An introduction to Epigenetics and Psychology

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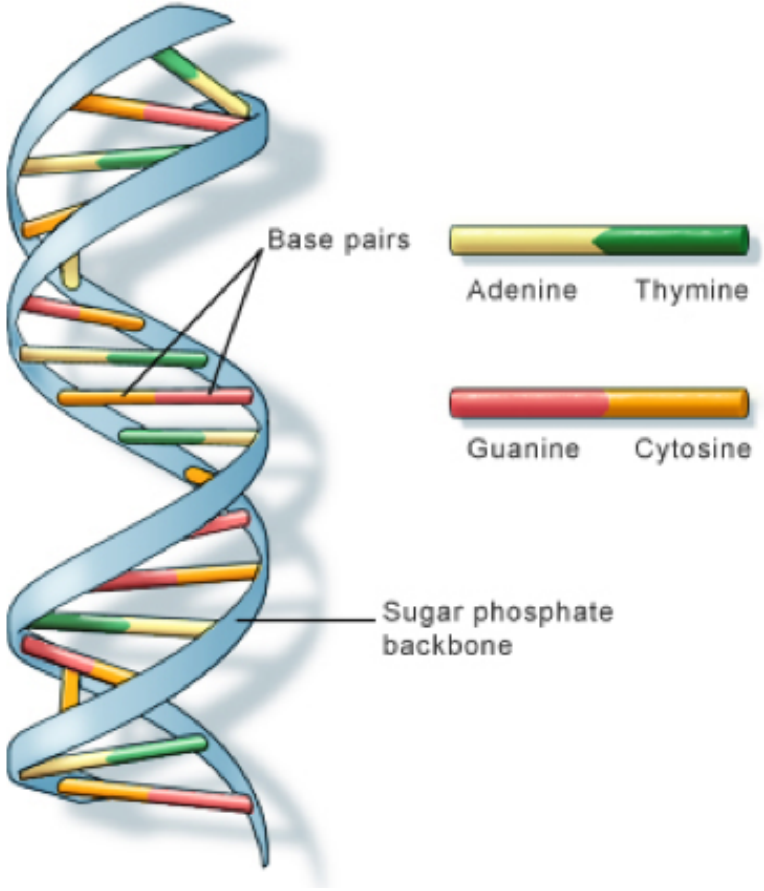
Learning outcomes

- By the end of the lecture, you should
  - Know what the terms ‘epigenetics’ and ‘epigenome’ means
  - Know the key factors in mammalian epigenetic machinery
  - Understand the nature and extent of epigenetic variation
  - Understand how epigenetic variation might contribute to human behaviour
  - Key issues and challenges for epigenetic research

Nucleotide:
Sugar + phosphate + base



DNA: Deoxyribonucleic acid

Structure: double helix

DNA molecule is made up of *sugar* residues *phosphate* groups and *bases*

Attached to carbon atom 1' of each sugar is a nitrogenous base:
Adenine (**A**)
Cytosine (**C**)
Guanine (**G**)
Thymine (**T**)

Watson-Crick base-pairing rules: A:T, C:G (hydrogen bonds)

The human genome

We know what the average human genome looks like

3.1 x10^8 bases (3 billion)

22,000 protein coding genes (~3%)

Broadly conserved sequence…..why?

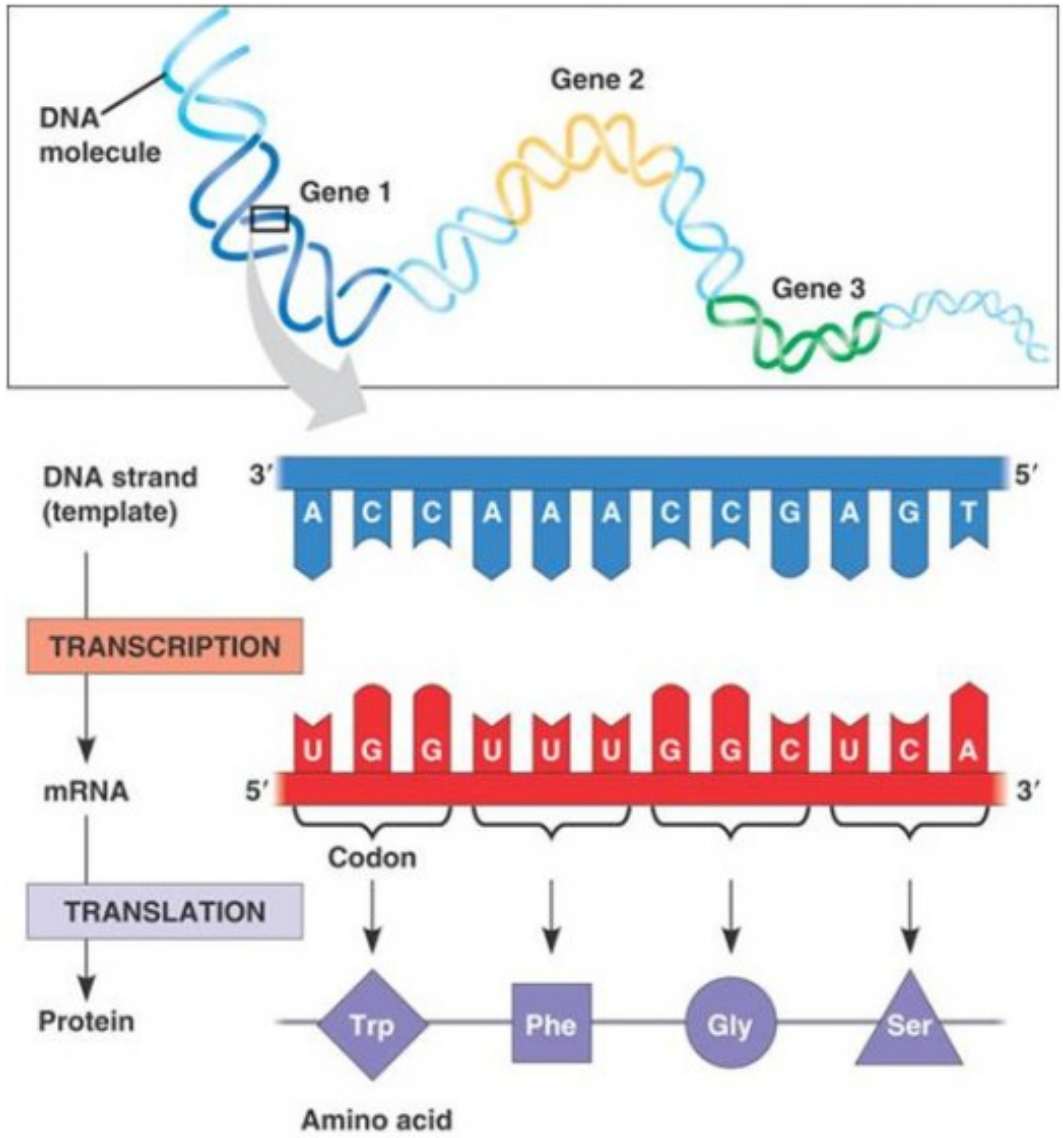
DNA sequence determines product

Principally proteins:

- Build (e.g., structural support)
- Control (e.g., enzymes)
- Protect (e.g., antibodies, waste clean-up)
- Signal (e.g., hormones, cell signaling pathways)

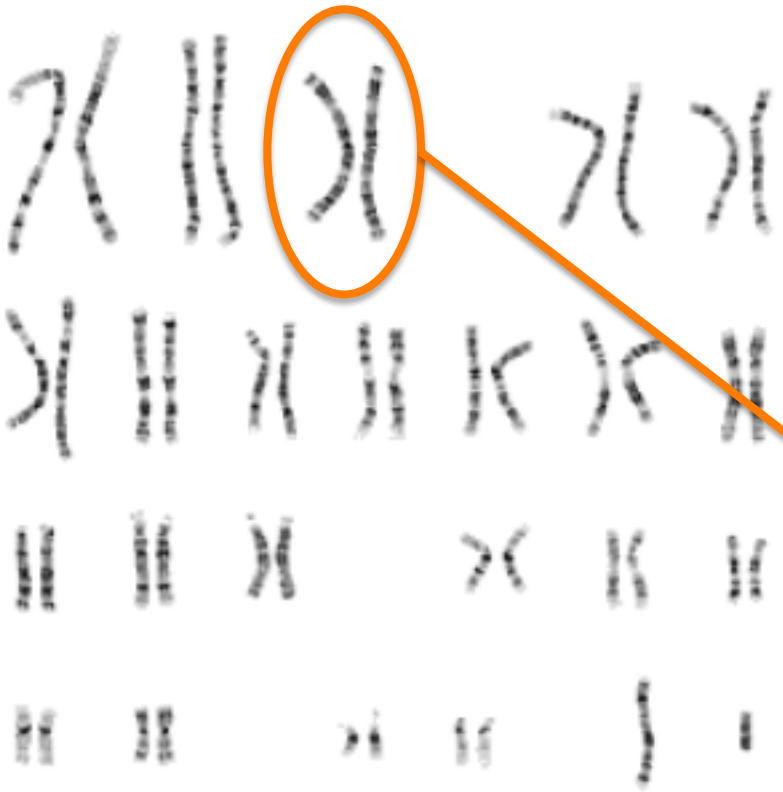
...they determine the function and activity of the cell

Protein production



The ‘Central Dogma’ of molecular biology – one way?

The human genome is organized into discontinuous chromosomes

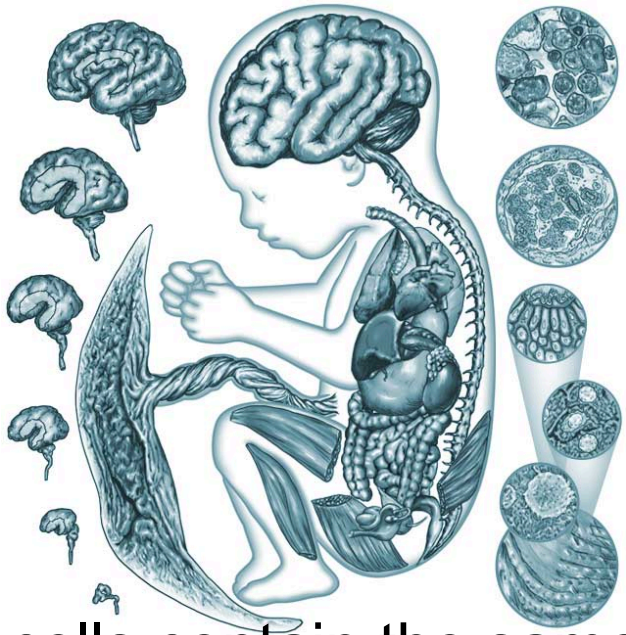


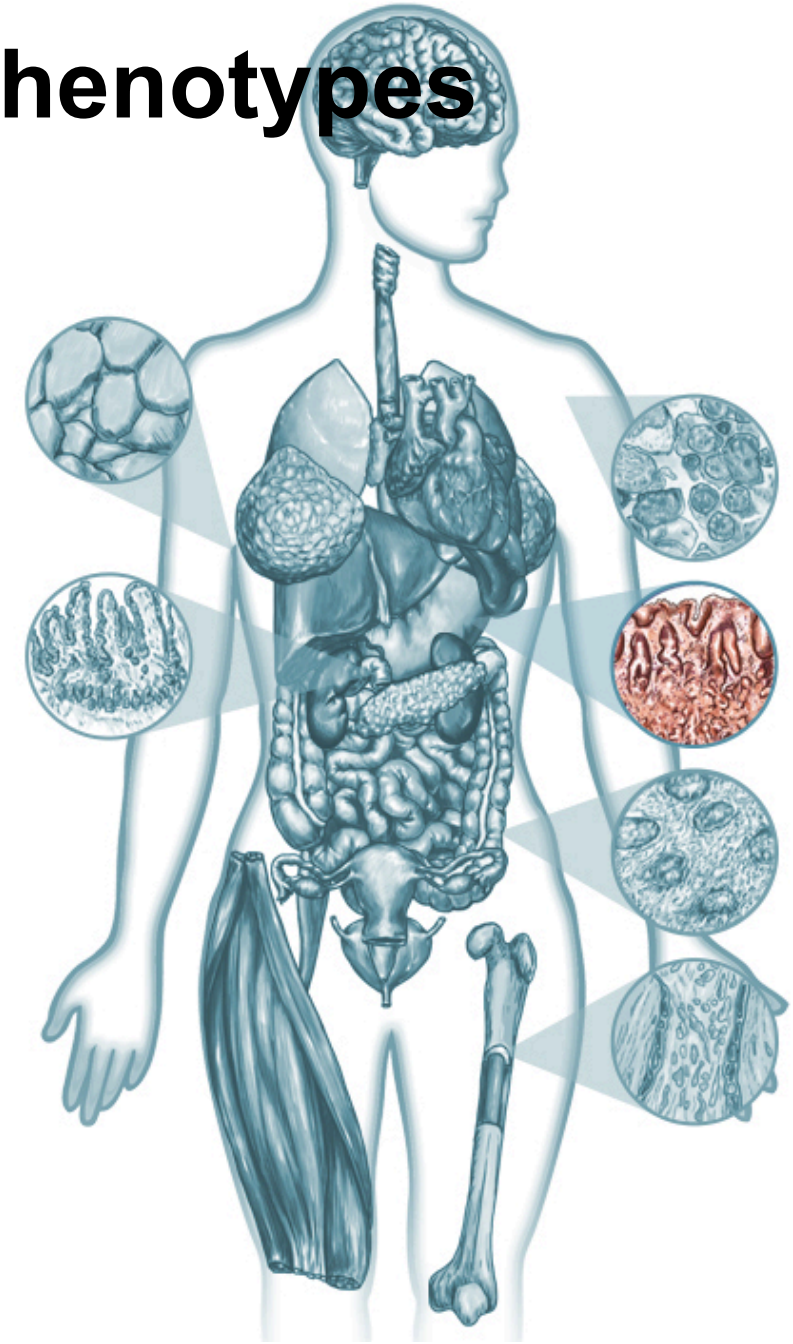
Normal Karyotype:
46, XY (male) or 46, XX (female)

