UNIVERSITY OF Southampton
School of Medicine

The role of epigenetics in the regulation of gene transcription

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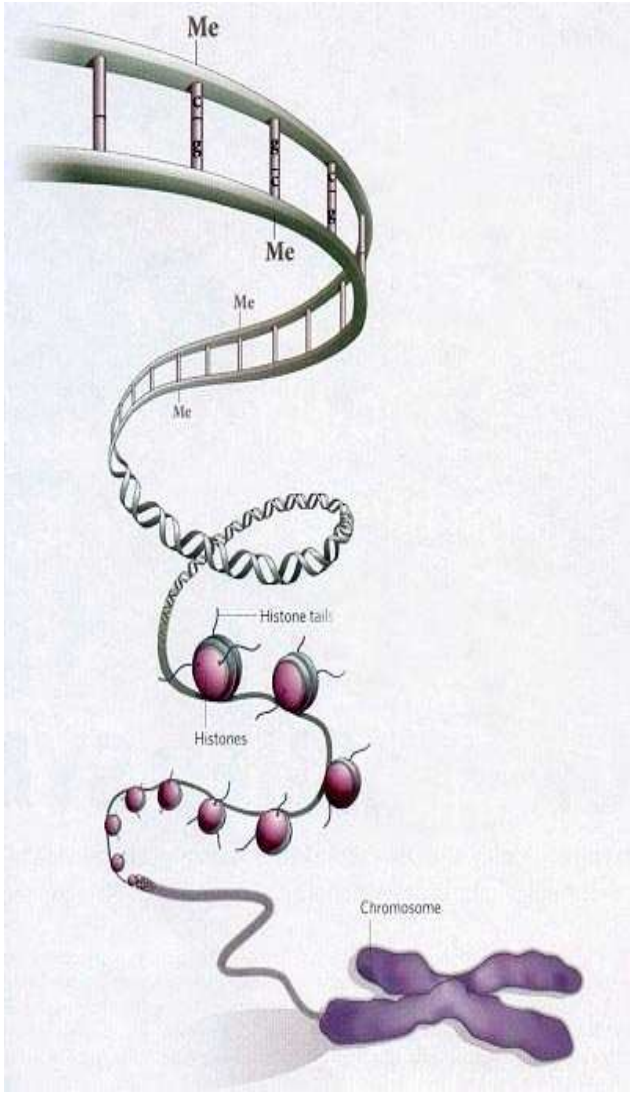
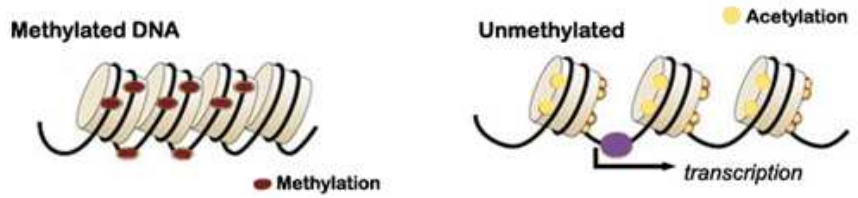
Epigenetics



♦ **Epigenetics is the study of mitotically heritable changes in gene expression that occur without changes in the DNA sequence (*Wolffe & Matzke, 1999*)**

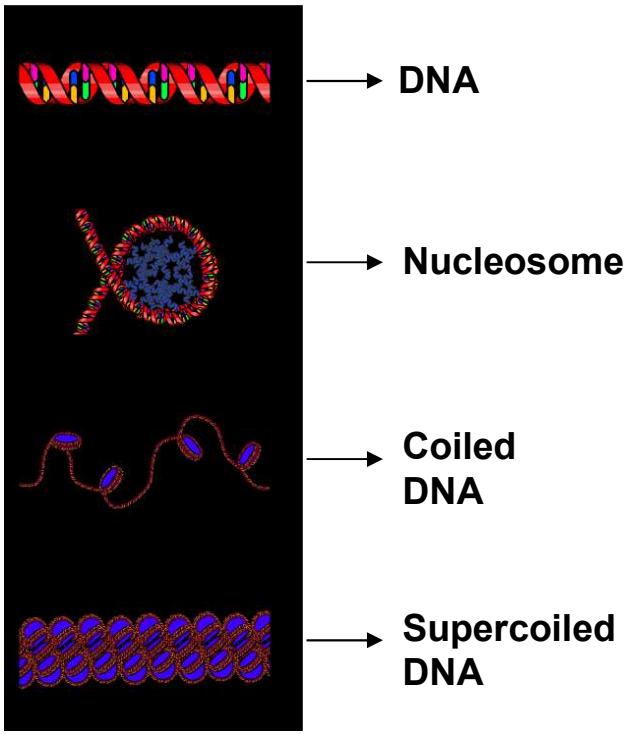
♦ **The term 'epigenome' describes epigenetic modifications all over the genome**

Scope of this talk:



- How is DNA packaged into the nucleus of an eukaryotic cell?
- Enlist the different covalent modifications to chromatin that constitute the diverse epigenetic mechanisms
- DNA methylation and demethylation, and their role in regulation of gene transcription
- Histone modifications and their role in epigenetic regulation
  - Histone acetylation
  - Histone methylation
- Importance of epigenetic programming during mammalian development
- Role of epigenetics in disease
  - Osteoarthritis

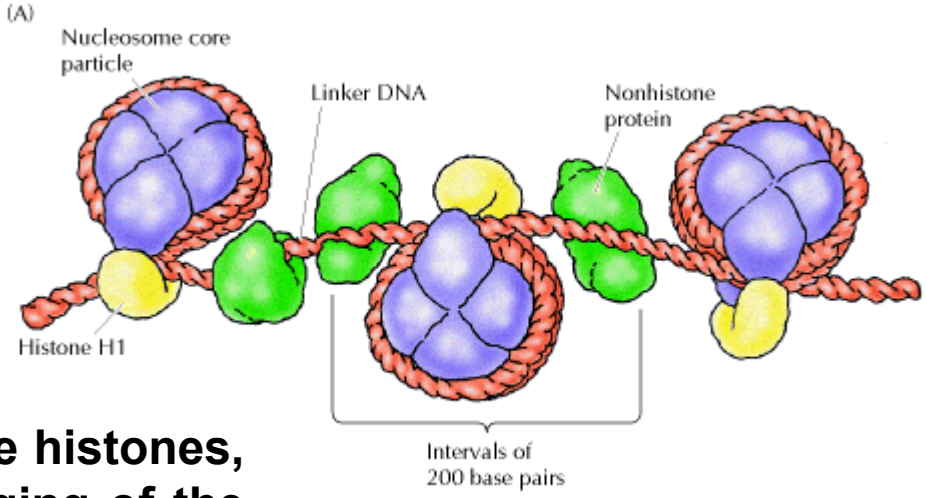
How is DNA packaged into the nucleus of an eukaryotic cell?



♦ Length of extended human DNA is nearly 2 m, while the diameter of the nucleus is 5-10 μm

♦ DNA of eukaryotic cells is tightly bound to small basic proteins, the histones, that package DNA in an orderly manner to form chromatin

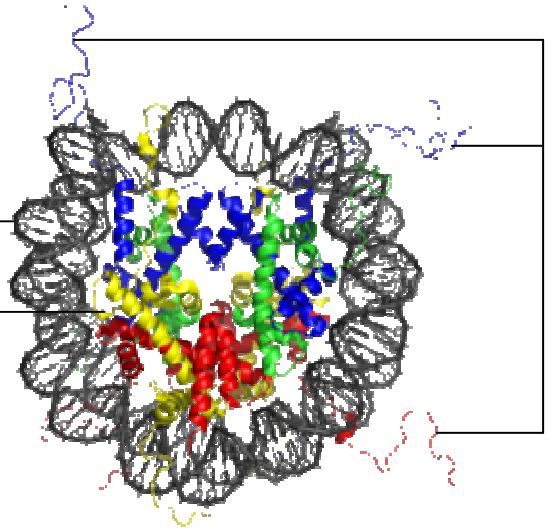
♦ Nucleosomes – Fundamental repeating units of eukaryotic chromatin



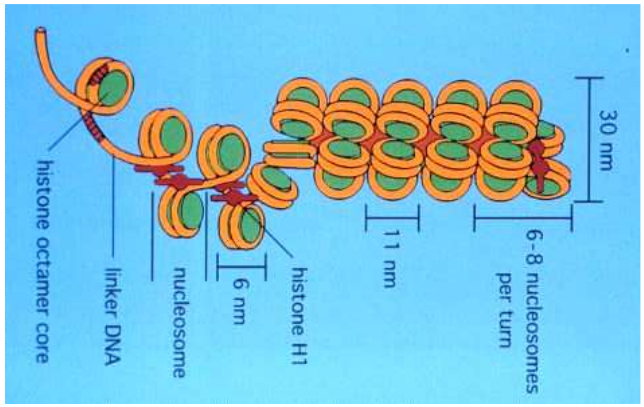
♦ The nucleosome is composed of a short length of DNA (146 bp) wrapped around a core of histone proteins in 2 turns

♦ H2a, 2b, 3 and 4 constitute the core histones, while histone H1 helps in the packaging of the nucleosomes on each other

