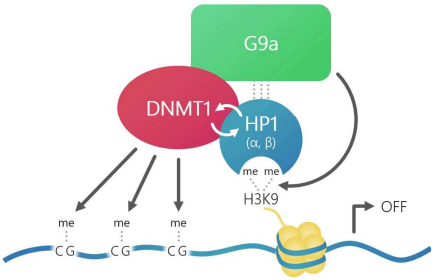
Model for action of histone code



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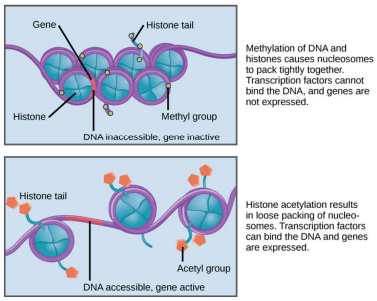
Model for action of histone code



Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.

Histone acetylation results in loose packing of nucleo-somes. Transcription factors can bind the DNA and genes are expressed.

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Non-coding RNA: X inactivation

- Non-coding RNA is not translated
- Dosage compensation: one X chromosome in females is inactivated
- In mammals X-inactivation in the embryo is random

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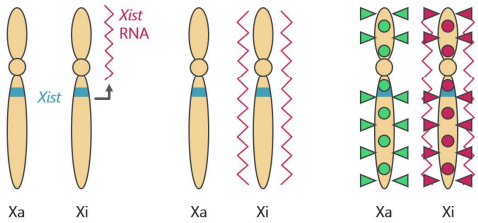
Non-coding RNA: X inactivation



Xist is an ncRNA that acts as the initial repressor

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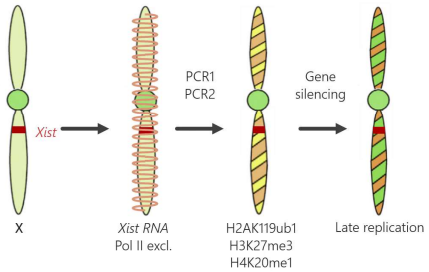
X chromosomes undergo global changes

Early phases of X inactivation are *Xist*-dependent



Xist recruits Polycomb complexes, which modify histones on the inactive X

Adapted from Wutz and Gribnau, *Curr. Opin. Genet. Dev.* (2007) 17 387-393.

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Polycomb group proteins

- Polycomb repressor complexes: PCR 1 and PCR2
- Polycomb repression group proteins (PRC1 and PRC2 complexes) are major epigenetic transcriptional repressors
- They act on several thousands of genes controlling differentiation pathways during development in most eukaryotes
- They maintain established gene repression patterns for the rest of the organism's life
- In addition to roles of PRC proteins in cell fate determination during development and X-inactivation, they are also important in cell cycle control, cancer, senescence and stem cell differentiation

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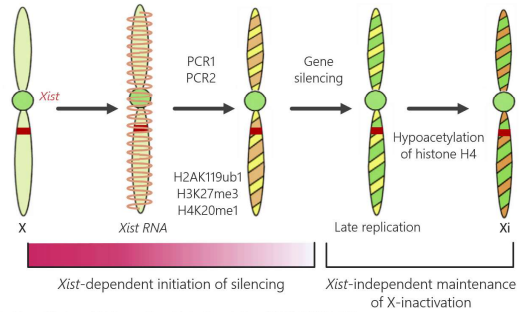
X-inactivation illustrates the histone code

X chromosomes inactivation late phases are *Xist*-independent



Adapted from Wutz and Gribnau, *Curr. Opin. Genet. Dev.* (2007) 17 387–393.

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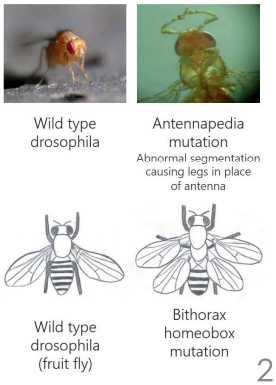
Polycomb repression PcG

- Discovered in *Drosophilia* as regulators of developmental homeobox *(HOX)* genes but now shown to be present in most eukaryotes
- HOX genes are master regulatory transcription factors responsible for anterior and posterior body patterning
- Expression patterns of HOX genes are maintained throughout life by the PcG complexes



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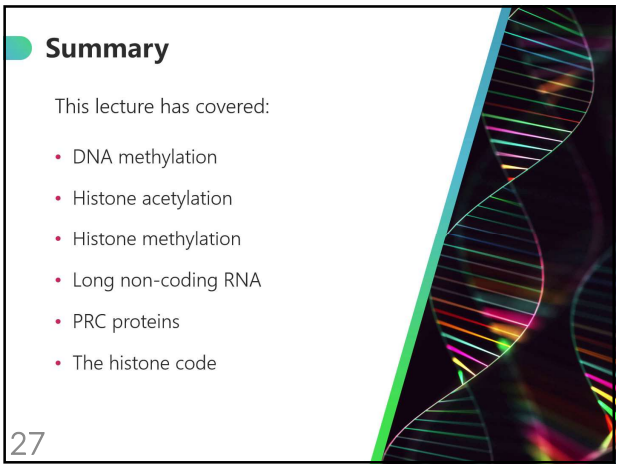

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