Haploid genome:
One copy of the genome (gametes)

Diploid genome: **6.2×10^8 bases**
Two copies of the genome; one inherited from each parent (somatic cells)
-22 pairs of autosomes (ordered by size; 1-22)
-1 pair of sex chromosomes; XX or XY

One genome, multiple phenotypes





Our cells contain the same DNA sequence but do very different things. This is achieved via different timings, patterns and levels of gene expression (usually at the level of transcription)

Epigenetics: the regulation of genome activities

Something other than DNA sequence determines when/where genes are transcribed and at what levels

We know that regulation of transcription (expression of genes) is -- for the most part -- controlled and influenced by epigenetic factors

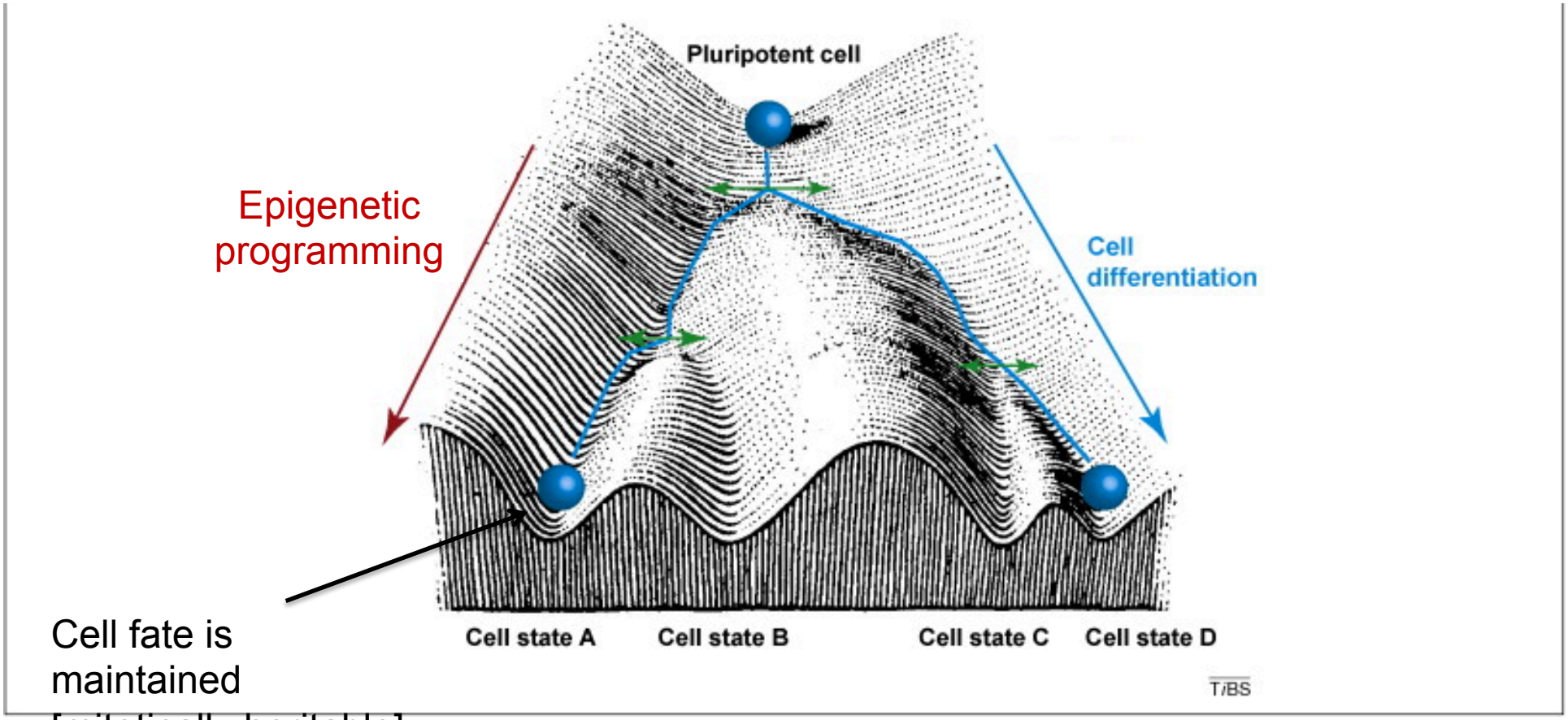
‘Epigenetics’

The mitotically heritable chemical modifications to DNA that alters the expression of a gene without changing the underlying sequence

Waddington, 1942; Wolfe & Matzke 1999

(‘epi’ – on top off)

Epigenetic patterning



Cell fate is maintained [mitotically heritable]

‘Epigenome’

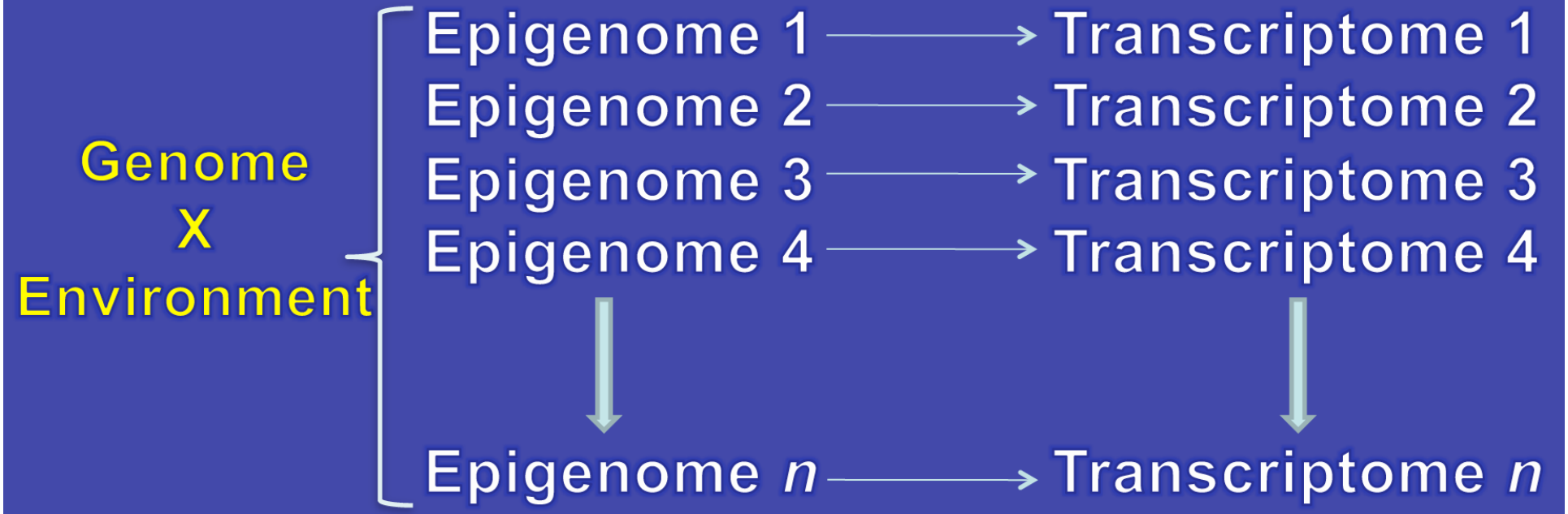
- The collection of epigenetic marks across the genome

‘Epigenomics’

- Research into the epigenetics of the genome
- Read, locate, and interpret the complete set of epigenetic marks across the genome

One genome...multiple epigenomes

Same genotype → many phenotypes!!



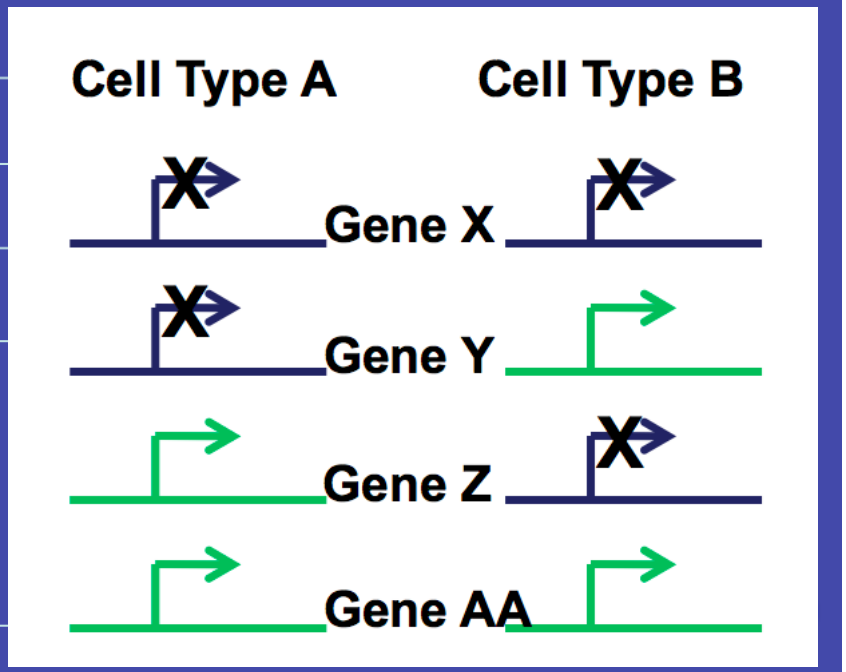
All our cells contain the same DNA sequence but do very different things

One genome…multiple epigenomes

Same genotype → many phenotypes!!