Crystal structure of the nucleosome core



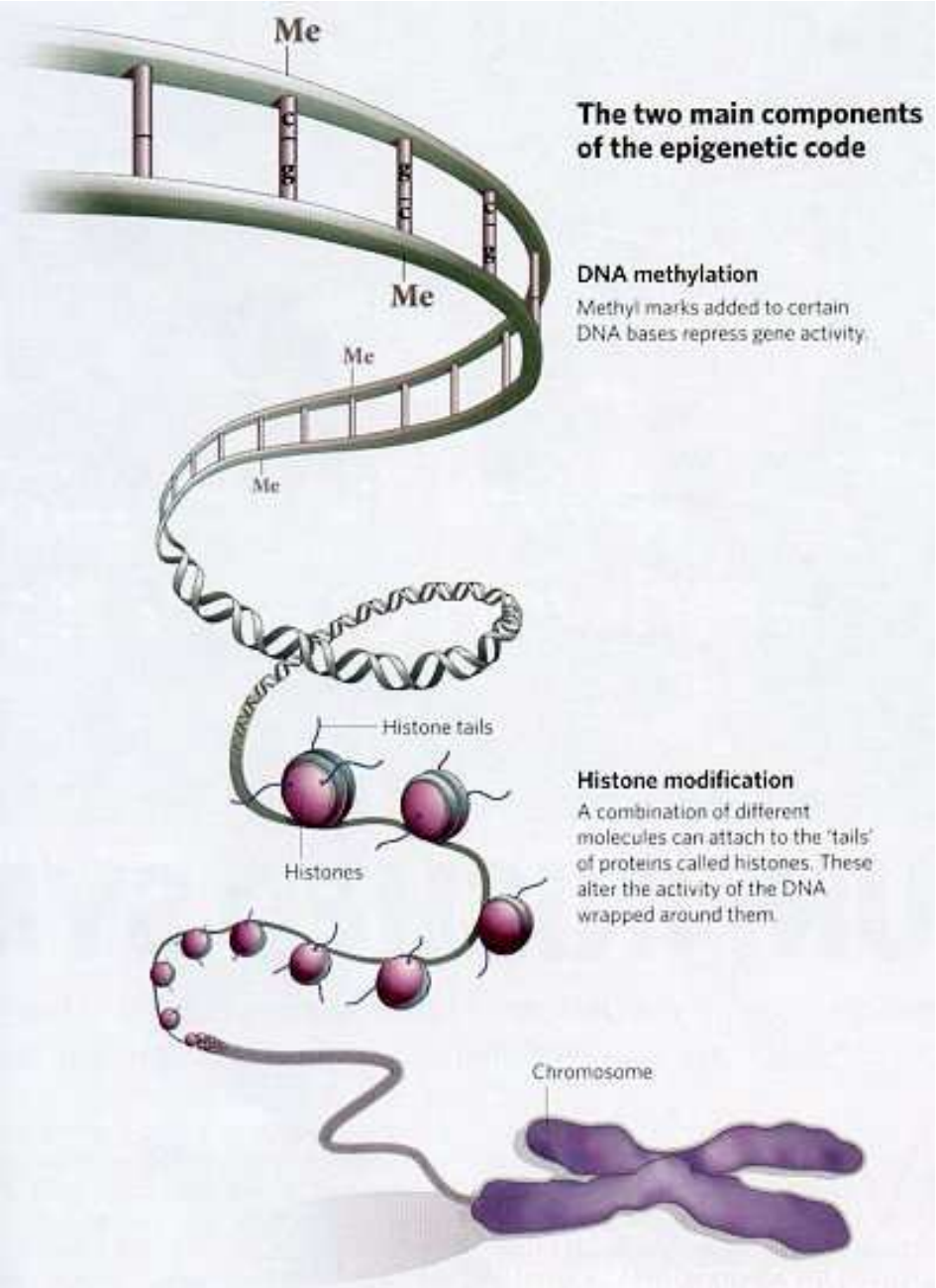
♦ Each core histone is associated with an amino-terminal tail, of 25-40 amino acid residues, that extends through the DNA into the space surrounding the nucleosome



♦ Thus, the coiling and supercoiling condenses the length of the DNA some 10,000-fold

♦ However, such a compact structure would block the transcriptional machinery from approaching its target and silence gene transcription

♦ To facilitate the transcription of certain genes and silence the expression of others, the DNA and the core histone tails undergo covalent modifications, which are collectively studied under the heading of 'epigenetic mechanisms of gene regulation'

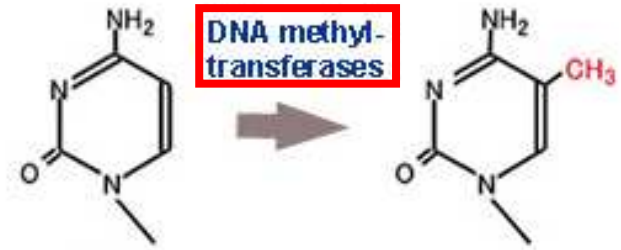


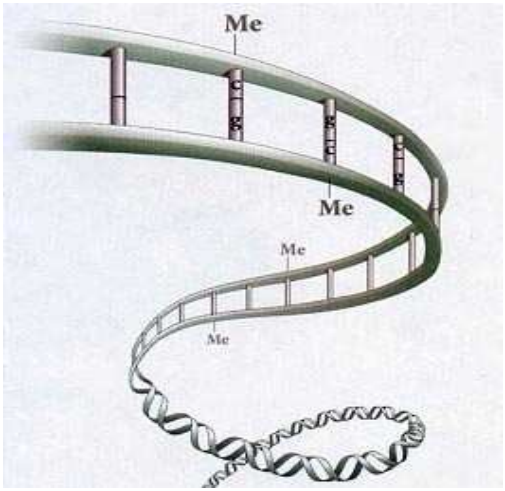
Mechanisms of epigenetic regulation,

- **DNA methylation**
- **Histone modifications**
  - **Histone acetylation**
  - **Histone methylation**
  - **Histone phosphorylation**
  - **Histone ubiquitination**
  - **Histone sumoylation**
  - **Histone ribosylation**
- **Small RNA control**
  - **siRNA**
  - **miRNA**

DNA methylation

♦ **In eukaryotes, it refers to the process by which a methyl group is covalently added to the carbon (at position 5) of cytosine in the DNA strand**





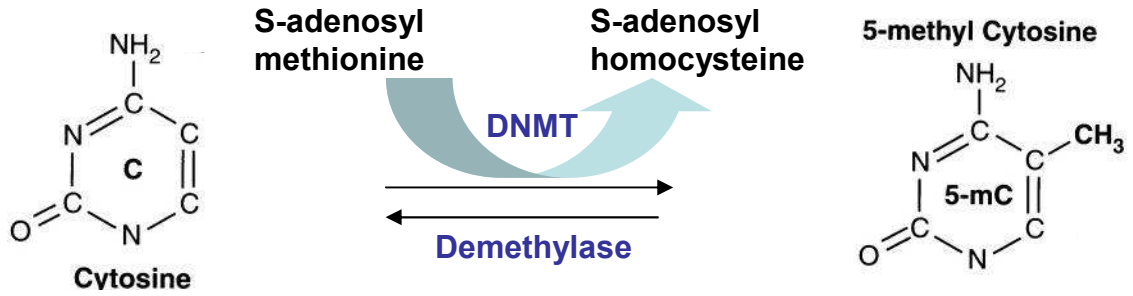
♦ **Only those cytidine residues that are adjacent to guanidine i.e. the CpG sites (cytidine bound through a phosphate molecule to guanidine) in the DNA strand are targets for the methylation-inducing enzymes**

♦ **These CpG sites may occur in multiple repeats and are known as CpG islands**

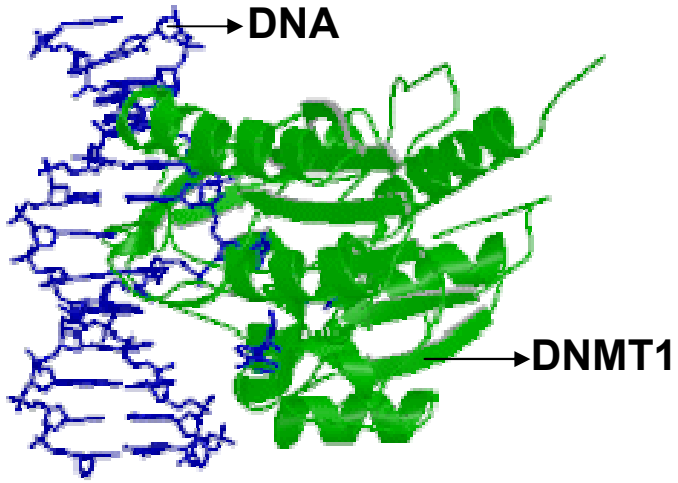
♦ **The most important location for DNA methylation is in the promoter region of the gene, where extensive methylation (hypermethylation) of the CpG sites causes gene silencing**

How is DNA methylated?

- **DNA methyl transferases (DNMT)**



- **4 DNA methyl transferases**
  - **DNA methyl transferase 1 (DNMT 1)**
  - **DNA methyl transferase 2 (DNMT 2)**
  - **DNA methyl transferase 3a (DNMT 3a)**
  - **DNA methyl transferase 3b (DNMT 3b)**



Functions of the DNMTs

DNMT1: Maintenance methyltransferase, component of DNA replication complex

***Functions* – a. 'Copies' the methylation pattern from the template DNA strand to the newly synthesized strand after cell division**

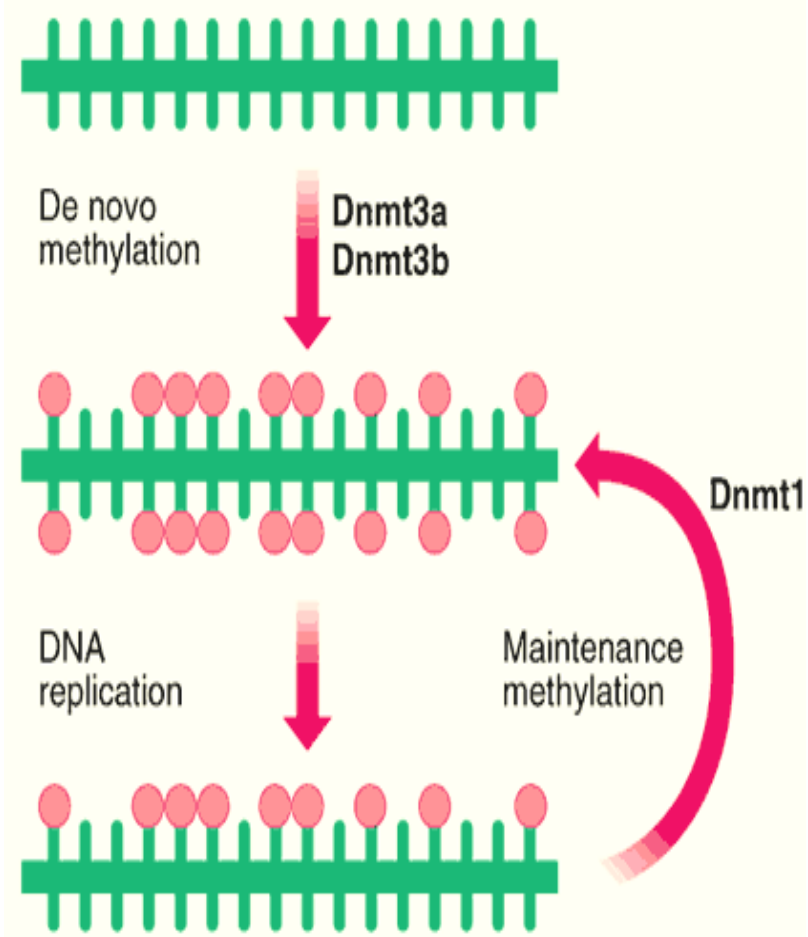
b. Supports long-term silencing of non coding DNA in addition to the epigenetic silencing of particular genes

DNMT3a & 3b:

***Functions* – a. Responsible for the de novo methylation of the non methylated DNA in response to environmental challenges and during development**

b. Important role in epigenetic silencing of particular genes

DNMT2: Displays negligible evidence of transmethylase activity



How does DNA methylation affect gene transcription?

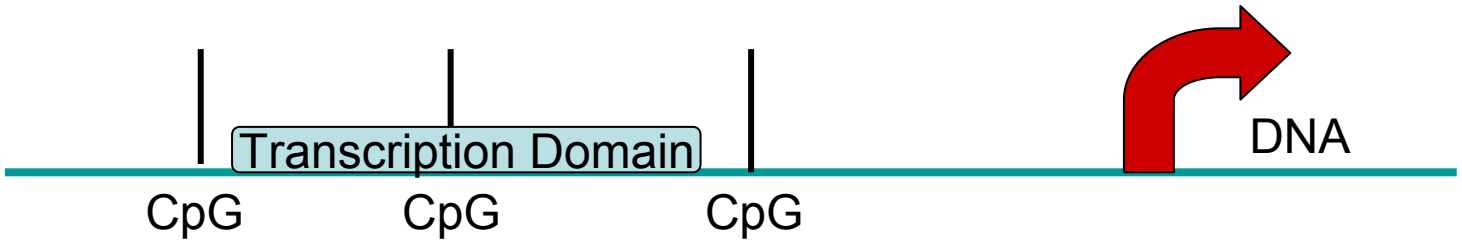
How are genes transcribed?

