



Dr. Suzanne Ruddy – University College London, UK

Introduction to Epigenetics
Dr. Suzanne Ruddy
Senior Teaching Fellow
Faculty of Life Sciences
University College London, UK

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Introduction to Epigenetics
Epi (from Greek) means **upon** or **close to**
Epigenetic therefore means **above genetics**
Inheritance, but not as we know it
Inherited phenotypic changes caused by chromatin changes other than base changes in the DNA sequence

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Mechanisms include

Histone modifications → DNA Methylation → Non-coding RNA (ncRNA) → Transcription on/off

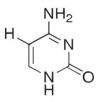
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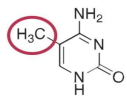
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DNA methylation

- DNA methylation is commonly found in eukaryotes, including animals, plants, fungi and bacteria
- Addition of methyl groups (CH₃) at CpG sites on the DNA



Cytosine



Methylated cytosine

5'-CpG-3'
3'-GpC-5'

5' GGAACCTCGACGGACTTG 3'
3' CCTTGGAGCTGCTCTGAAC 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

```

CATTG CCTCTCTCC AGATGG TGGGA
GGTGTTCCTT GGTTCGTAAAGATAGGCCAGG
CAGCTTCC GQATG CTATCCCTCT G
GGTTC CTCCAC C TT GC GTT
C CCTG AGATGTTTC A GACAATGATC
CACTCT G CCTCCCATGTTGATCCAGCTCCT
CTG GG TCAGACCCCTGGGCCC CCC
CTCCACTAGTCAATCTTTTCTCC TATAAGG
GATTAI GGTGGCTGGGG GCTGATTO A
ATGGCCCTGGGGTCCCG GGAGGAACTC
GGCTC GCTTGGCCAGCC CACCCCTGGT
TGGAG GGC AGGGCCACCGGGGG CT
ATGTTCCGACGCCCC CAGCAGCCCCACTCC
C GCTCACCTA ATGGCTGGC CCC AG
CTGTCTCTGTGATGTCACAGC TGTG T
GG C GGG GATA AGGTGA CA
GAGGCCGAGCT GGG GTGTCC G
ACTG GG GAGTTT AGGCC AAG
GGCAGTGTGA GCAG GTCCTGGGAGG C
C GAGCAGCTCC TCCTC CA
GC TCAC C GC T C CCTTGGCC
TCC CACT CACTCCTGT C CCAC
CCCACTCCCACT ATG GTGG GCTGTG
TG TGATGGGDTG GAG G CCTG G
CT G GC CTGCT GTGAGGTG T
GTGCC GCCCCC CCCC C
GCTCCTGTTGACC gtc CC T gTCTGC
AG GGTGAGTAAAG G GGGCTGGC
GTTGG C GT GGGTTGGGAGGG
GGC CTTG GGGAGGAG GC GGCCTG
GGTC GG GGGTGTAGGGGA
                    
```

Main body of gene

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CTCTTAGTTTTGGGTGCAITTTGCTAGCTTCCMA
CTAGATTGAAAGCTCTGAAAAAAAACATCTTTG
GTTCTATCTGTTGAGCTCATAGTAGTATCCAGGA
AGTAGTAGGGTTGAOTGCATTGANTTGGGACTCAC
TGGGAGTTTTCTT CCATCCCTTTAGTTTTCTT
TTTTCTTCTTTCTTTCTTTCTTTTCTTTTCTTTT
TTGAGATTG TTTTCTCAGTCCCGAGCTGGA
GTGCAGTGGTG ATCTGGCTCACTGTAGCCTCC
ACCTCCAGGTTGAGCAATCTAGCTGCTTAGCCT
CC AGTGTCTGGGATTCAGAGCAC CCACAT
TCCTGGCTAATTTTTTTTTGTATTTTTAGTTGAGA
GAGGGTTCCAGCATTTGGTGTGGTGTCTGAGA
CTCCTGGGGCCTAG ATCCCTGCTCAGCT
CCGAGAGTGTAGGATACAGCCATGAGCCACTGT
AGC GCGTCTCCAGTTTTCCAGTTGGAATCCA
GGGAAAGTAAAGTTAAGATAAGTTA ATTTGAAAT
CTTTGGATTGAGAAATTTGTACCTTTAAGACT
AGAGTTGAA TTCATACCTGGAGGCCCTAACATT
AAGCCTAGCCAGCCTCCAGCAAGTGGADTTGGT
CAGGTTTTGGCAGATT TCCTCGAAGTGGACT
GAGAGCCACACCCTGGCCCTGTACCATTACCATCC
CCATCCTTAGTGAAGAAAAGTCTTTGTTCCCTT
CTCCCTTCCGATGAGAGAAAATTTGTGCTCTA
AAGATGAAAATAGCTTGTACCT TGGCCTCAG
GGCCTTGTGACTTCAAG GTTCTGTTTATCAAGT
GACATCTCC AGGCTCCCTGAATGTGGCAGATG
AAGAGACTAGTTCAACCTGACCTGAGGGGAAAG
CCTTTGTGAGGGTCAAGGA
                    