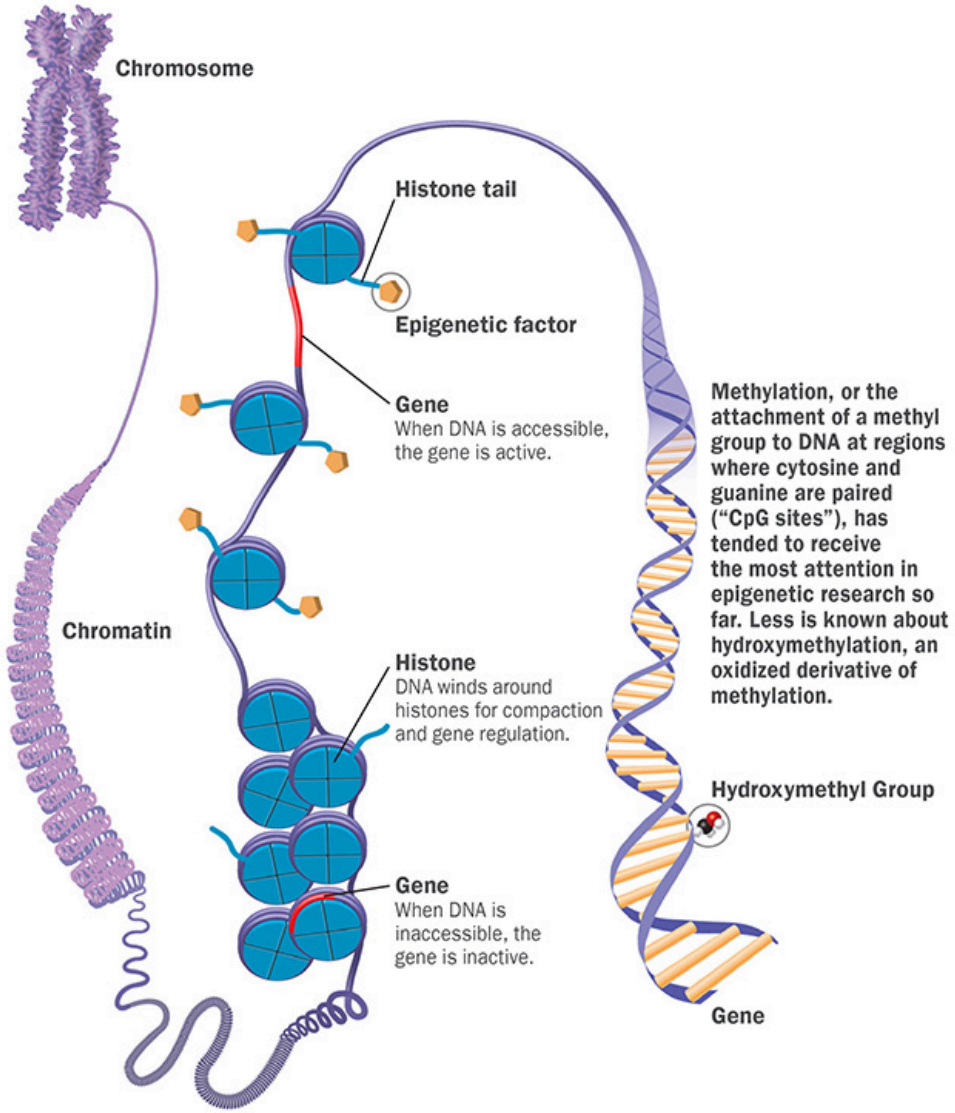
Genome
X
Environment

Epigenome 1
Epigenome 2
Epigenome 3
Epigenome 4
Epigenome *n*



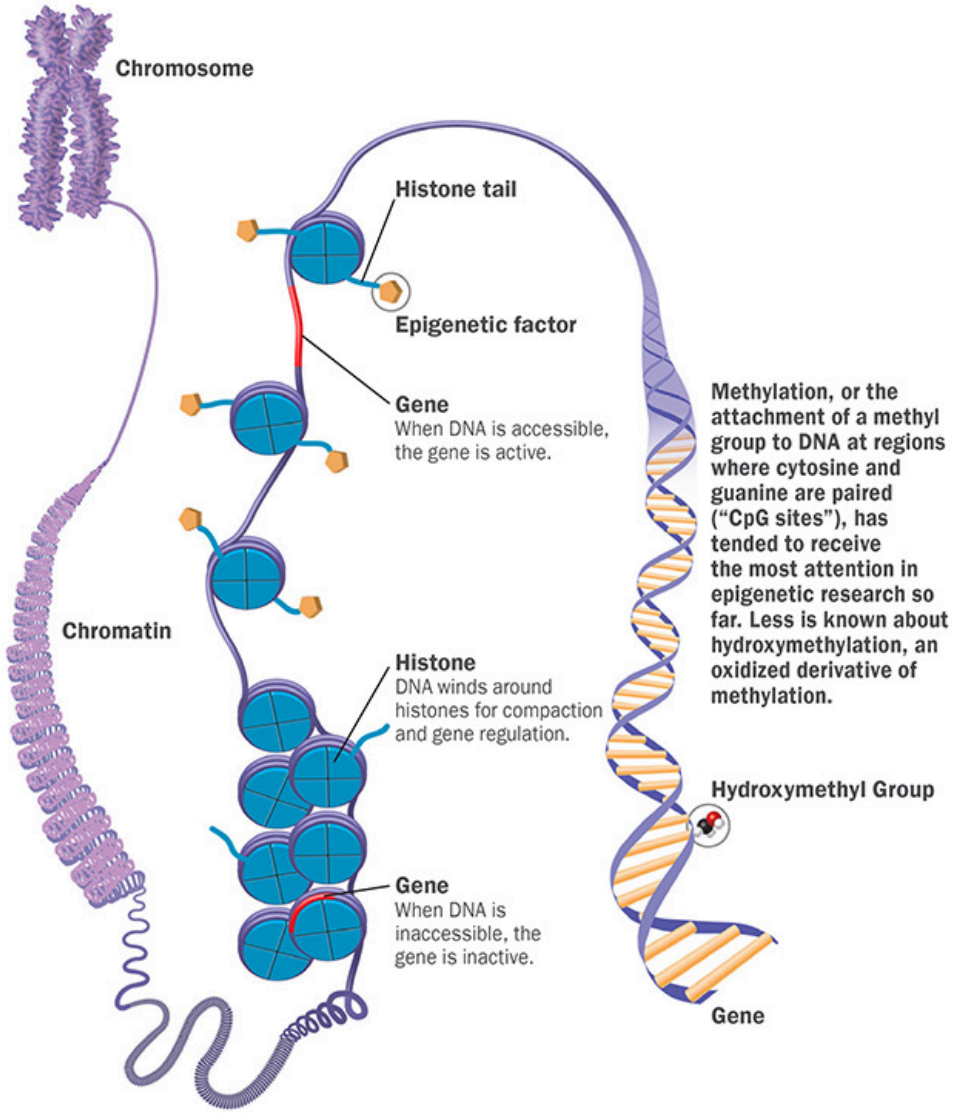
All our cells contain the same DNA sequence but do very different things

Epigenetics: regulation of genome activities



- The DNA sequence determines what specific mRNA molecules are synthesized
- Epigenetic factors determine how much of the mRNA is made, and where and when it is synthesized

Chromatin packaging affects accessibility of gene expression machinery



DNA + histone proteins = nucleosomes

Nucleosomes are packaged together to form **chromatin**

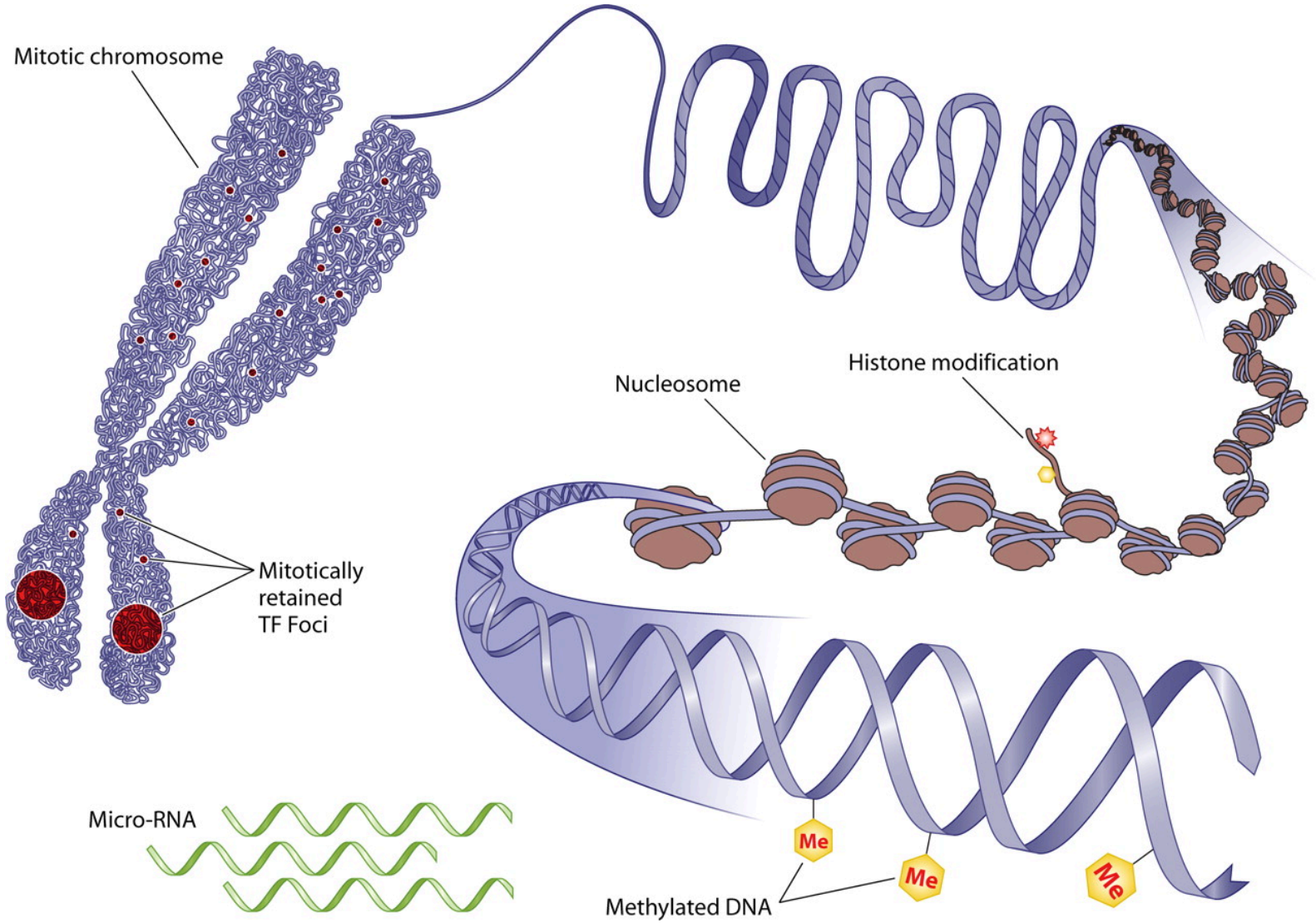
<u>Euchromatin</u>:

'Open DNA' readable genes and transcriptional activity

<u>Heterochromatin</u>:

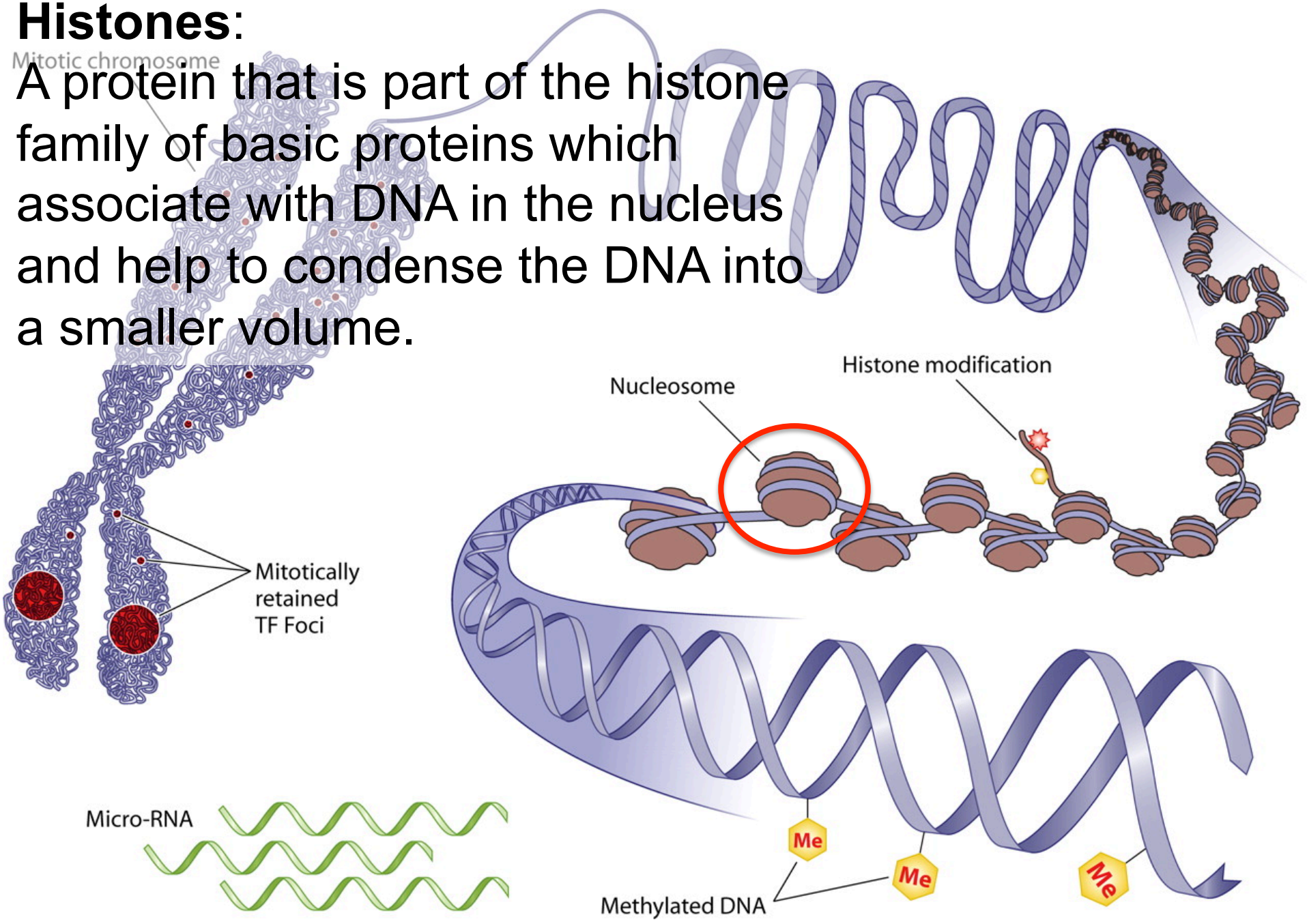
'Closed DNA' and repression of transcription

Epigenetic machinery



Histones:

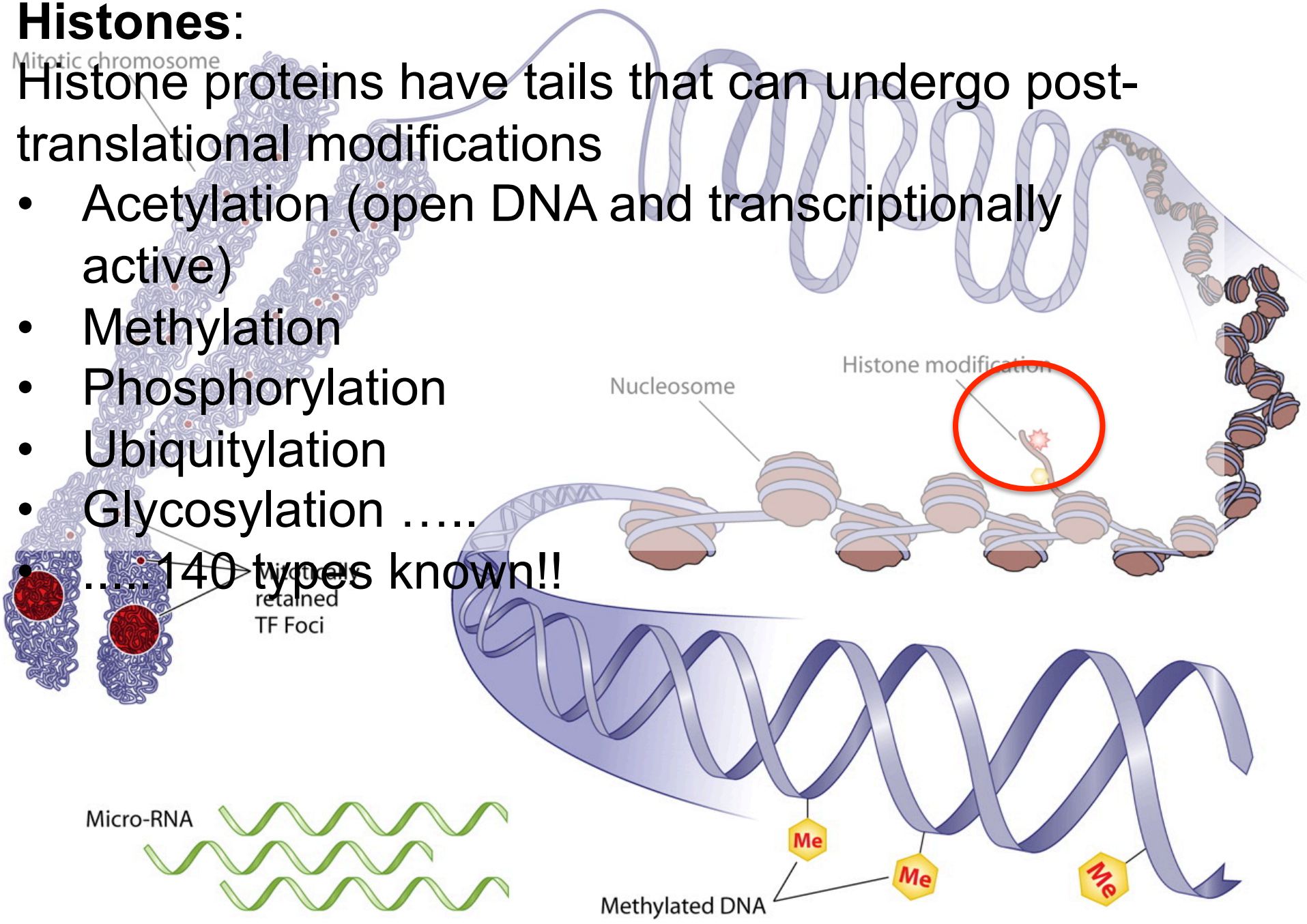
A protein that is part of the histone family of basic proteins which associate with DNA in the nucleus and help to condense the DNA into a smaller volume.



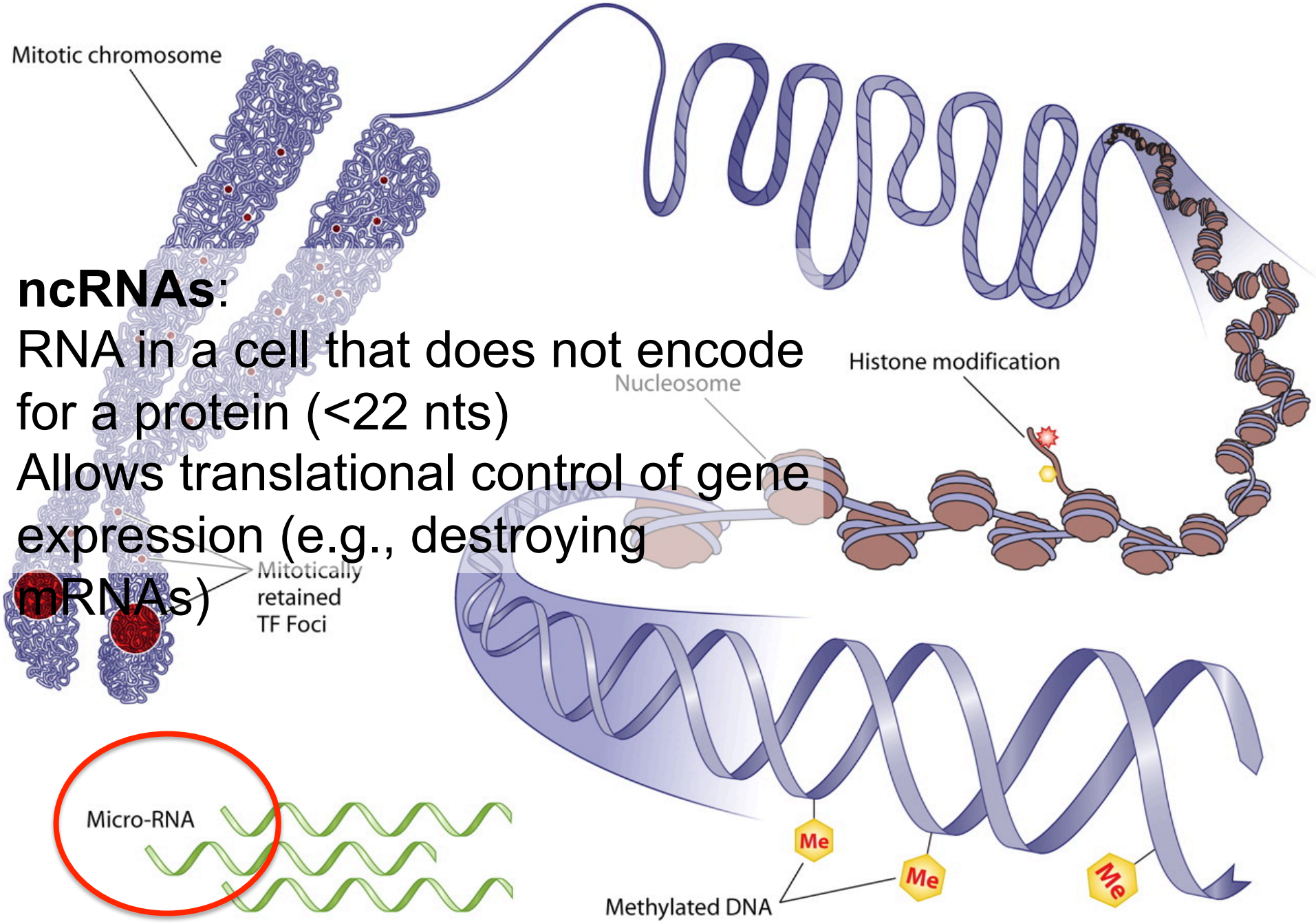
Histones:

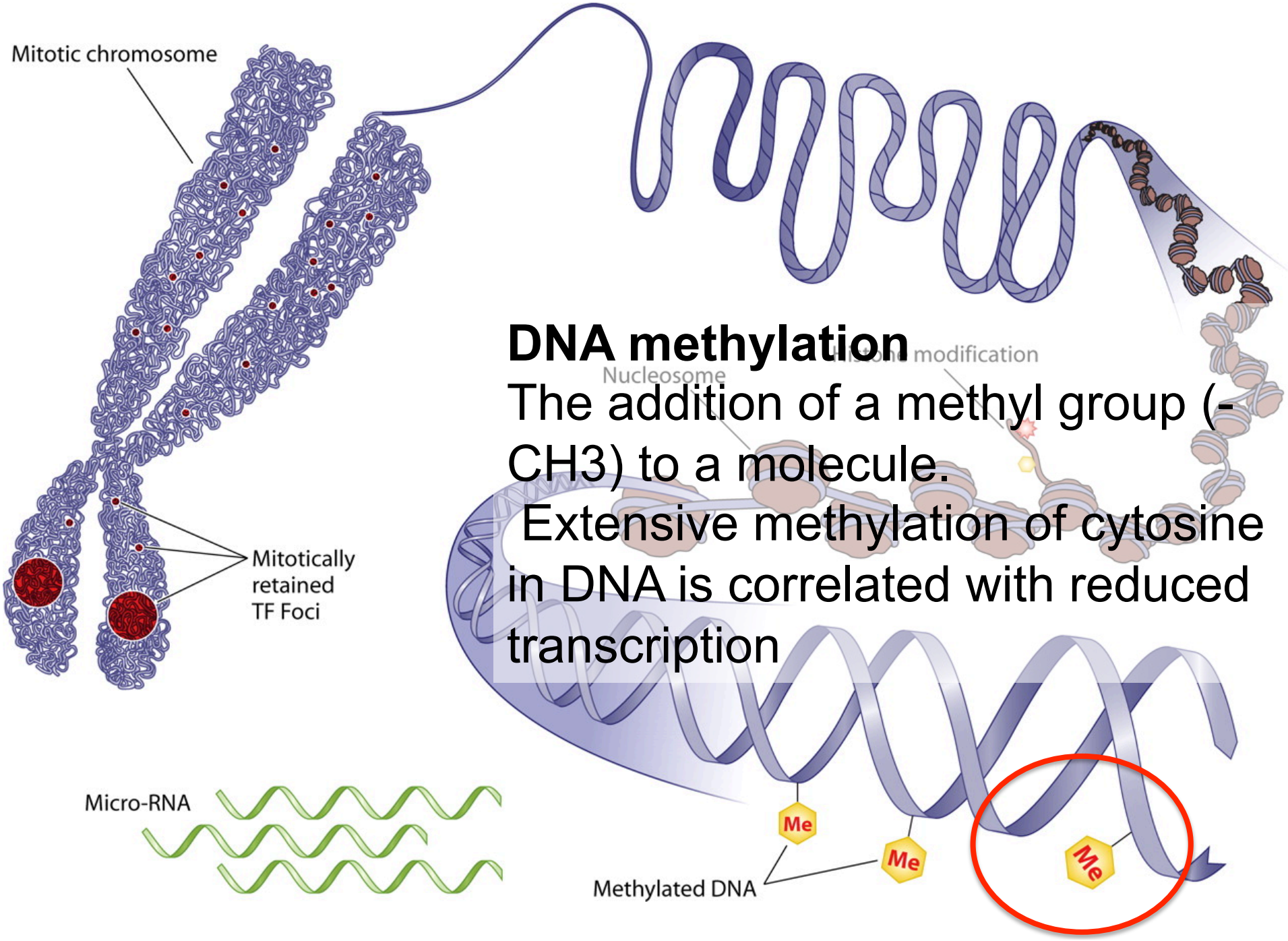
Histone proteins have tails that can undergo post-translational modifications

- Acetylation (open DNA and transcriptionally active)
- Methylation
- Phosphorylation
- Ubiquitylation
- Glycosylation …..
- ….140 types known!!



ncRNAs:
RNA in a cell that does not encode for a protein (<22 nts)
Allows translational control of gene expression (e.g., destroying mRNAs)

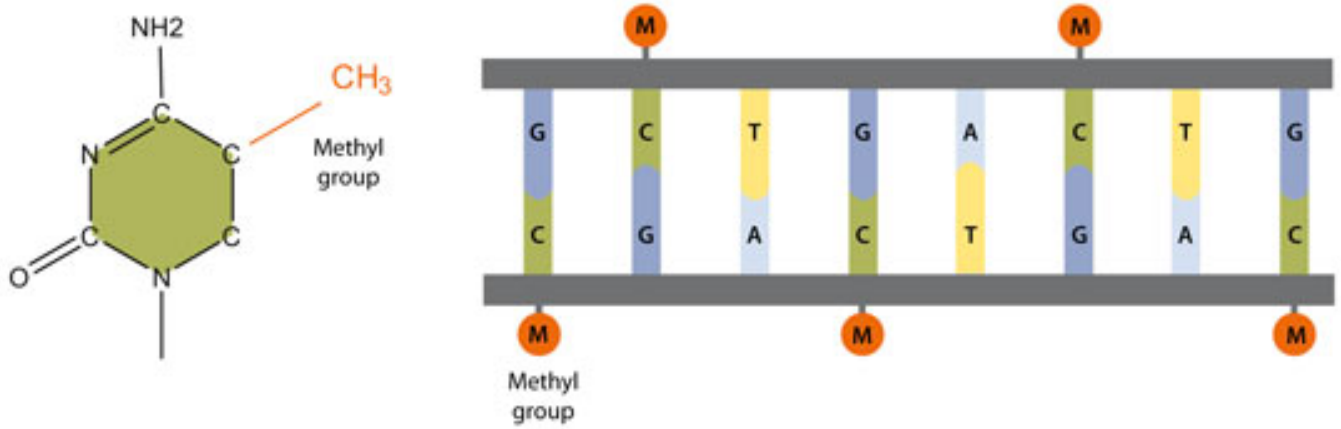




DNA methylation

The addition of a methyl group (-CH3) to a molecule.