♦ **Actively transcribed genes are characterised by the presence of CpG sites in their promoter regions that are <u>hypomethylated</u>**

Transcription Factors

♦ **As the CpG sites in the promoter region are hypomethylated, it is possible for appropriate transcription factors to bind to their recognition sequences in the promoter**

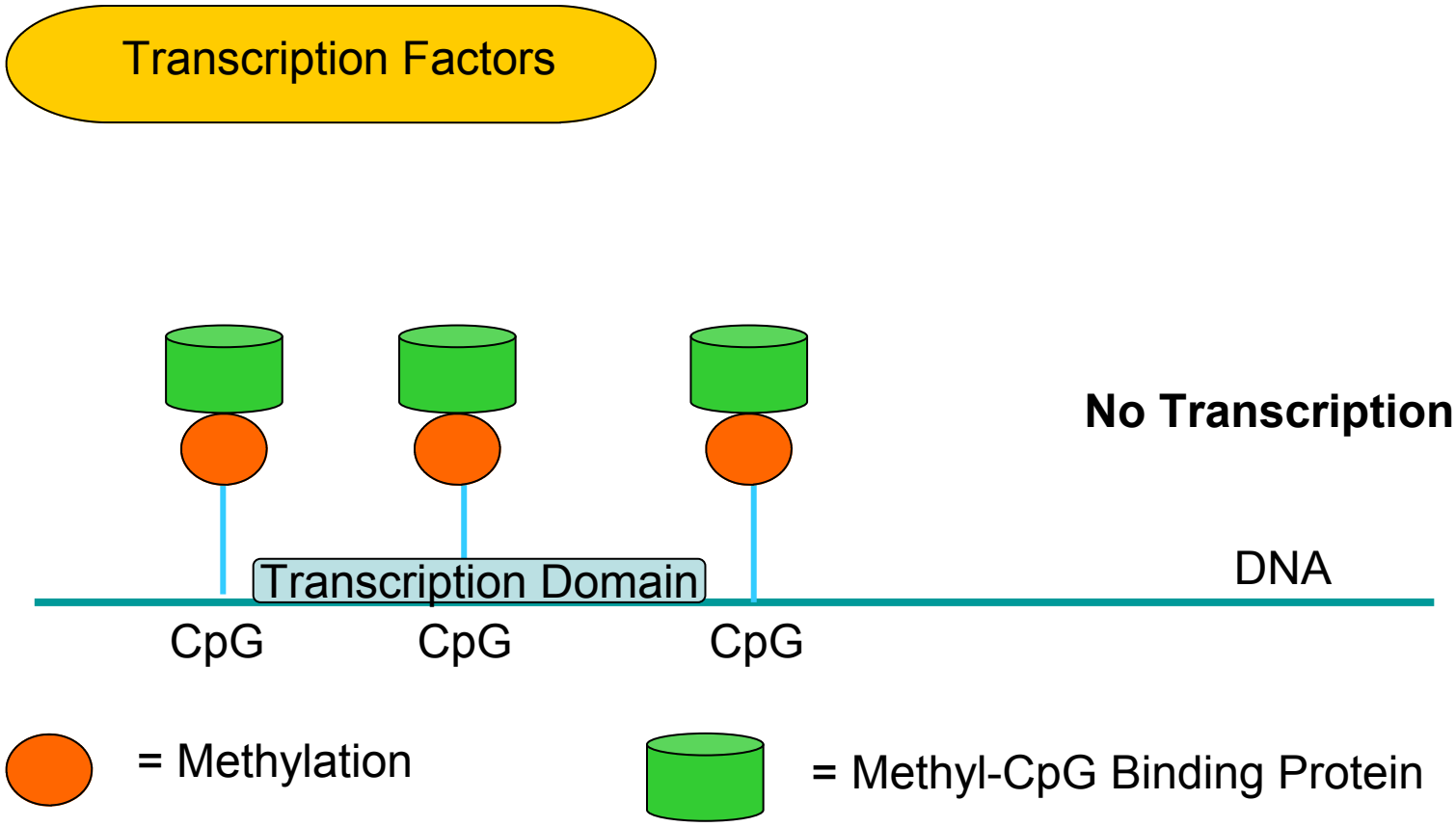
ACTIVE TRANSCRIPTION



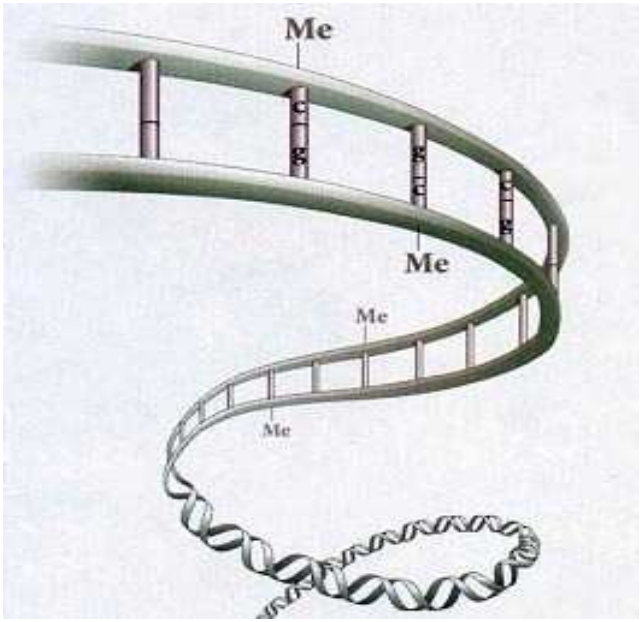
"Simplified version of mechanism of active gene transcription"

How does DNA methylation silence gene transcription?

- **The nuclear matrix proteins, methyl-CpG-binding proteins 1 and 2, bind preferentially to the methylated cytosines**
- **Presence of the methyl-CpG-binding proteins, bound to the methylated cytosines in the promoter region, obstructs binding of appropriate transcription factors to their transcription domains**



DNA demethylation



♦ **DNA demethylation is an important component of the epigenetic control as methylated genes may need to be 'unsilenced' by demethylation in response to different environmental signals or during development**

♦ **Passive demethylation:**

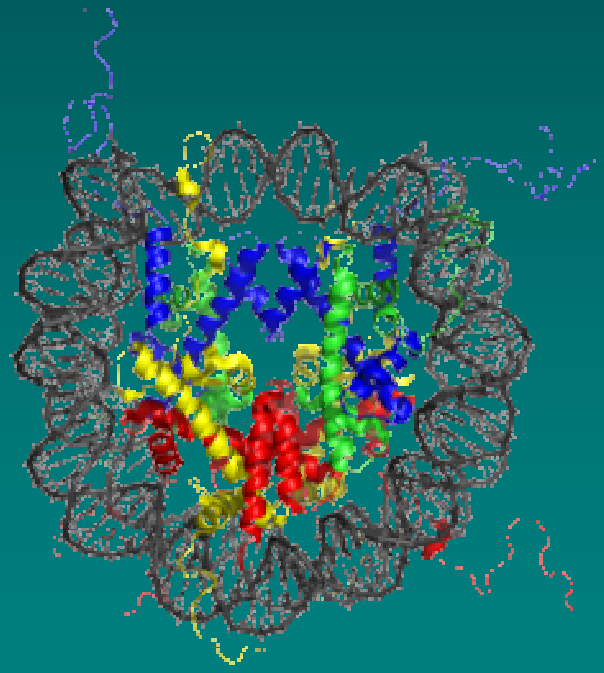
∗ **Occurs during DNA replication i.e. cell division and involves the inhibition of DNMT1 activity**

♦ **Active demethylation:**

∗ **Glycosylase-dependent: Enzymes known to have 5-Methylcytosine-DNA-glycosylase activity cleave the bond between the DNA backbone and the methylated cytosine base (Zhu *et al*., 2000, *Nucleic Acid Res*)**

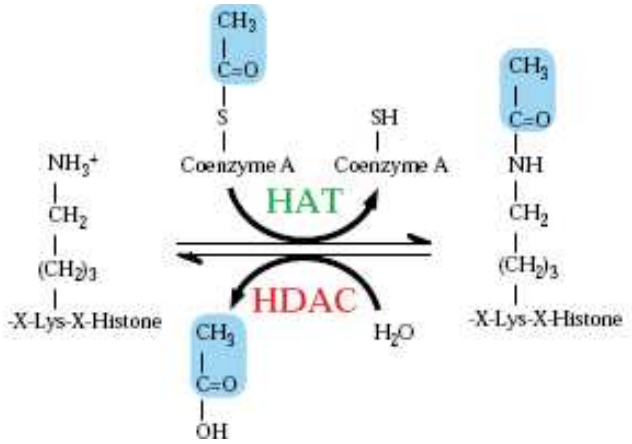
∗ **Direct removal of the methyl moiety from the methylated DNA (Ramchandani *et al*., 1999, *PNAS*)**

Histone modifications and their role in epigenetic regulation



Histone acetylation

♦ **The enzymes histone acetyltransferases (HATs) catalyse the transfer of acetyl groups from acetyl coenzyme A to the amino groups of conserved lysine residues located in N-terminal tails of the core histones**



♦ **The enzymes histone deacetylases (HDACs) catalyse the removal of acetyl groups from histones. Many HDAC inhibitors are currently involved in clinical trials as chemotherapeutic agents**

♦ **Dynamic process**

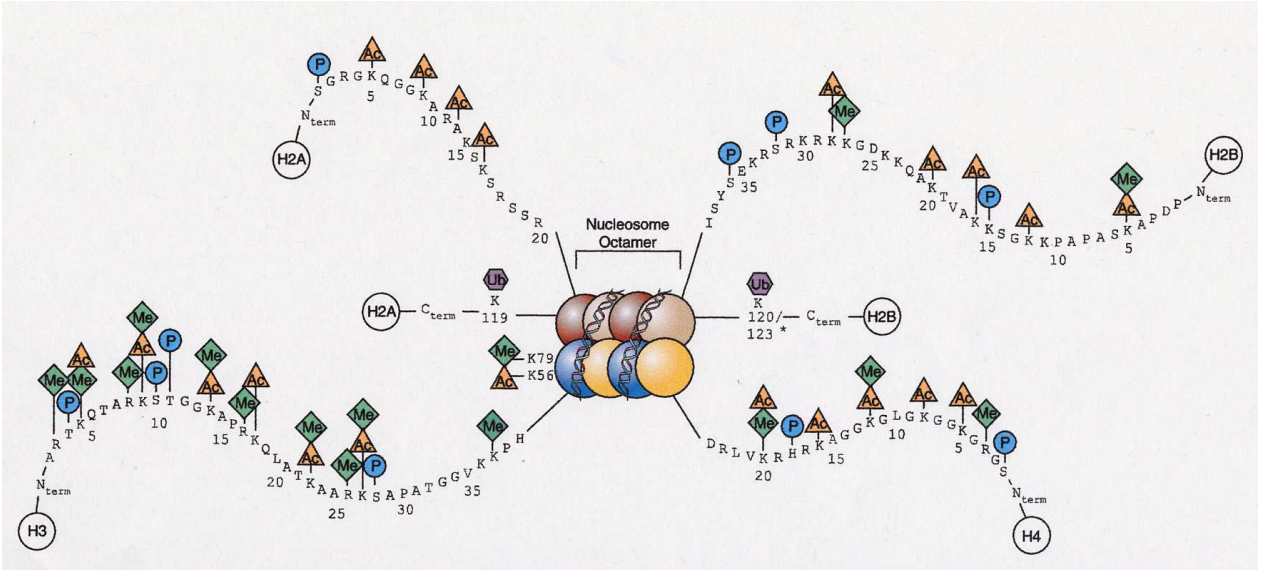
Histone methylation

♦ **The enzymes histone methyltransferases (HMTs) catalyse the transfer of methyl groups from S-adenosylmethionine (SAM) to the amino groups of conserved lysine residues located in N-terminal tails of the core histones**