```

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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

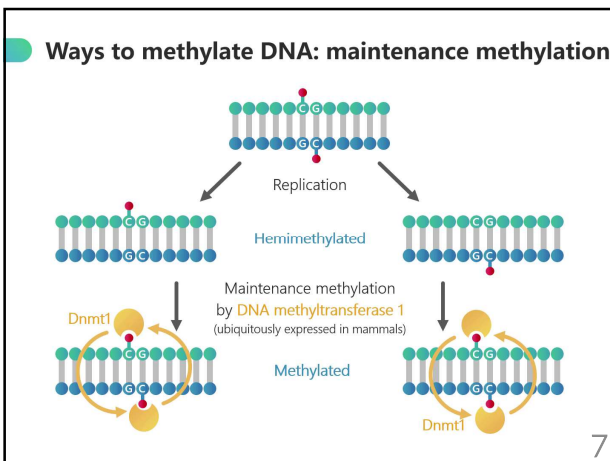
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Three mechanisms for interaction

- 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: *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:

- Whole view of wild type embryo (left) and two homozygous mutant littermates
- Whole view of the yolk sac of a wild-type embryo (left) and that of a homozygous mutant littermate
- 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 (CH₃CO) 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*

Acetylation destabilizes chromatin

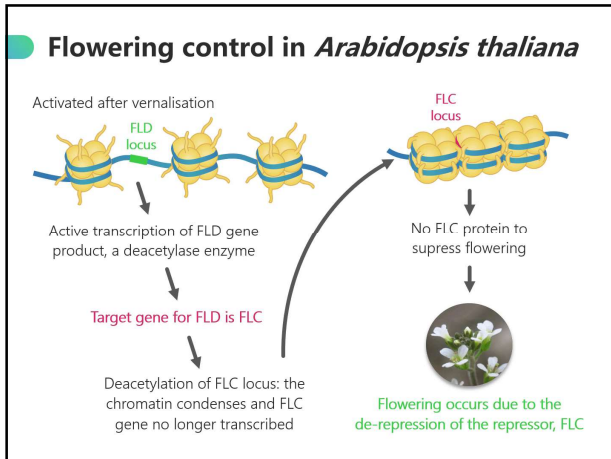
Active transcription of FLC gene product, a flowering repressor protein

No flowering

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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:

Indirect modifications can:

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

The diagram illustrates a repressed state where DNMT1 (red) and G9a (green) methylate DNA at CpG sites (me-CG) and histone H3K9 (me-H3K9). HP1 (blue) binds to these methylated sites, leading to a condensed nucleosome structure and a repressed state labeled 'OFF'.

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Model for action of histone code

The diagram compares two states of DNA packaging. The top part shows DNA methylation (me) on CpG sites and histone tails, leading to tightly packed nucleosomes and an inactive gene. The bottom part shows histone acetylation (Acetyl group) on histone tails, leading to loosely packed nucleosomes and an active gene.


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Non-coding RNA: X inactivation

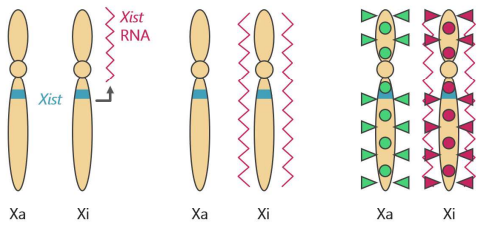
- Non-coding RNA is not translated
- Dosage compensation: one X chromosome in females is inactivated
- In mammals X-inactivation in the embryo is random



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Non-coding RNA: X inactivation

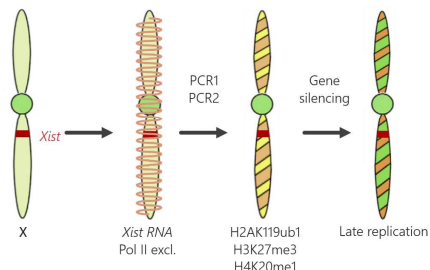


Xist is an ncRNA that acts as the initial repressor

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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

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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 *Drosophila* 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

Wild type drosophila

Antennapedia mutation
Abnormal segmentation causing legs in place of antenna

Wild type drosophila (fruit fly)

Bithorax homeobox mutation

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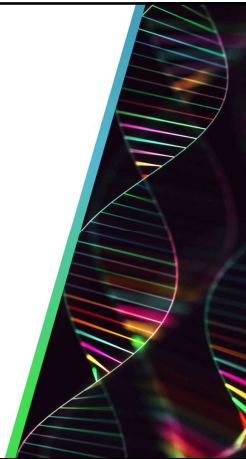
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Summary

This lecture has covered:

- DNA methylation
- Histone acetylation
- Histone methylation
- Long non-coding RNA
- PRC proteins
- The histone code

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