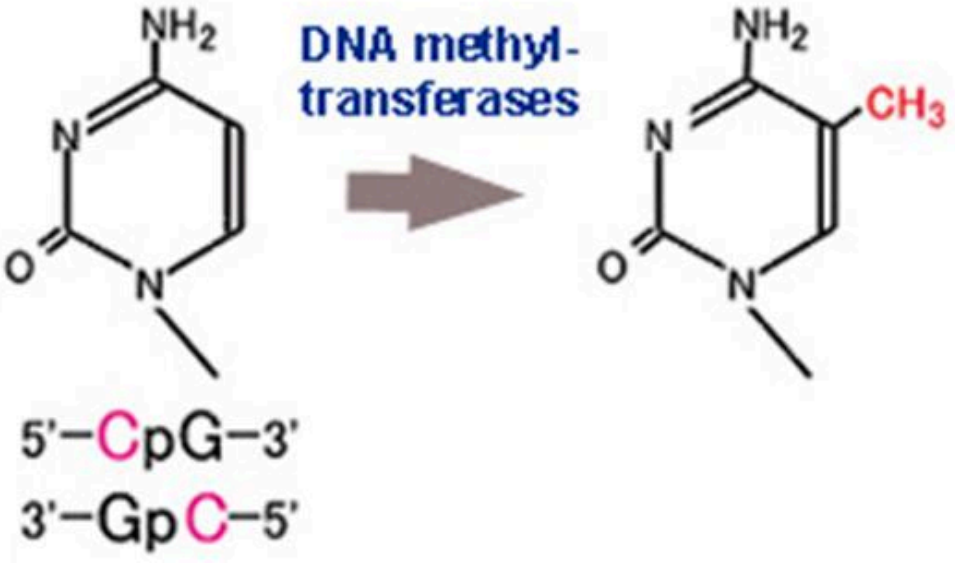
Extensive methylation of cytosine in DNA is correlated with reduced transcription

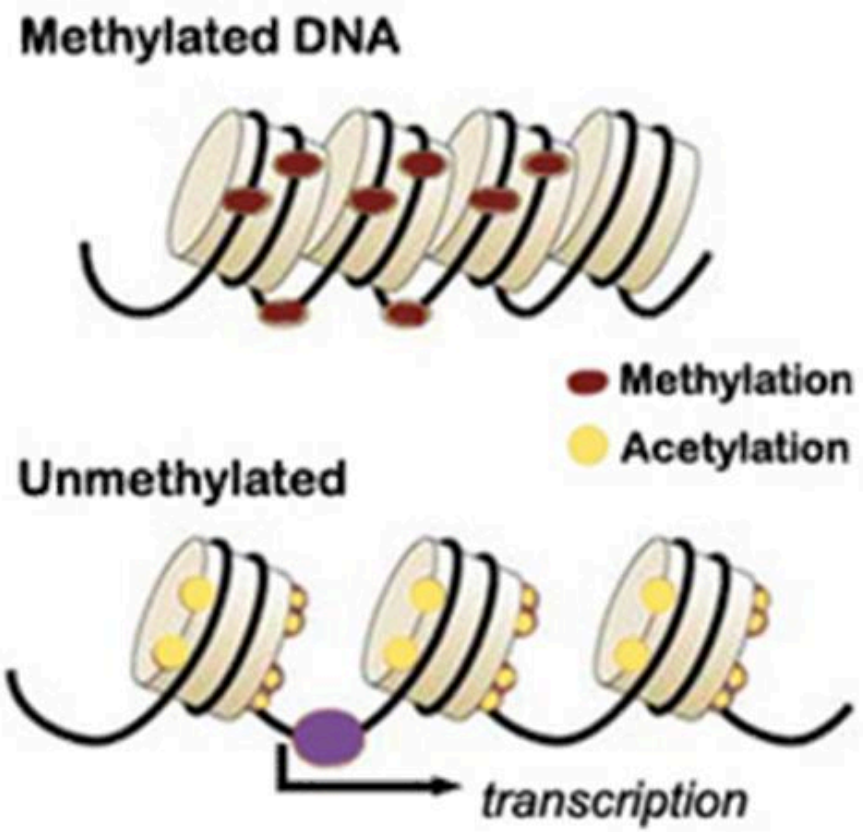


DNA methylation is the most widely studied epigenetic mechanism

- The addition of a methyl group (-CH3) to a cytosine
- Occurs primarily in CpG dinucleotides
- [cytosine-phosphate-guanine]
- Heritable by somatic cells after division
- Extensive methylation is correlated with reduced transcription

DNA methylation





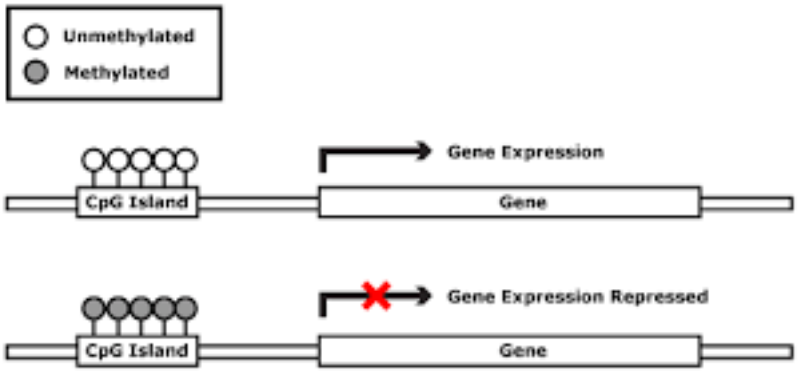
DNMT1: 'maintenance'
DNMT3a & DNMT3b: 'De novo'

- Addition of methyl to the the 5th carbon position of Cytosine ('5MeC') catalyzed by DNA methyltransferases

Awesome video (or two) here:

https://www.youtube.com/watch?v=JMT6oRYgkTk

DNA methylation is non-random



~28 million CpG sites in the genome with a non-random distribution

CpG 'Islands'

Clusters of CpGs located near promoter regions of genes (2/3 of genes contain CGIs in their promoters)

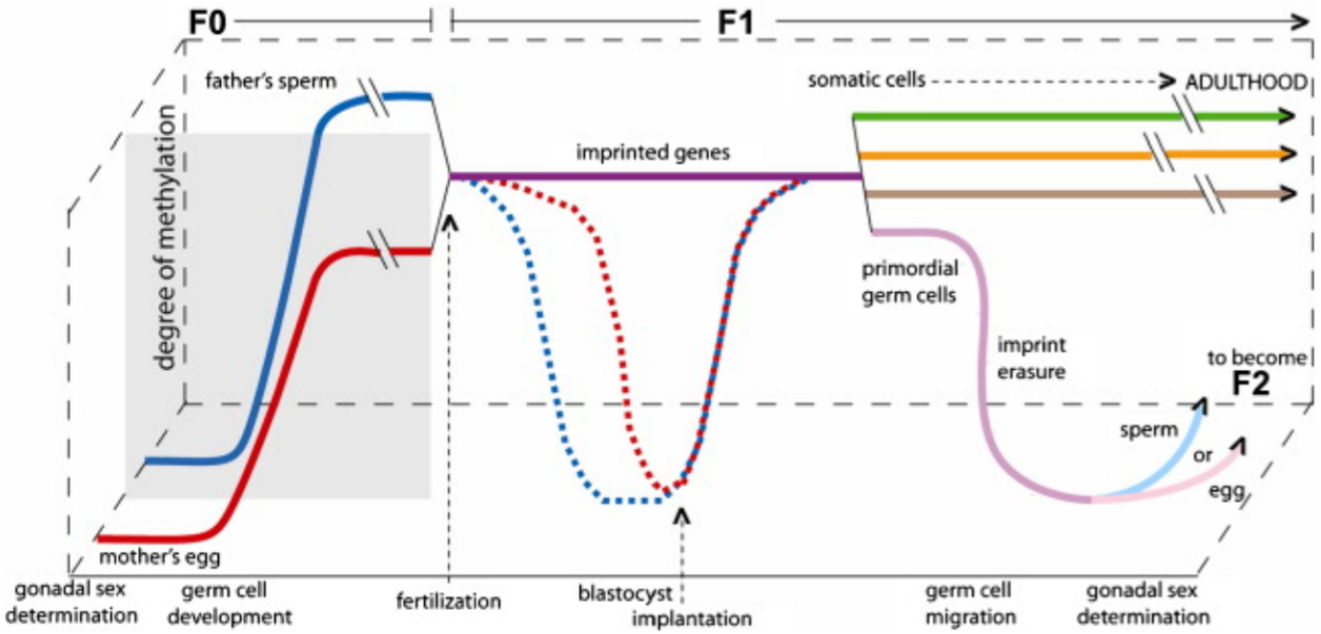
Promoters

Usually unmethylated and associated with transcriptionally active state ('house keeping' genes, etc)

Non-promoter regions

Usually methylated and transcriptionally repressed

Epigenome established early in development



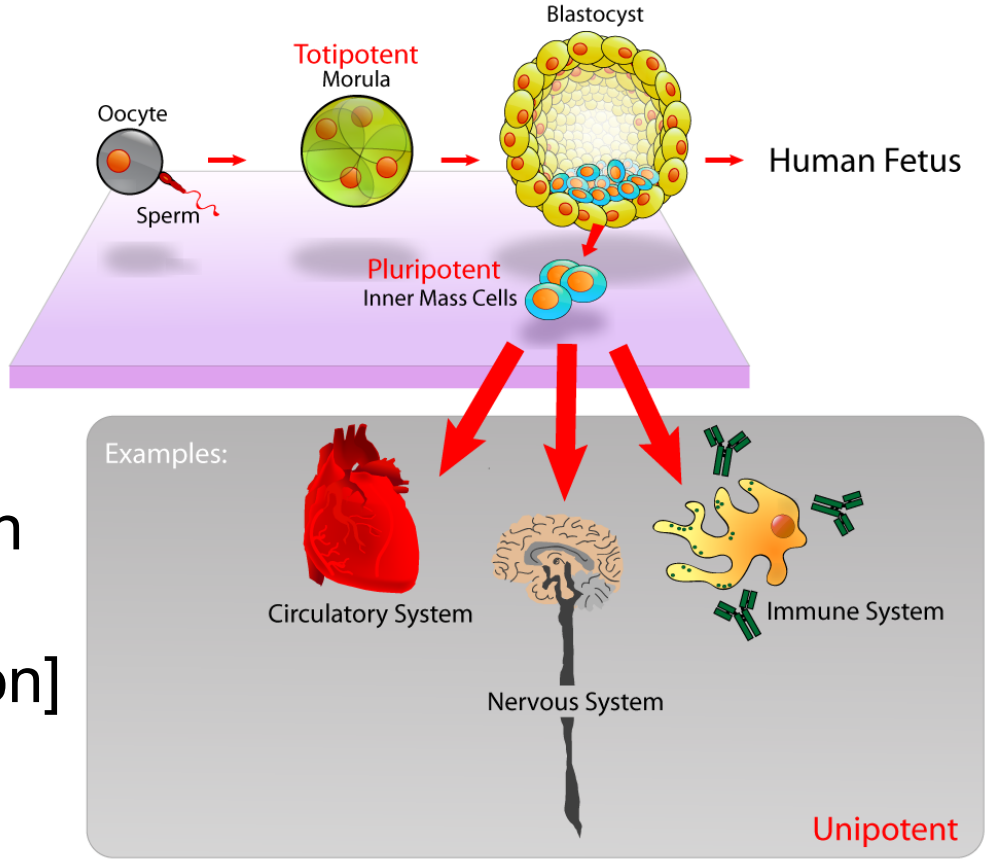
During mammalian development, two major waves of epigenetic reprogramming take place

- During embryogenesis DNA synthesis rate is high
- Time when elaborate DNA methylation patterns and chromatin structure required for normal tissue development are established

Regulation of gene expression is critical for normal cellular development and differentiation