Note: Arginine residues in N-terminal tails of core histones can also be methylated

♦ **Existence of a global histone demethylase seems unlikely as very little evidence exists for large-scale decreases in methylated histones from bulk chromatin**

♦ **Relatively stable, turnover of methyl groups is relatively less**

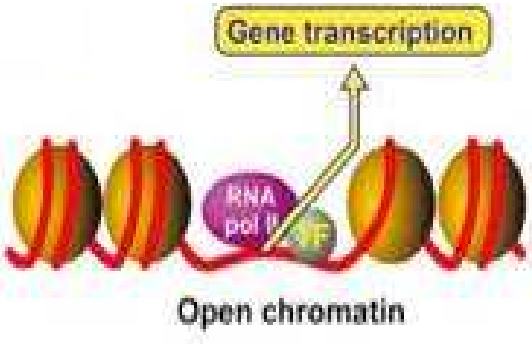


Conserved lysine residues in the N-terminal tails of H3 and H4 are the major targets for acetylation and methylation

However, lysine residues in N-terminal tails of H2a and H2b have been reported to also undergo these modifications

Histone acetylation

♦ **Addition of acetyl groups (CH_3CO^-) to the lysine residues neutralizes the positive charge of the histone tails, decreasing their affinity for the negatively charged DNA, thereby 'opening' the chromatin, allowing access to the transcriptional machinery and facilitating gene transcription**



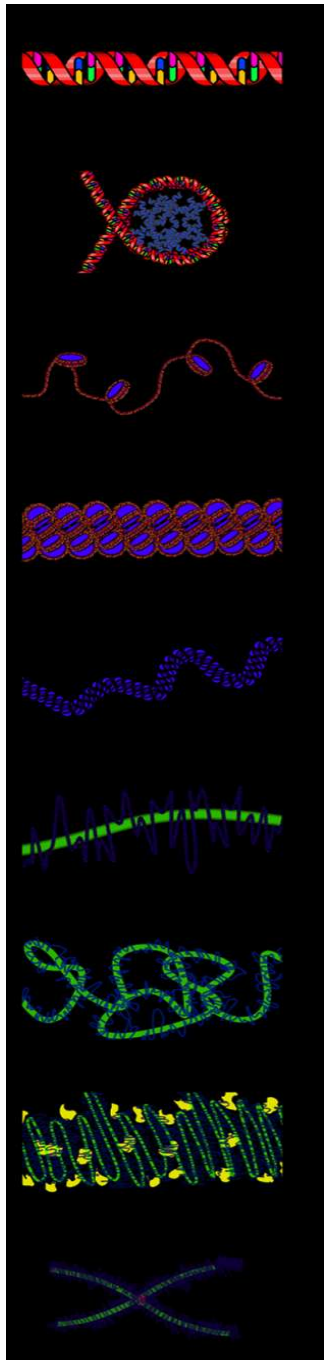
Histone methylation

♦ **Addition of methyl groups (CH_3+) to the lysine residues (particularly at positions 9 and 27 in H3) increases the affinity of the histone tails for anionic DNA, thereby 'closing' the chromatin, obstructing access to the transcriptional machinery and silencing gene transcription**

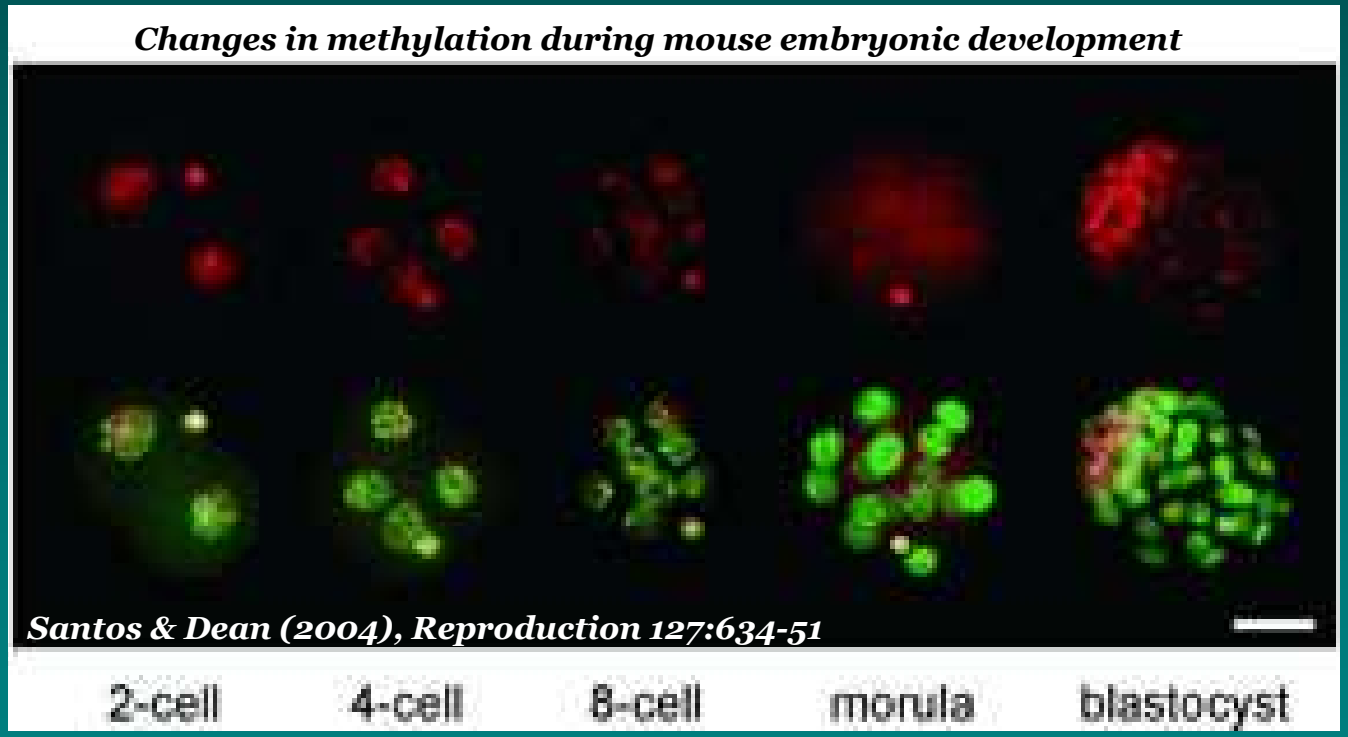
Note: Methylation of lysine (4) and arginine (17) residues in the H3 tail = transcriptional activation

Epigenetic modifications

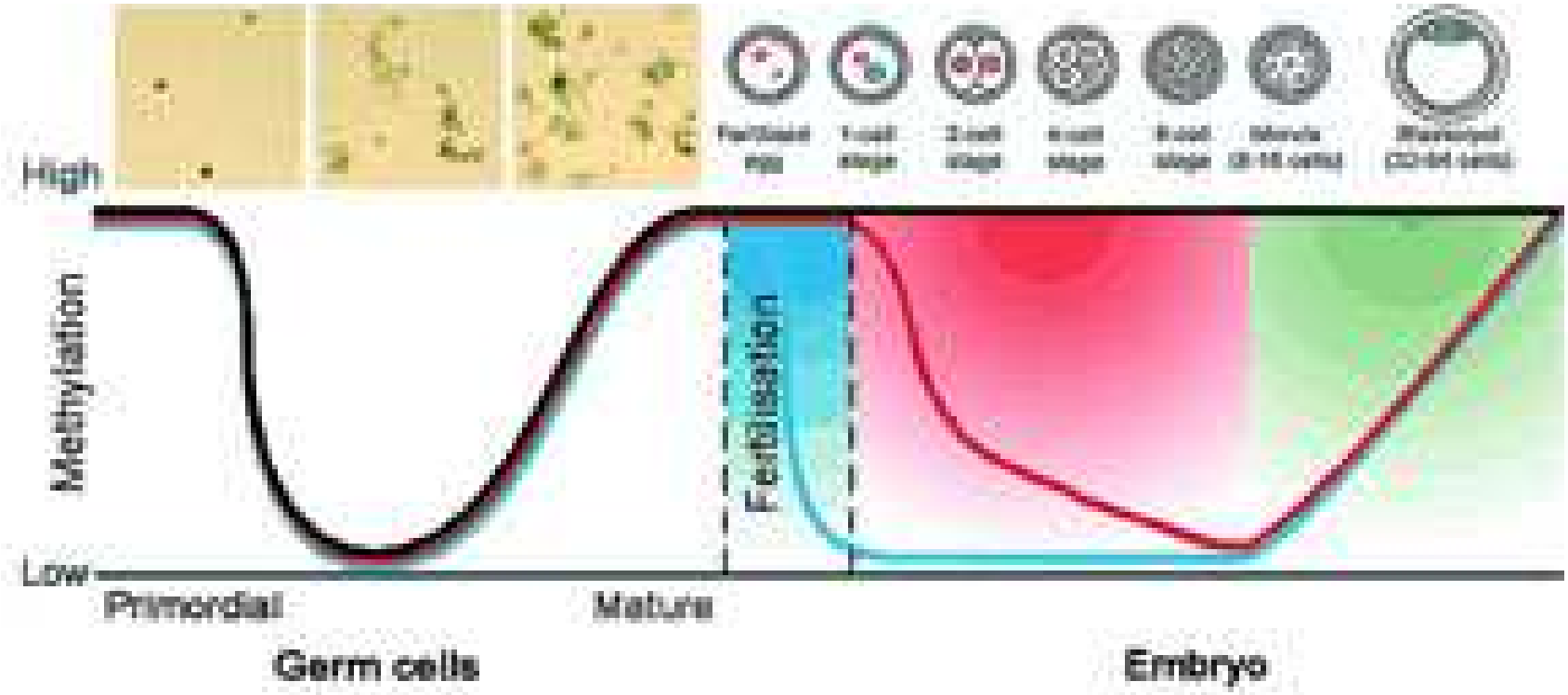
- DNA methylation = gene silencing
- DNA demethylation = gene unsilencing
- Histone acetylation = open chromatin, active transcription
- Histone methylation = closed chromatin, silencing of transcription (generally)



Importance of epigenetic programming during mammalian development



Epigenetic programming during gametogenesis, post-fertilization & implantation



Black line = imprinted genes, Blue line = paternal genes, Red line = maternal genes

♦ **Primordial germ cells: DNA is completely demethylated**

♦ **Mature germ cells: DNA methylation gradually restored**

♦ **Post-fertilization: Another wave of demethylation occurs in maternal (passive gradual process) and paternal (active, very fast) genomes with exception of imprinted genes**

♦ **Lineage-specific de novo methylation is apparent at the blastocyst stage**

Santos & Dean (2004), Reproduction 127:634-51

Aberrant epigenetic programming can cause failure of mammalian cloning

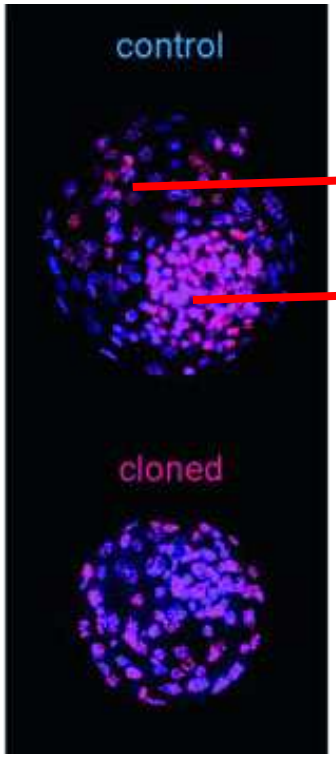
♦ In cloning experiments, very few animal embryos survive to birth, most die postnataly, or at best, before their normal siblings as a result of multiple abnormalities

♦ The asymmetry of both DNA and H3K9 methylation, characteristic of unmanipulated normal blastocysts, is lacking in cloned blastocysts

♦ Cloned blastocysts are highly homogeneous with regards to their methylation pattern

♦ Aberrant hypermethylation of the trophectoderm in cloned embryos maybe an early marker of placental abnormalities frequently reported

Merged channels of DNA and H3K9 methylation in normal and cloned blastocysts

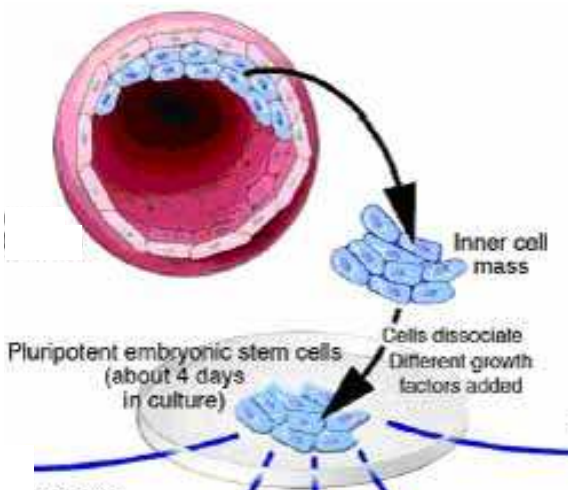


Trophectoderm (extra-embryonic)

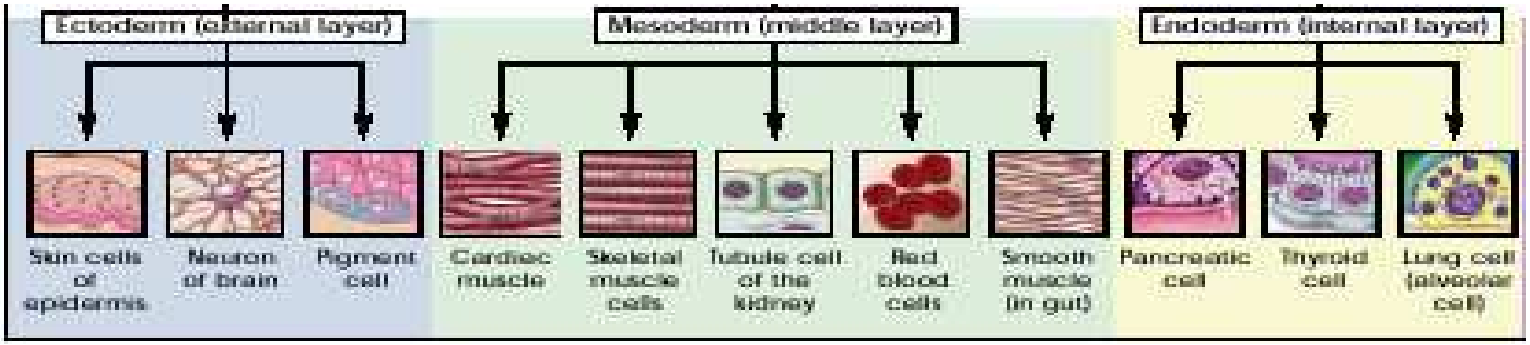
Inner cell mass (embryonic)

Santos & Dean (2004), Reproduction 127:634-51

Differentiation of stem cells into different lineages involves progressive demethylation of cell type-specific lineage genes



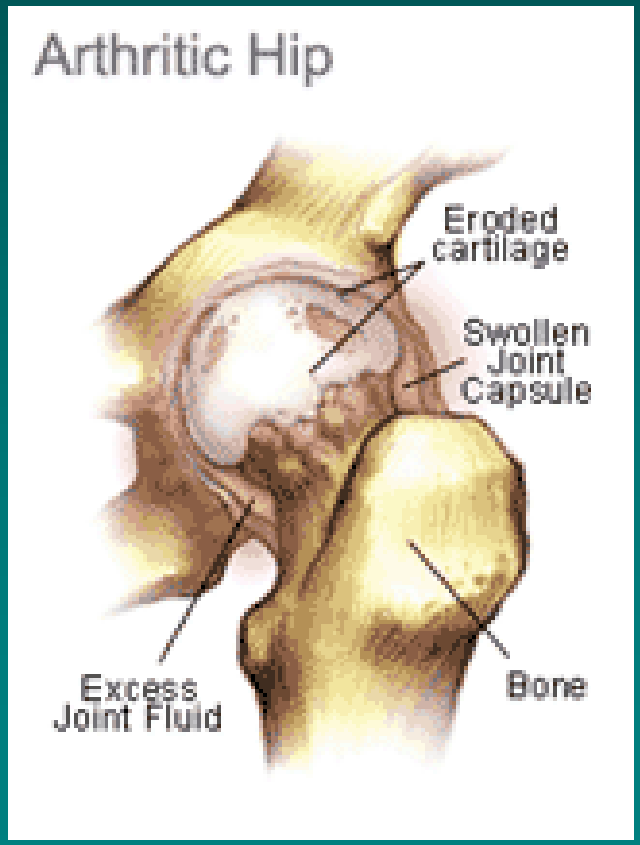
♦ **Embryonic stem cells: Cytosine residues in the promoters of genes responsible for maintaining pluripotency and the undifferentiated state are kept hypomethylated, allowing transcription factors such as Oct4 and Nanog to maintain the undifferentiated pluripotent state**



♦ **As stem cells differentiate, promoters of cell type-specific lineage genes become hypomethylated and promoters of pluripotency genes become hypermethylated**

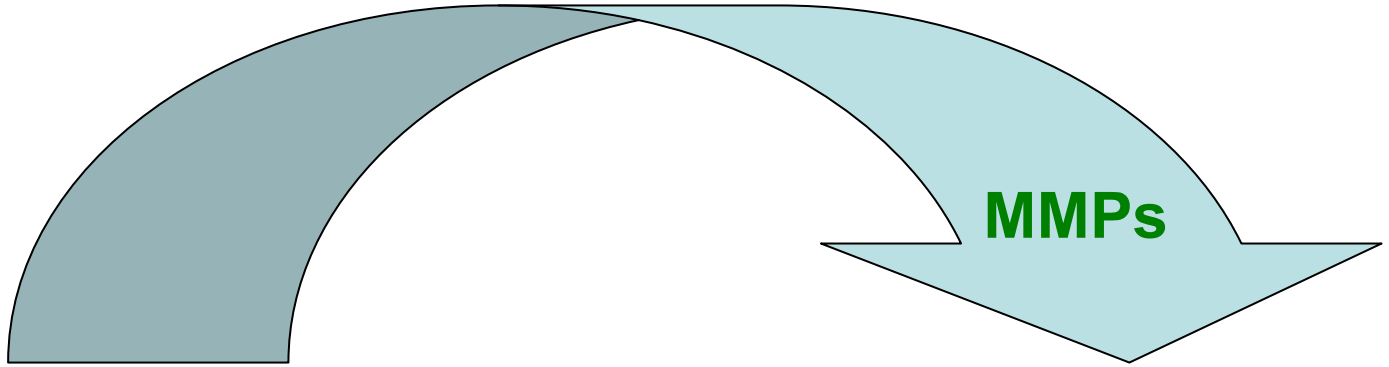
Role of epigenetics in disease e.g. Osteoarthritis (OA)

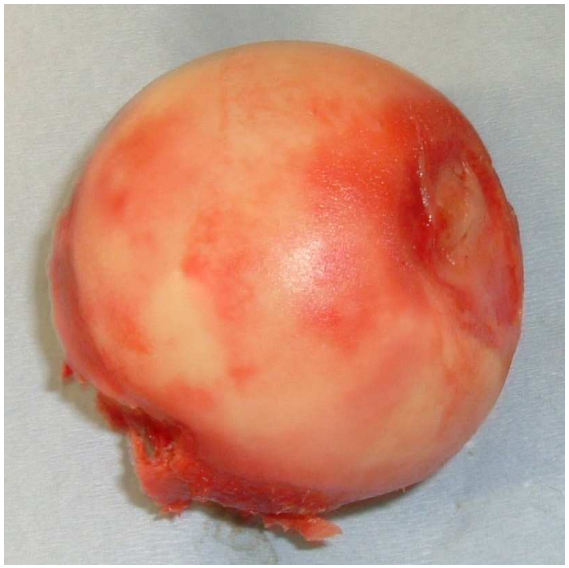
Research by Trudy Roach's group



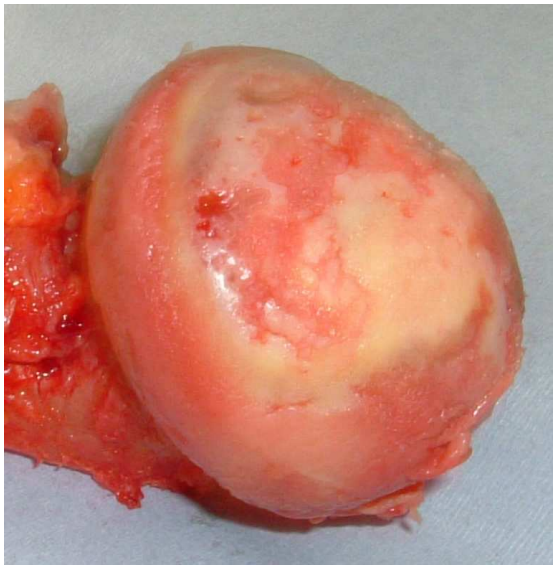
What causes degradation of articular cartilage in OA?