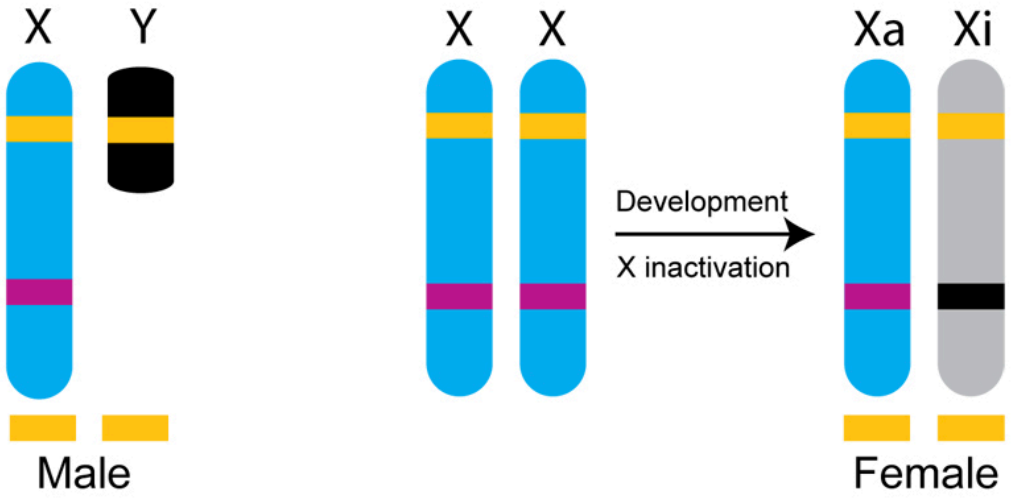
Establishment of epigenetic marks is essential

- Nuclear organization
- Genome stability
- Cell-linage specification
- Regulation of gene expression [transcription]
- X-chromosome inactivation



Reik, W. (2007) Stability and flexibility of epigenetic gene regulation in mammalian development. Nature 447, 425–432

X-chromosome inactivation: dosage compensation



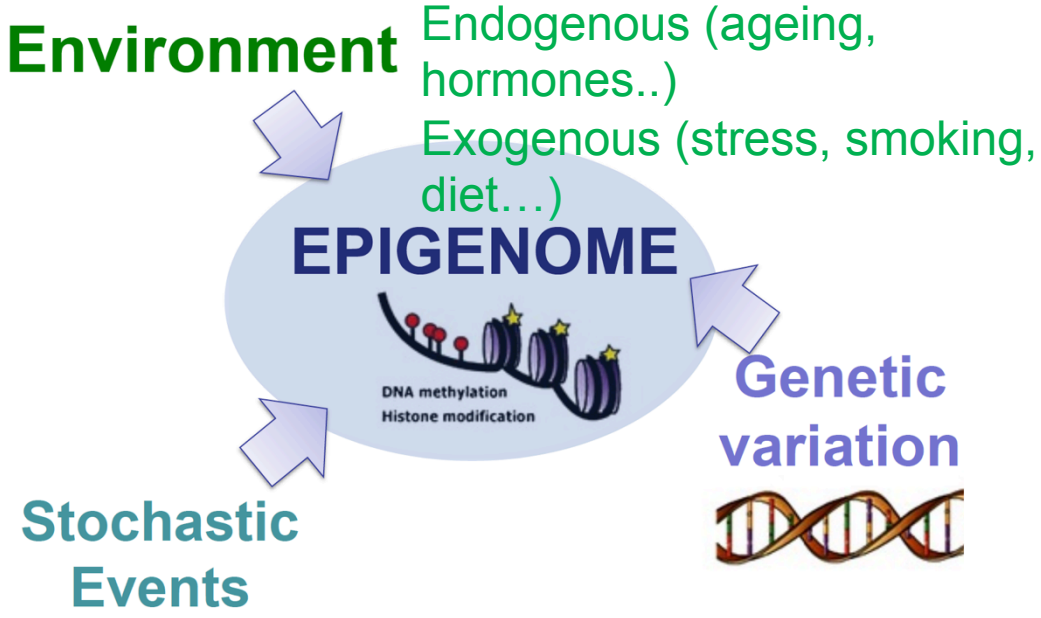
Dosage compensation
Y=70-200 genes
X=~2,500 genes

Mammals adjust genetic imbalance of X-linked genes via a process of hypermethylation of CpG islands on the X chromosomes

Occurs randomly early on in female embryonic development and is stable once established

THE DYNAMIC (AND REVERSIBLE) EPIGENOME

The epigenome: an interface between the genome and our environment



Unlike the genome, DNA can acquire or lose a methyl group more readily than it can change its sequence

Provides a dynamic mechanism for mammals to respond to the environment without changing its hardware

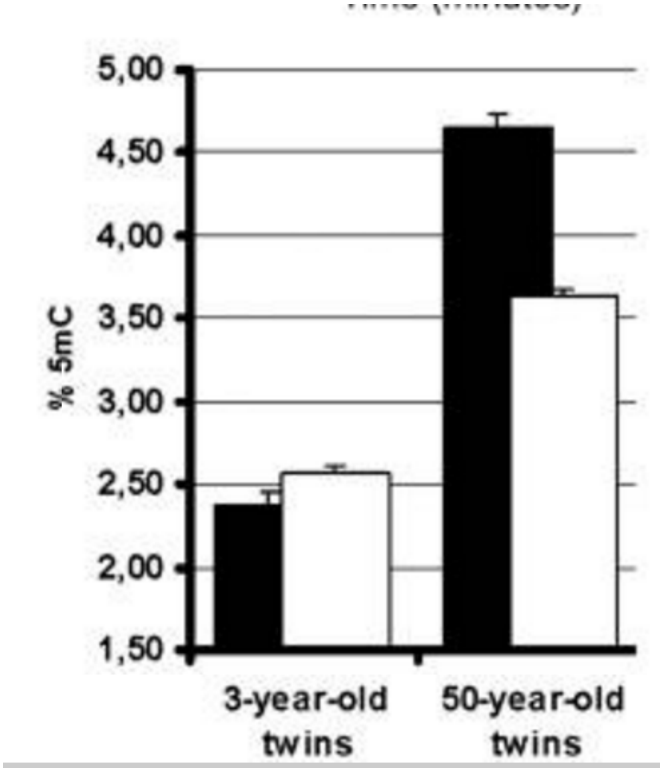
[contrast to natural selection!]

Same genome, different epigenome!

Healthy identical (MZ) twins have been shown to differ in their DNA methylation profiles

<u>Fraga et al (2005):</u>

- landmark study of epigenome in 40 pairs of MZ twins (3-74 years of age)
  - Found discordance in epigenetic profiles
  - Greatest differences found in older twins – '**epigenetic drift**'
  - Differences related to phenotypes (?)



Fraga et al, Proc Natl Acad Sci U S A. 2005 Jul 26;102(30)

Epigenetic differences increase with age

Table 1 Studies of CpG methylation discordance in monozygotic (MZ) twins

Study	Type	Method	Tissue	MZ pairs, n	Conclusion
Fraga *et al.* [29]	Cross-sectional, age-stratified	High-performance capillary electrophoresis of total methyl-cytosine content	Peripheral lymphocytes; buccal epithelial cells; muscle biopsy; adipose tissue	40	Young MZ twins are nearly identical epigenetically; discordance progresses with age, mediated by a combination of external and/or internal factors
Kaminsky *et al.* [85]	Cross-sectional	Human 12 K CpG island microarrays	White blood cells, buccal epithelial cells, rectal biopsy	57[1]	Methylation discordance in MZ twins confirmed; monochorionic MZ twins significantly more discordant than dichorionic MZ twins. Epigenetic drift suggested as the main cause of discordance
Saffery *et al.* [86]	Cross-sectional, taken at birth	Bis-seq (*IGF2/H19*)	Cord blood, mononuclear cells, buccal epithelial cells, placental cells, umbilical vein cells, endothelial cells	56	CpG methylation discordance can arise in newborn twins by combination of environmental and/or stochastic factors acting *in utero* and varies depending on the type of the tissue
Wong *et al.* [87]	Longitudinal, with single 5-year interval	High-throughput mass spectrometry (*DRD4, SERT, MAOA*)	Buccal cells, epithelial cells	46	CpG methylation discordance is present in early childhood and susceptibility to epigenetic change is highly locus-specific. Environmental influence is the main cause of discordance, with various loci having differential susceptibility to shared and non-shared exposures
Talens *et al.* [88]	Cross-sectional and longitudinal with single 10-year interval	High-throughput mass spectrometry; global methylation and selected loci (*IGF2, LEP, CRH, ABCA1, INS, KCNQ1OT1, GNASAS*)	Whole blood	230	Global and locus-specific methylation increases gradually with age, owing to unique environmental and stochastic factors

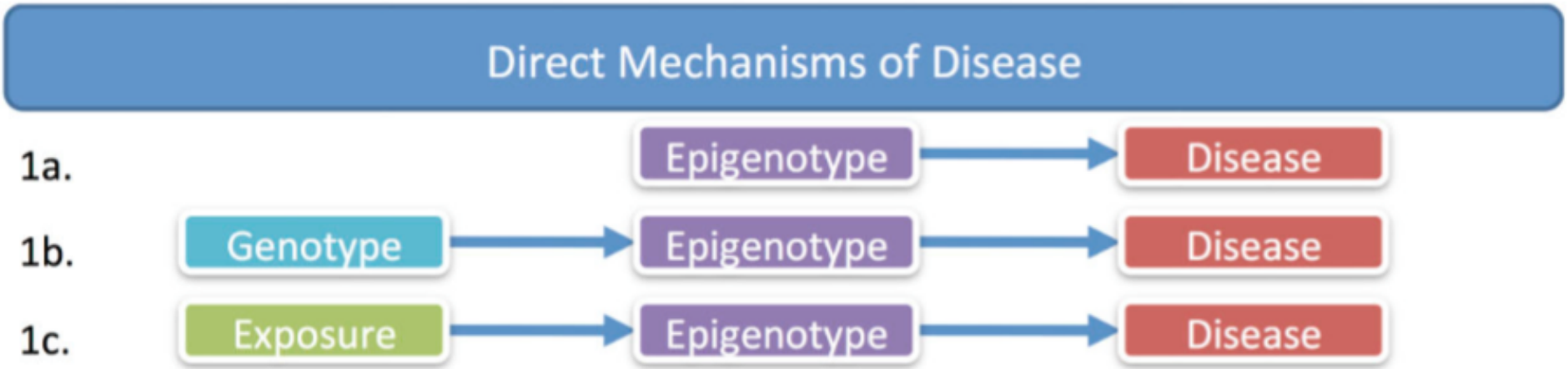
EPIGENETICS AND COMPLEX HUMAN TRAITS AND DISEASES

Epigenetics and human complex traits and diseases

Epigenetics is emerging as important to study when examining the etiology of disease

- Can provide mechanistic insights into genetic and environmental risk factors for disease
- Act as a 'biomarker' for disease or environmental exposures
- Will help localize disease relevant regions in the genome
- Might provide intervention/treatment targets

Epigenetics as a biological mechanism for disease



Mediation: Epigenetic factors are causally involved by directly mediating the genetic (genotype) and/or environmental ('exposure') risk

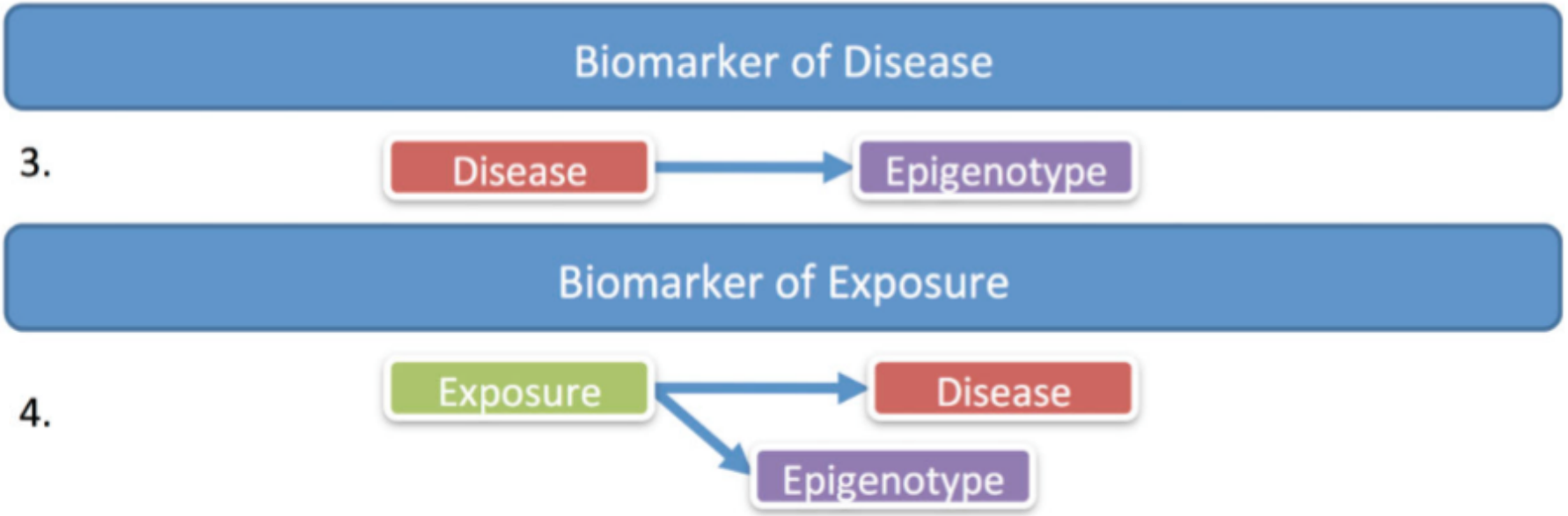
Ladd-Acosta & Fallin, 2016 (Epigenomics); Bakulski & Fallin, 2014 (Environ Mol Mutagen)