Aberrant production and secretion of matrix metalloproteinases (MMPs) by OA chondrocytes





Normal cartilage

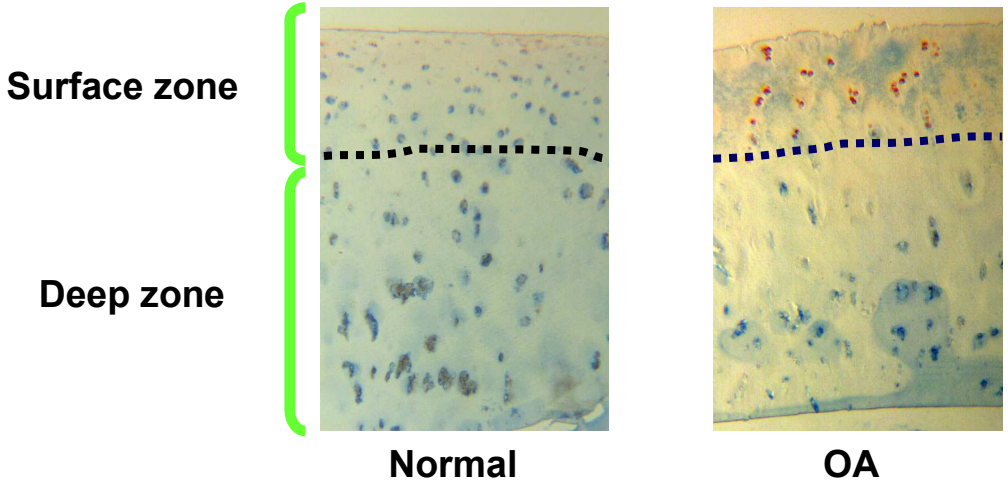


Degraded OA cartilage

Aberrant expression of MMPs by OA chondrocytes and their effects

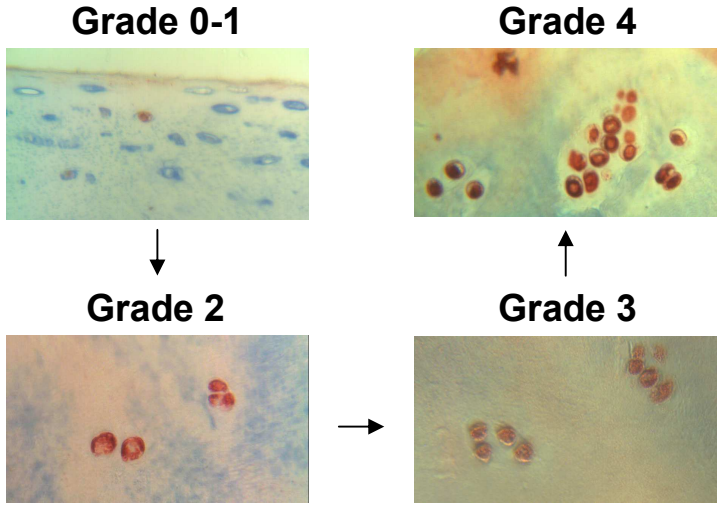
Aberrant expression of MMPs in the superficial zone of OA cartilage

Immunostaining for MMP-13



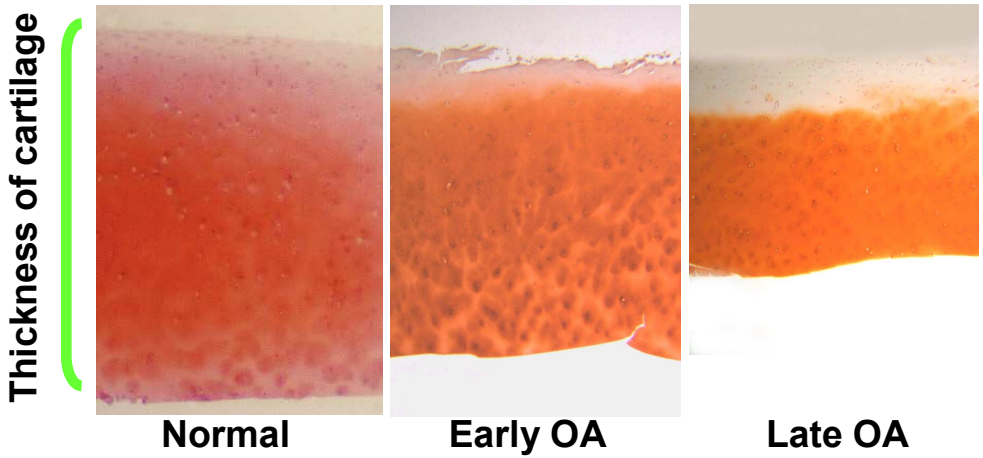
Aberrant expression of MMPs is transmitted to daughter cells

Immunostaining for MMP-9

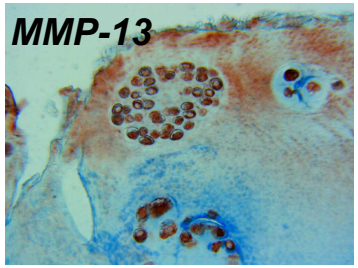


Loss of matrix proteoglycans and articular cartilage thinning

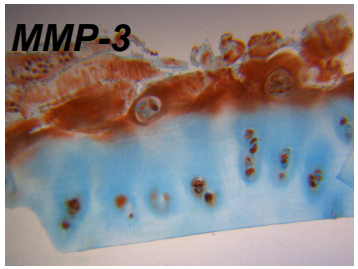
Safranin O staining for proteoglycans



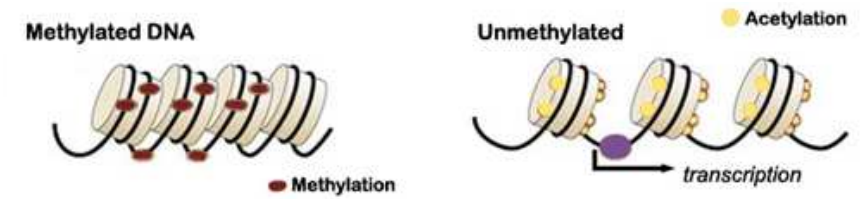
Massive clones of chondrocytes expressing MMPs in late-stage OA

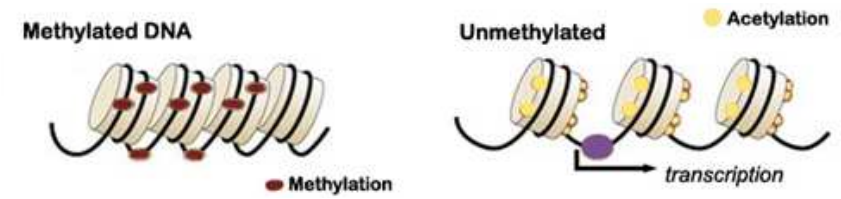


Secretion of MMPs and consequent degradation of the cartilage



Role for epigenetics in the pathophysiology of OA





Epigenetic regulation of gene transcription results in,

Osteoarthritic cartilage

- **stable/ semi-permanent induction or silencing of gene expression**
- **Aberrant expression of MMPs is stably induced in the articular chondrocytes**
- **change in the cell phenotype**
- **Chondrocyte phenotype is changed from a cell normally not expressing MMPs to a cell expressing MMPs**
- **transmission of the altered expression and phenotype to the daughter cells**
- **Aberrant expression of MMPs and the altered phenotype transmitted to daughter chondrocytes**

Hypothesis

MMP genes are silenced by DNA methylation in normal chondrocytes, therefore, loss of DNA methylation in the MMP gene promoters induces their expression in OA chondrocytes

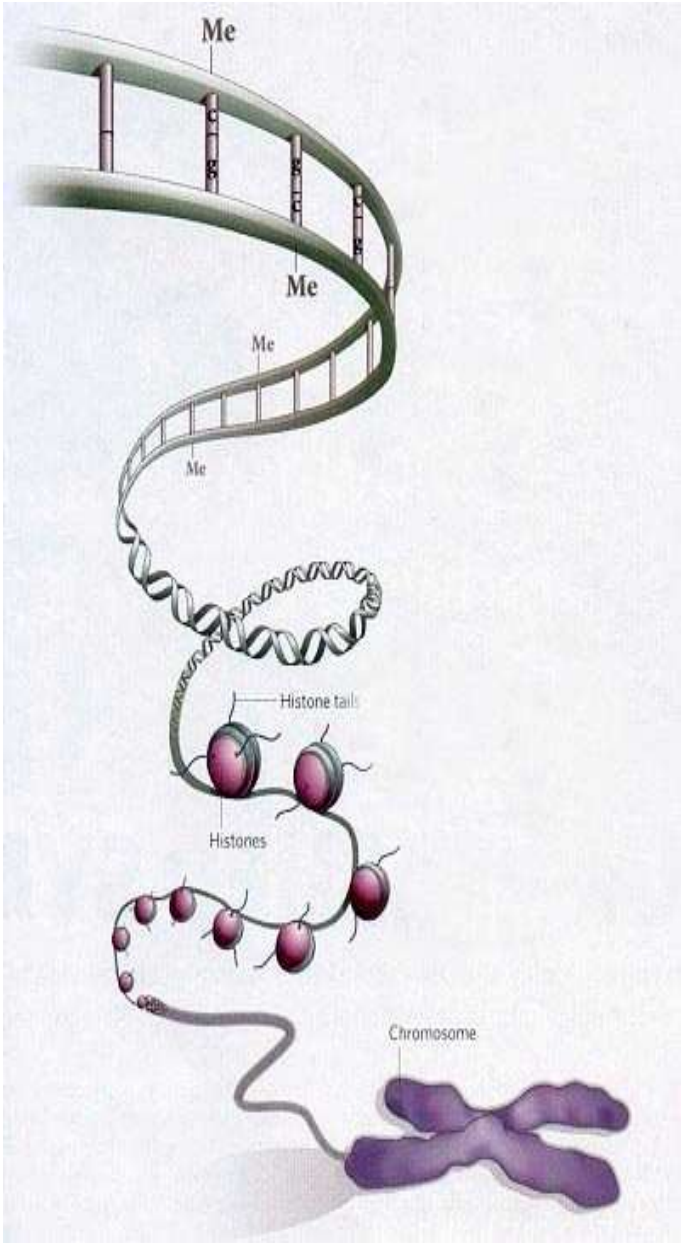
Methods to assess DNA methylation status of MMP promoters

1. Methylation-sensitive restriction enzyme (MSRE) method

2. Bisulphite modification method

Material

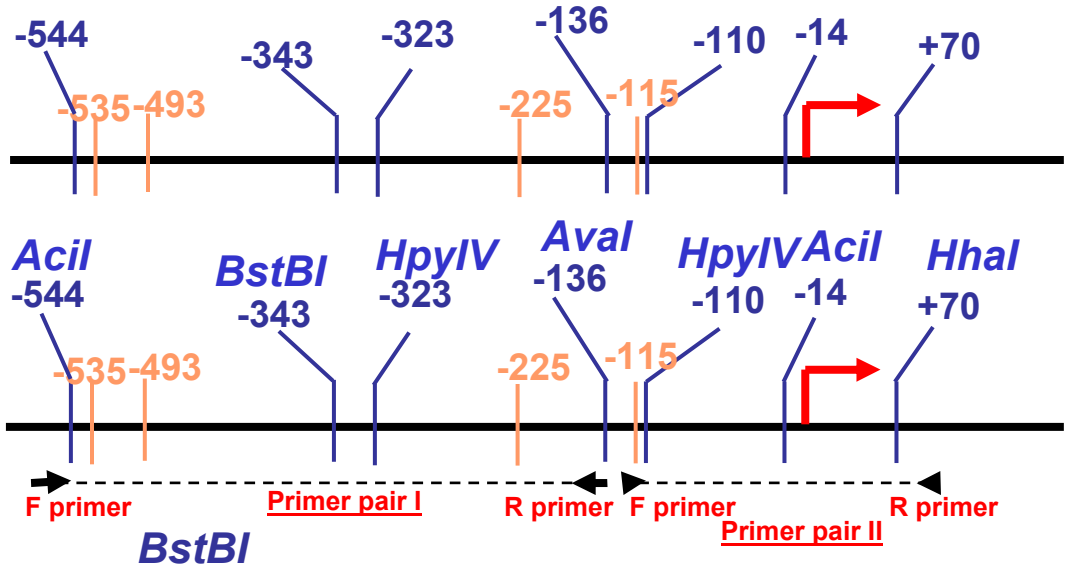
DNA isolated from chondrocytes in the superficial layer of articular cartilage from OA and # NOF (control) femoral heads



MSRE method to detect methylation status of MMP-13 promoter in OA chondrocytes

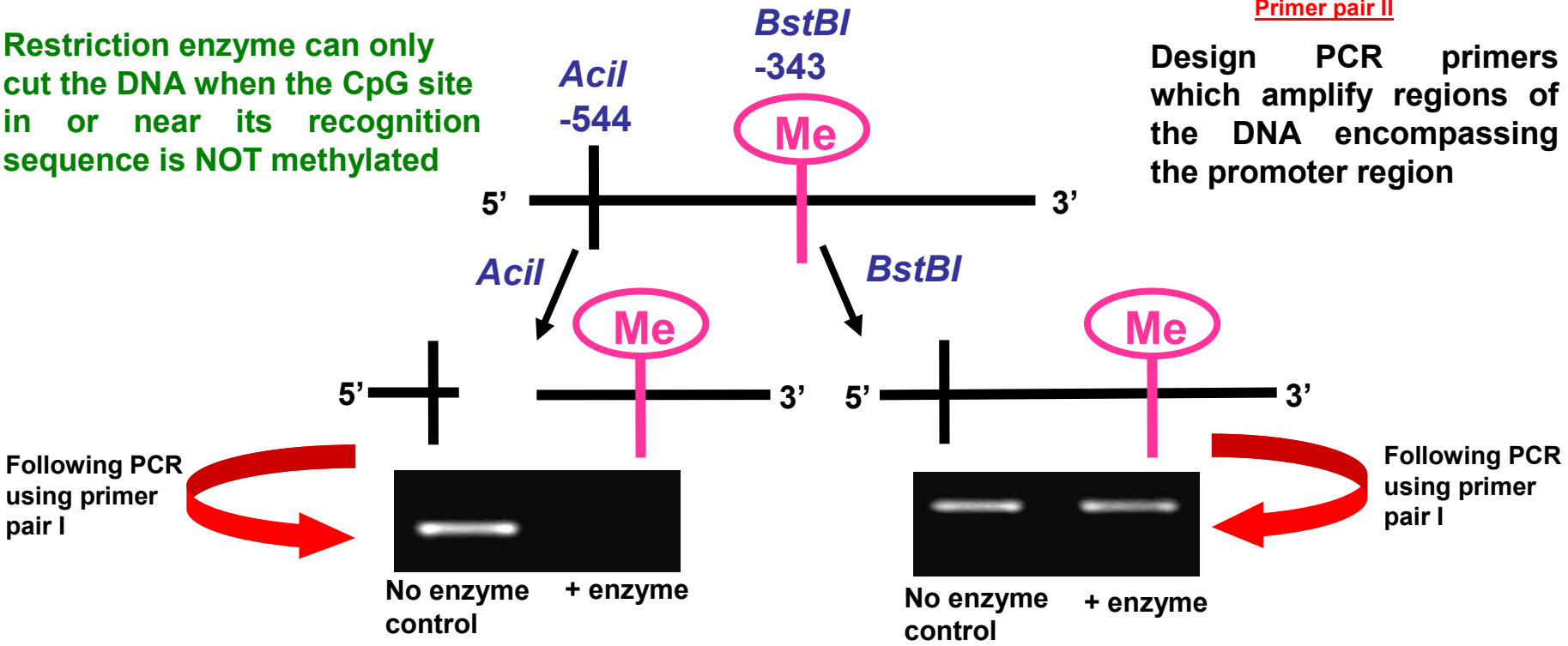
Get sequence for the human MMP13 gene promoter, identify CpG sites in the promoter

Identification of restriction enzymes whose recognition sequences contain these CpG sites or the recognition sequences are in close vicinity of the CpG sites



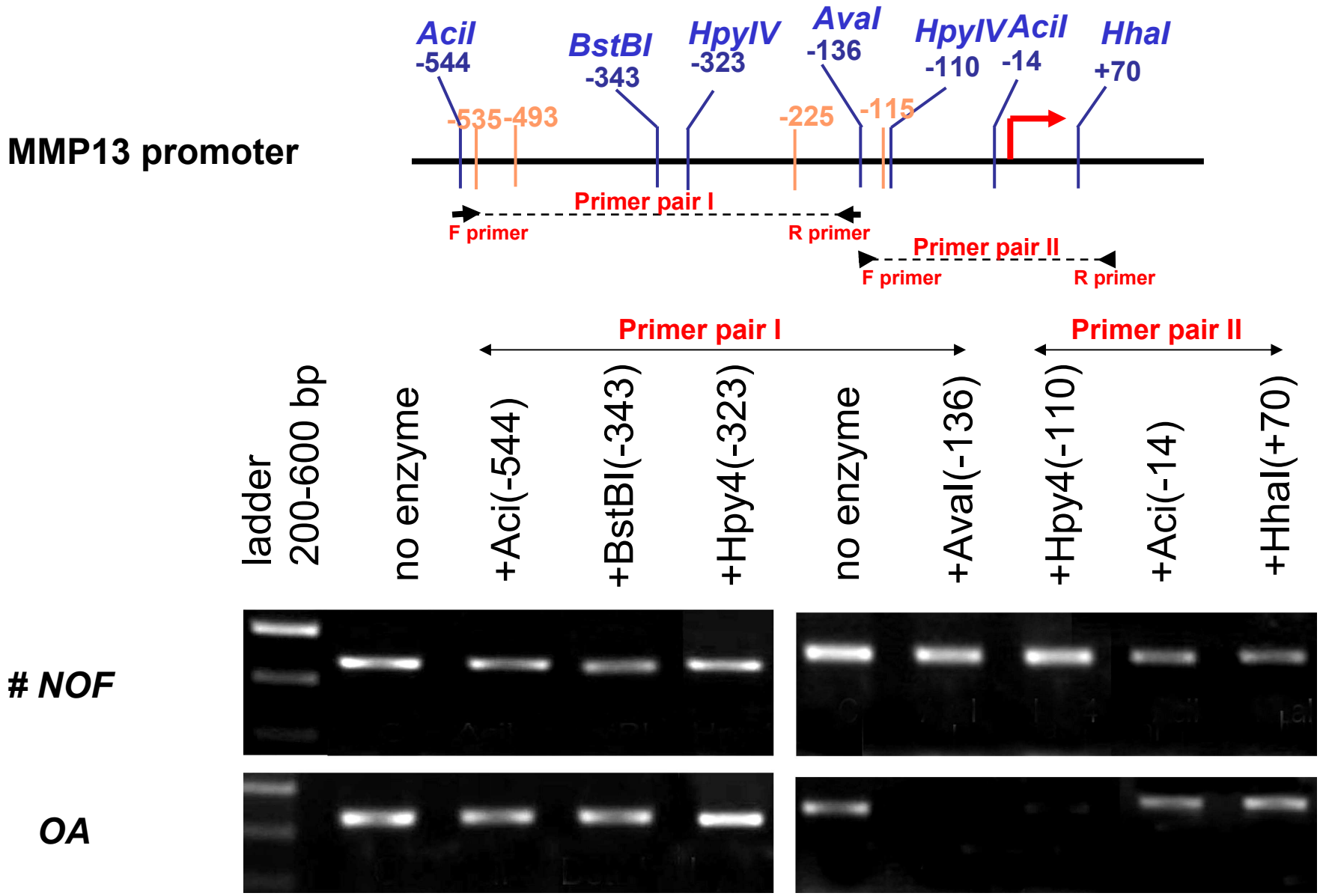
Restriction enzyme can only cut the DNA when the CpG site in or near its recognition sequence is NOT methylated

Design PCR primers which amplify regions of the DNA encompassing the promoter region



ABSENCE OF BAND INDICATES ABSENCE OF METHYLATION AT THAT CpG SITE

Loss of methylation at CpG sites -136 and -110 of the MMP-13 promoter is sufficient to 'unsilence' the expression of MMP13 in OA chondrocytes



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Association Between the Abnormal Expression of Matrix-Degrading Enzymes by Human Osteoarthritic Chondrocytes and Demethylation of Specific CpG Sites in the Promoter Regions

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[Epigenetics 2:2, e1-e1, EPUB Ahead of Print: http://www.landesbioscience.com/journals/epigenetics/abstract.php?id=4203; April/May/June 2007]; ©2007 Landes Bioscience

Research Paper

Improved Quantification of DNA Methylation Using Methylation-Sensitive Restriction Enzymes and Real-time PCR

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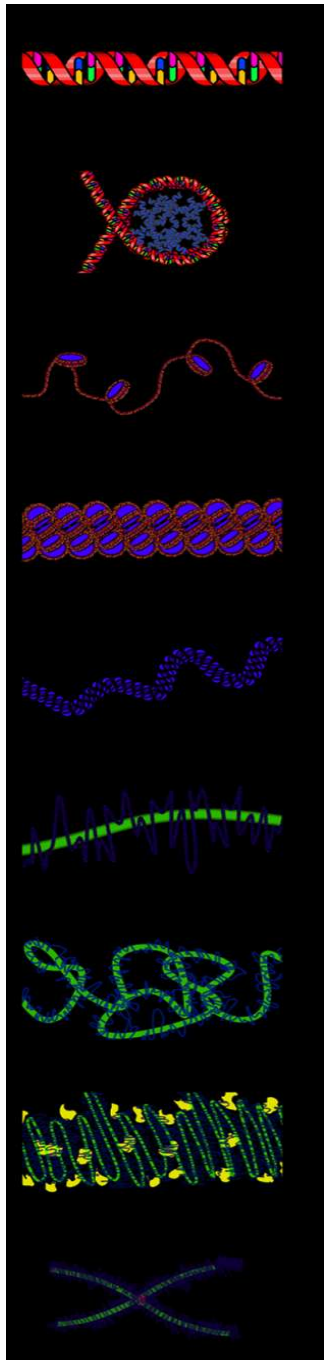
ABSTRACT