Epigenetics as a biological mechanism for disease

Modification of risk: Epigenetic factors could act as a modifier of genetic (genotype) and/or environmental ('exposure') risk

Ladd-Acosta & Fallin, 2016 (Epigenomics); Bakulski & Fallin, 2014 (Environ Mol Mutagen)

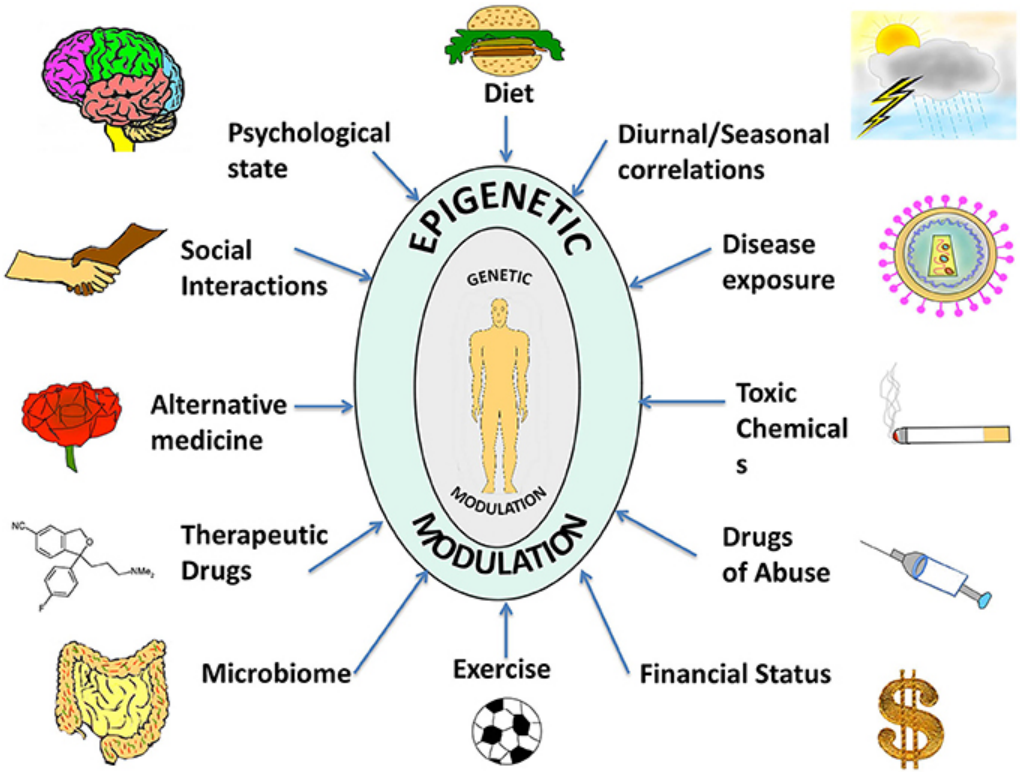
Epigenetics as a biomarker for disease



Epigenetic factors <u>are not causally related to disease</u>, but might serve as ‘biomarkers’ for disease (disease state, or subtype) or ‘tombstones’ for exposure to environmental risks factors in early life

EVIDENCE FOR ENVIRONMENTAL EFFECTS ON THE EPIGENOME

Environmental (non-genetic) influence on DNA methylation



. Kanherka et al,. (2014)
Epigenetics across the human lifespan
Front. Cell Dev. Biol

Mounting evidence that epigenetic factors are sensitive to stochastic events in the cell and can vary *systematically* with specific environmental factors

It provides a biological mechanism to explain how environmental exposures (in utero and/or early life) can cause disease

Timing of environmental effects likely to be important

Note also: 'Epigenetic drift'

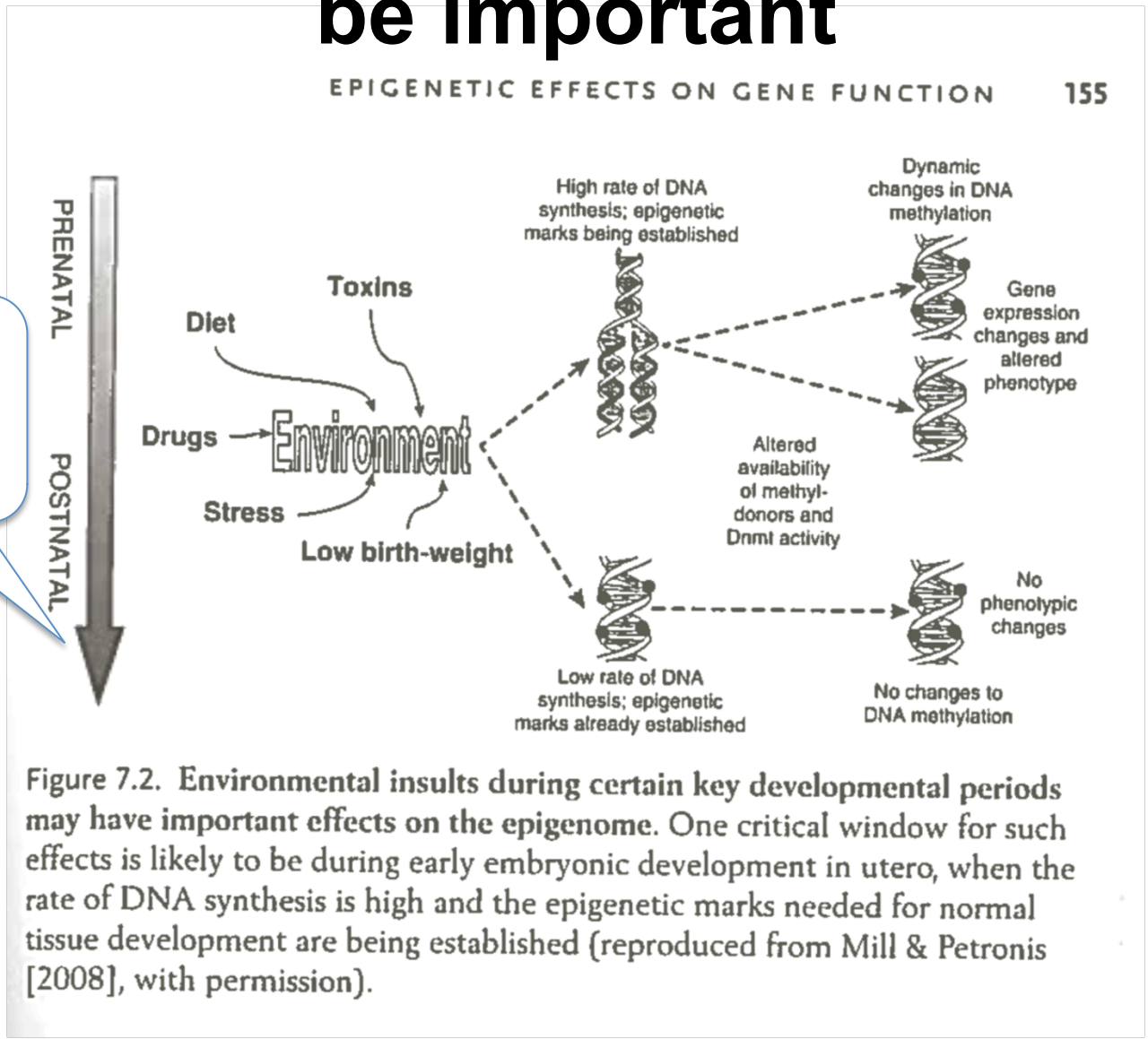


Figure 7.2. **Environmental insults during certain key developmental periods may have important effects on the epigenome.** One critical window for such effects is likely to be during early embryonic development in utero, when the rate of DNA synthesis is high and the epigenetic marks needed for normal tissue development are being established (reproduced from Mill & Petronis [2008], with permission).

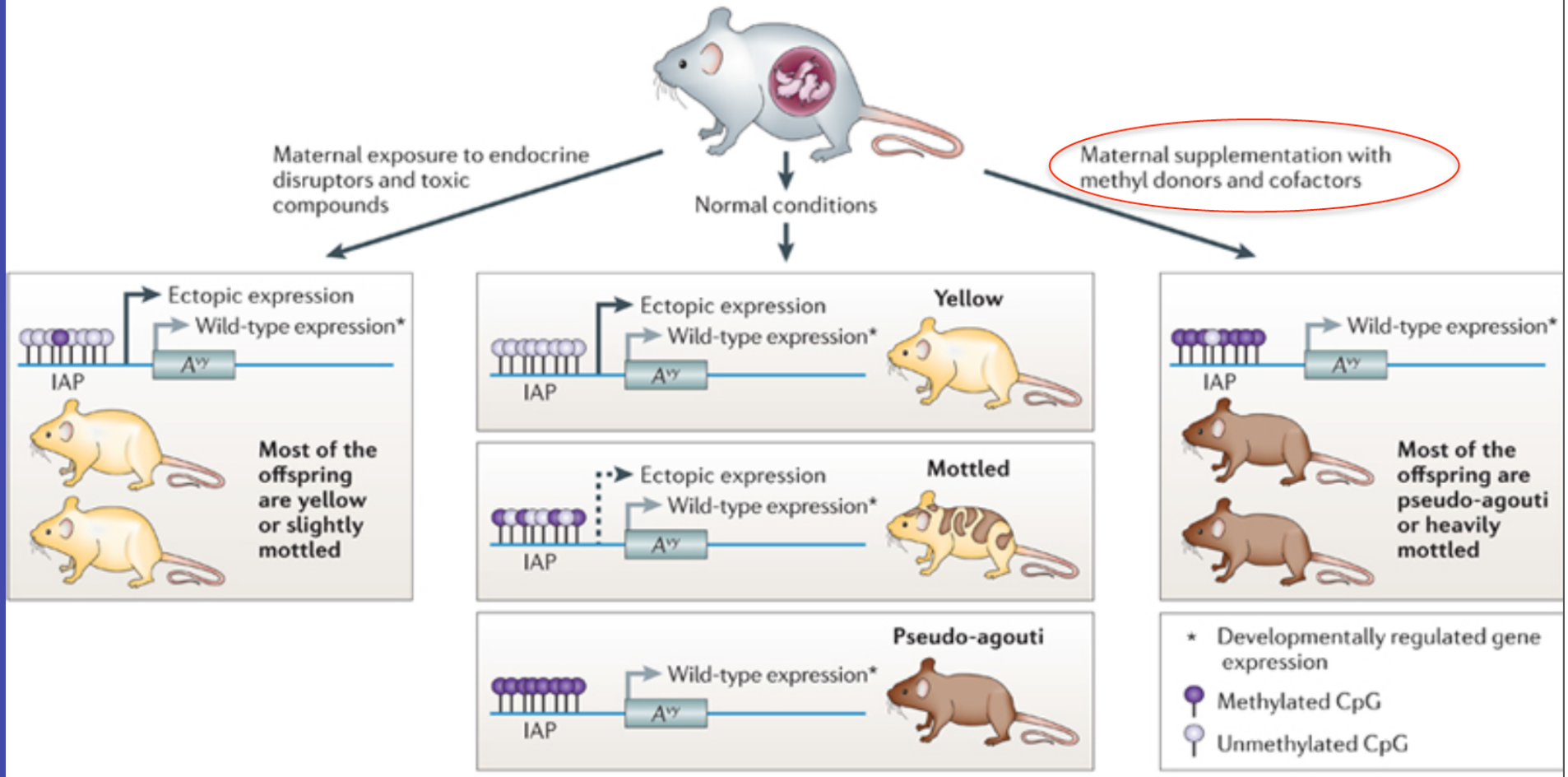
Maternal diet in pregnancy and offspring phenotype: Agouti Mouse experiment

- Agouti mice carry a gene ‘agouti variable yellow’ (Avy) that determines coat color and health
  - Under normal conditions methylation of the gene is highly variable
  - More methylated the gene, the darker the coat and healthier they are
- Pregnant dams fed a diet that differed in methyl donor content (e.g., folic acid, B12)
- Their pups showed differential methylation at a metastable allele (‘IAP’) upstream of the agouti gene Avy
- Associated levels of expression of the agouti gene resulted in observable phenotypic differences
  - Coat color
  - Obesity

Epigenetics and Nutrition

Maternal Genistein Alters Coat Color and Protects A^{vy} Mouse Offspring from Obesity by Modifying the Fetal Epigenome