Heterogeneity of cells with respect to the DNA methylation status at a specific CpG site is a problem when assessing methylation status. We have developed a simple two-step method for the quantification of the percent of cells that display methylation at a specific CpG site in the promoter of a specific gene. The first step is overnight digestion of genomic DNA (optimal conc. 20ng/5ul) with a relevant methylation-sensitive

Summary

- Epigenetics plays an important role in normal development and pathophysiology of disease states
- DNA methylation = gene silencing
- DNA demethylation = gene unsilencing
- Histone acetylation = open chromatin, active transcription
- Histone methylation = closed chromatin, silencing of transcription (generally)



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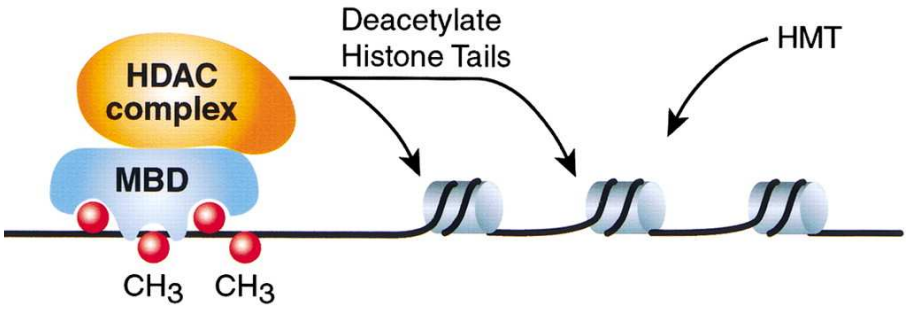
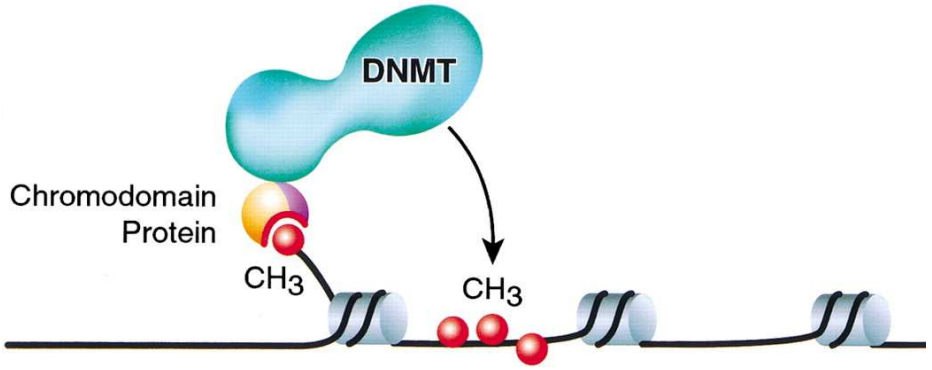
Models depicting the relationship between DNA methylation, histone deacetylation and histone methylation



Methyl CpG-binding proteins (MBD) bound to methylated Cytosines recruit the HDAC complex, which deacetylates the histone tails, which then become available for methylation by the histone methyltransferase (HMT)



Methylated histone tails, inturn, recruit the DNA methyltransferases to methylate the DNA for long-term gene silencing

Zhang Y., Reinberg D. Genes Dev.;2001;15:2343-2360

Small RNA species: Role in epigenetic regulation

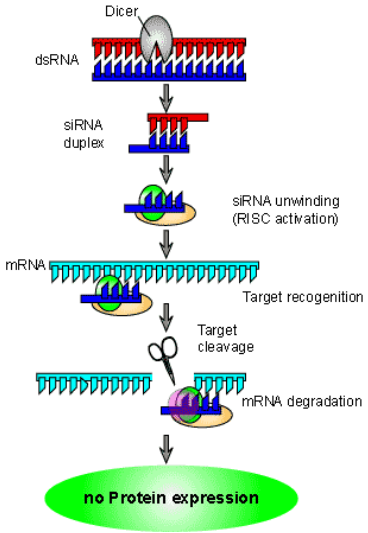
♦ 98% of the transcribed RNA is not translated into protein in humans i.e. transcriptional noise

♦ From this population of 'functionless' RNA, two types of RNA, with established roles in epigenetic regulation, have evolved

♦ As these two types of RNA are generally 21-25 nucleotides in length, they are collectively called small RNA

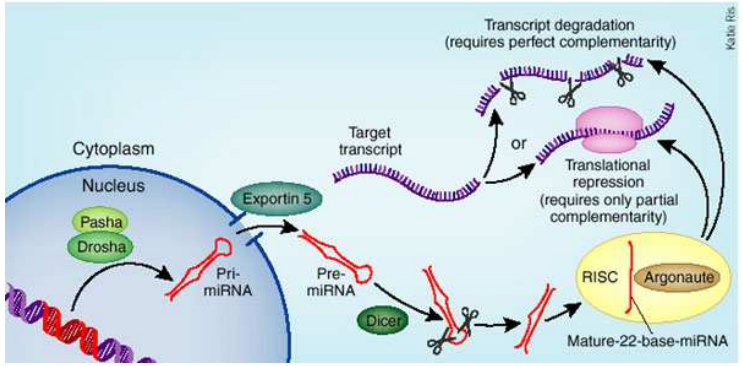
♦ **Small interference RNA/ siRNA**

siRNA binds to its complementary sequence in the target mRNA and guides the RNA-inducing silencing complex to the target mRNA, resulting in the endonucleolytic cleavage of the target mRNA

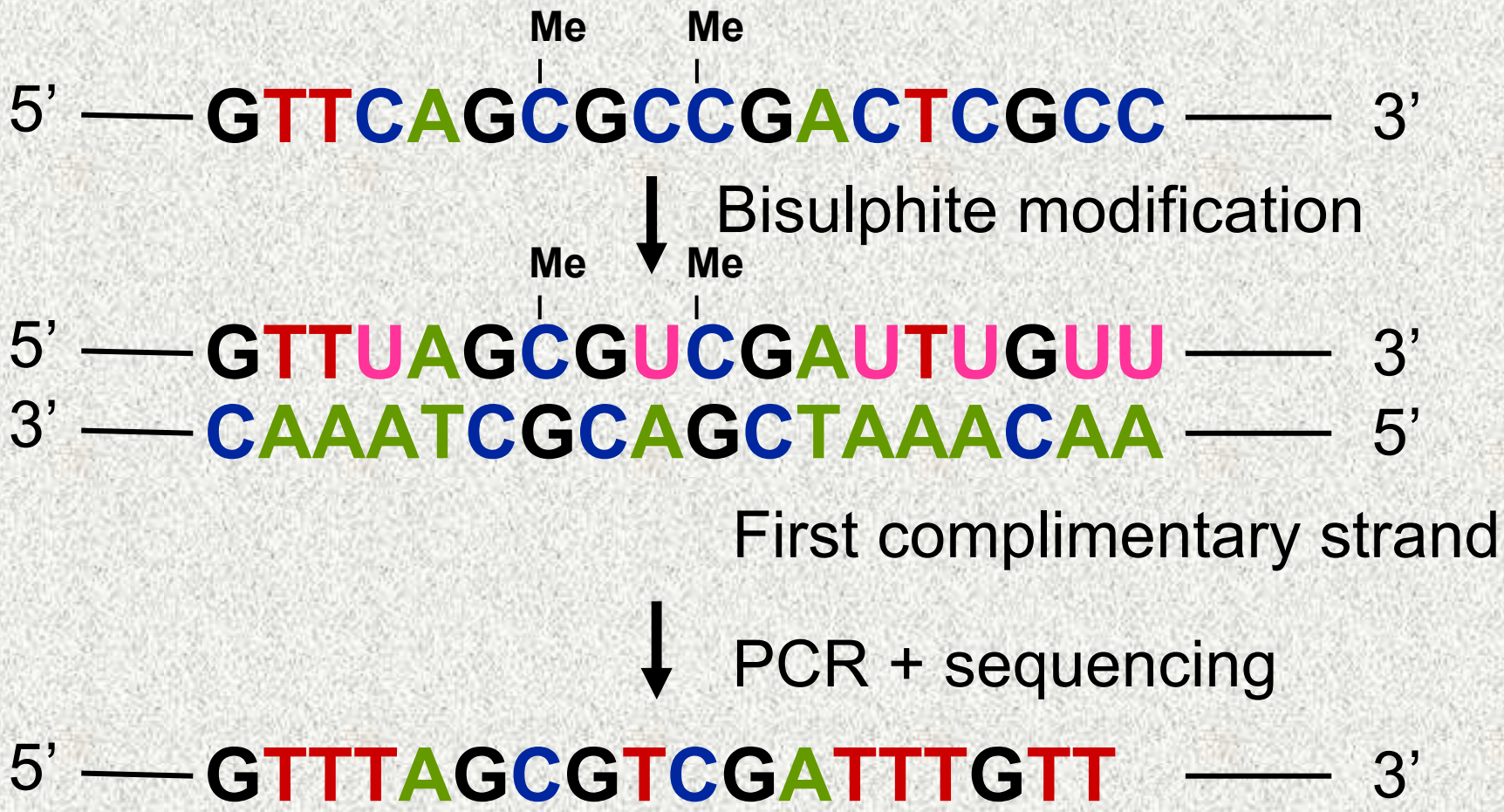


♦ **Micro RNA/ miRNA**

miRNA represses the translation of its target mRNAs and hence blocks protein production or it can cause transcript degradation



Bisulphite modification method



Methylated C = C
All other C = T

As a result of bisulphite modification, all Cytosine residues except those that are methylated are converted to Uracil

This modification to the DNA sequence is identified by DNA sequencing

How to assess methylation status?

Bisulphite modification

- Can identify methylation status of every CpG
- Expensive, lots of sequencing

Methylation-sensitive restriction enzymes

- Faster and cheaper
- Can only identify methylation status of some CpGs
- Can be quantified by real-time PCR

Role of environmental influences on epigenetic programming

Maternal dietary methyl supplements influence the phenotypes of the offspring by methylation-dependent epigenetic modulation

Cooney et al. (2002), The Journal of Nutrition 132(8 Suppl):2393S-2400S

♦ **Female mice were fed two levels of methyl supplements prior to and during pregnancy**

♦ **Extent of methylation in the regulatory region of the DNA governing the expression of the *agouti* gene (responsible for the yellow coat colour) was analysed in the offspring**

♦ **Y2 offspring: Mothers fed a diet high in methyl supplements, responsible for high degree of methylation in the regulatory region of the DNA, causes silencing of the *agouti* gene, offspring lose yellow coat colour and are black/ brown**

♦ **The level of methyl supplements fed to the mothers influences the extent of methylation in the regulatory DNA sequence governing the expression of the *agouti* gene, resulting in offspring with coat colours ranging from yellow (Y5), mottled (Y3) and black (Y2)**