Dana C. Dolinoy,[1,2,3] Jennifer R. Weidman,[1,2] Robert A. Waterland,[4,5] and Randy L. Jirtle[1,2,3]

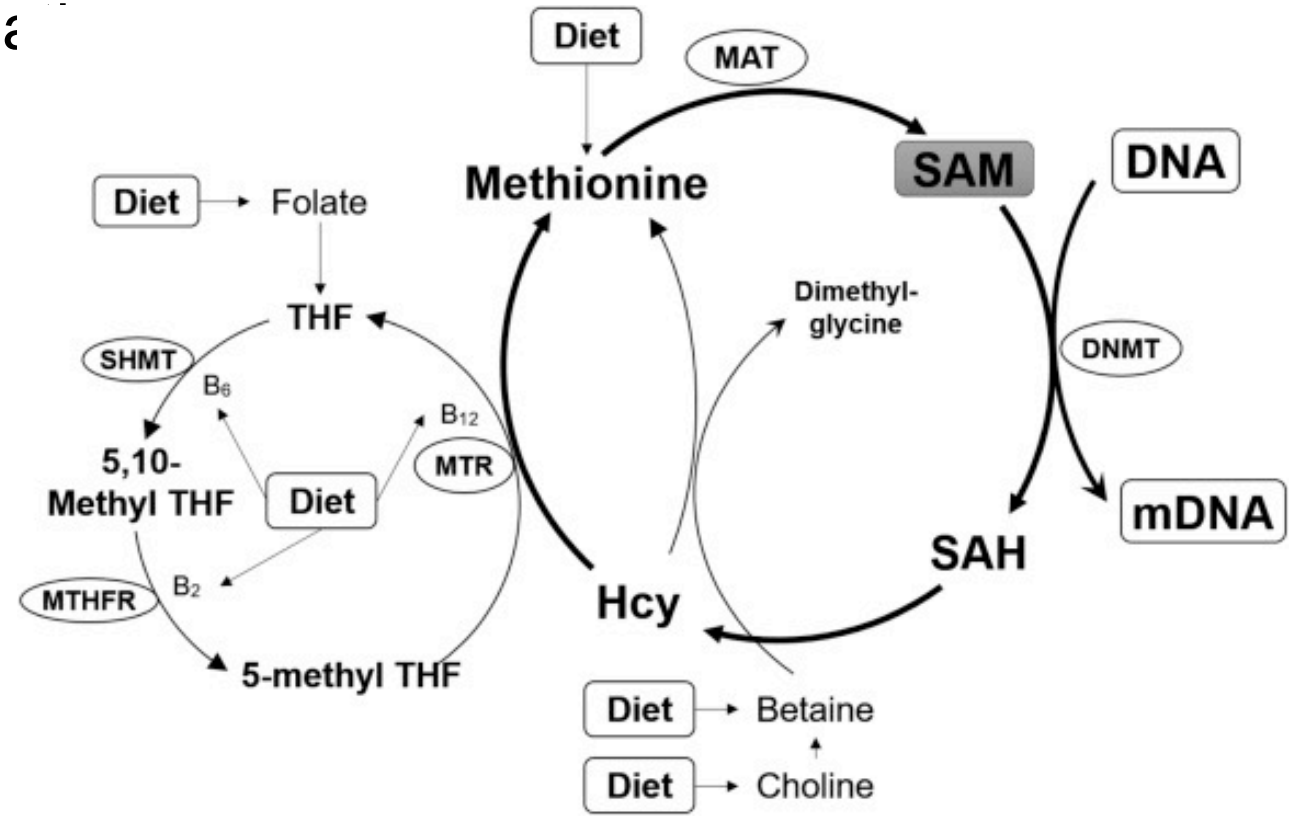


Epigenetic patterns in agouti locus modified by maternal diet

Nature Reviews | Genetics

Nutrition and epigenetic machinery

Deficiencies in key methyl donors (choline, folate, betaine) may disrupt the metabolic pathways responsible for DNA methyla



The Dutch winter famine study

Natural experiment

- Nov 1944 - May 1945: Famine due to harsh early winter and Nazi blockades
  - 4.5 million people affected [20,000 died]
  - 667 kcal a day rations (recommended 1,800-2,200)

- Exposure to famine associated with later health outcomes
  - Lower birthweight; increased adult blood pressure; obesity; risk of schizophrenia

Persistent epigenetic differences associated with prenatal exposure to famine in humans

Bastiaan T. Heijmans[a,1,2], Elmar W. Tobi[a,2], Aryeh D. Stein[b], Hein Putter[c], Gerard J. Blauw[d], Ezra S. Susser[e,f], P. Eline Slagboom[a], and L. H. Lumey[e,1]

Departments of [a]Molecular Epidemiology, [c]Medical Statistics, and [d]Gerontology and Geriatrics, Leiden University Medical Center, Leiden, The Netherlands; [b]Hubert Department of Global Health, Rollins School of Public Health, Emory University Atlanta, GA 30322; [e]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032; and [f]New York State Psychiatric Institute, New York, NY 10032

Edited by Charles R. Cantor, Sequenom Inc., San Diego, CA, and approved September 17, 2008 (received for review July 7, 2008)

Extensive epidemiologic studies have suggested that adult disease risk is associated with adverse environmental conditions early in development. Although the mechanisms behind these relationships are unclear, an involvement of epigenetic dysregulation has been hypothesized. Here we show that individuals who were prenatally exposed to famine during the Dutch Hunger Winter in 1944–45 had, 6 decades later, less DNA methylation of the imprinted *IGF2* gene compared with their unexposed, same-sex siblings. The association was specific for periconceptional exposure, reinforcing that very early mammalian development is a crucial period for establishing and maintaining epigenetic marks. These data are the first to contribute empirical support for the hypothesis that early-life environmental conditions can cause epigenetic changes in humans that persist throughout life.

a normally distributed quantitative trait that is largely determined by genetic factors in both adolescence and middle age, indicating that the methylation mark is stable up to middle age. Thus, if affected by environmental conditions early in human development, altered *IGF2* DMR methylation may be detected many years later.

Here we used our ongoing Hunger Winter Families Study (8) to investigate whether prenatal exposure to famine is associated with persistent differences in methylation of the *IGF2* DMR. Our primary focus was exposure during periconception, thus ensuring that the exposure was present during the very early stages of development that are critical in epigenetic programming. To further investigate the role of timing, we also studied individuals who were exposed late in gestation.

Mechanistic links for nutritional exposures in early life and disease risk?

- Found differences (<5.2%) in level of DNA methylation of gene IGF2 in prenatal exposed vs unexposed siblings
  - Note: measured 60 years after exposure
- Specific to early gestation (<10 weeks).
  - Epigenetic programming seems particularly susceptible in early pregnancy?
- Note: links maternal exposure to infant epigenetic patterns only

Table 1. *IGF2* DMR methylation among individuals periconceptionally exposed to famine and their unexposed, same-sex siblings

IGF2 DMR methylation	Mean methylation fraction (SD)				Relative change exposed	Difference in SDs	*P*
	Exposed ($n = 60$)		Controls ($n = 60$)				
Average	0.488	(0.047)	0.515	(0.055)	−5.2%	−0.48	5.9×10^{-5}
CpG 1	0.436	(0.037)	0.470	(0.041)	−6.9%	−0.78	1.5×10^{-4}
CpG 2 and 3	0.451	(0.033)	0.473	(0.055)	−4.7%	−0.41	8.1×10^{-3}
CpG 4	0.577	(0.114)	0.591	(0.112)	−2.3%	−0.12	.41
CpG 5	0.491	(0.061)	0.529	(0.068)	−7.2%	−0.56	1.4×10^{-3}

P values were obtained using a linear mixed model and adjusted for age.

The Dutch winter famine study

Natural experiment

- Nov 1944 - May 1945: Famine due to harsh early winter and Nazi blockades
  - 4.5 million people affected [20,000 died]
  - 667 kcal a day rations (recommended 1,800-2,200)
- Exposure to famine associated with later health outcomes
  - Lower birthweight; increased adult blood pressure; obesity; risk of schizophrenia
- **Are epigenetic factors the mechanism for risk exposure?**

Persistent epigenetic differences associated with prenatal exposure to famine in humans

Bastiaan T. Heijmans[a,1,2], Elmar W. Tobi[a,2], Aryeh D. Stein[b], Hein Putter[c], Gerard J. Blauw[d], Ezra S. Susser[e,f], P. Eline Slagboom[a], and L. H. Lumey[e,1]

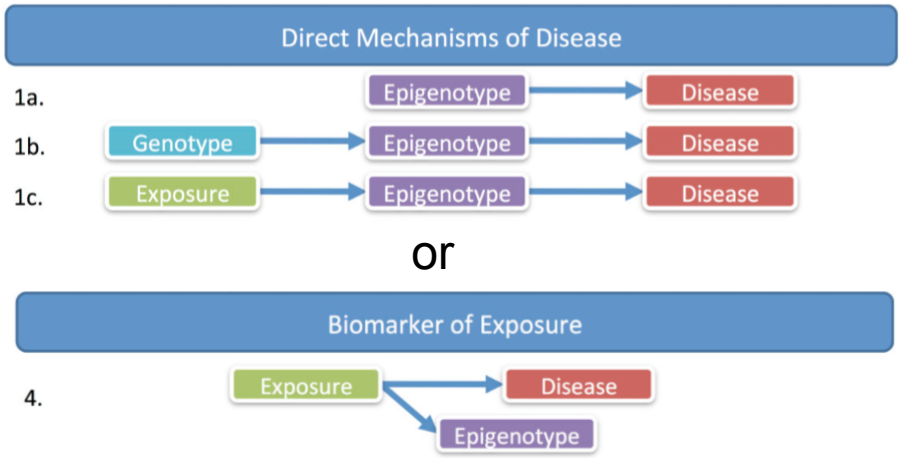
Departments of [a]Molecular Epidemiology, [c]Medical Statistics, and [d]Gerontology and Geriatrics, Leiden University Medical Center, Leiden, The Netherlands; [b]Hubert Department of Global Health, Rollins School of Public Health, Emory University Atlanta, GA 30322; [e]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032; and [f]New York State Psychiatric Institute, New York, NY 10032

Edited by Charles R. Cantor, Sequenom Inc., San Diego, CA, and approved September 17, 2008 (received for review July 7, 2008)

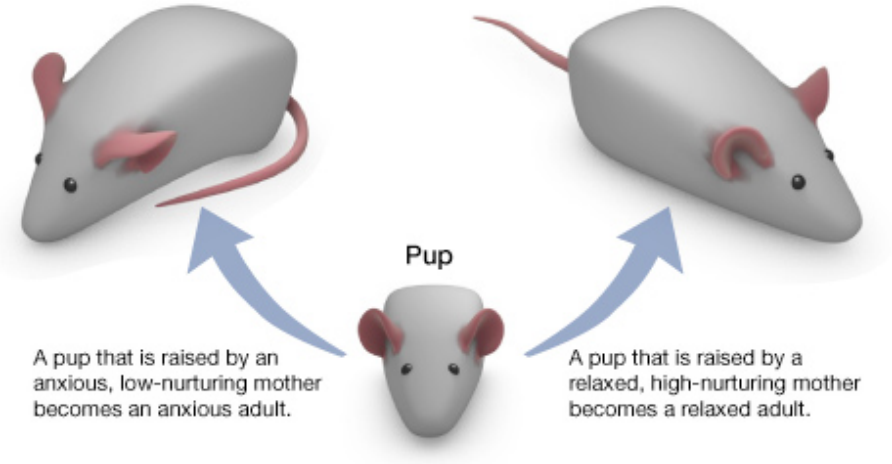
Extensive epidemiologic studies have suggested that adult disease risk is associated with adverse environmental conditions early in development. Although the mechanisms behind these relationships are unclear, an involvement of epigenetic dysregulation has been hypothesized. Here we show that individuals who were prenatally exposed to famine during the Dutch Hunger Winter in 1944–45 had, 6 decades later, less DNA methylation of the imprinted *IGF2* gene compared with their unexposed, same-sex siblings. The association was specific for periconceptional exposure, reinforcing that very early mammalian development is a crucial period for establishing and maintaining epigenetic marks. These data are the first to contribute empirical support for the hypothesis that early-life environmental conditions can cause epigenetic changes in humans that persist throughout life.

a normally distributed quantitative trait that is largely determined by genetic factors in both adolescence and middle age, indicating that the methylation mark is stable up to middle age. Thus, if affected by environmental conditions early in human development, altered *IGF2* DMR methylation may be detected many years later.

Here we used our ongoing Hunger Winter Families Study (8) to investigate whether prenatal exposure to famine is associated with persistent differences in methylation of the *IGF2* DMR. Our primary focus was exposure during periconception, thus ensuring that the exposure was present during the very early stages of development that are critical in epigenetic programming. To further investigate the role of timing, we also studied individuals who were exposed late in gestation.



Epigenetics and maternal care



Early maternal care influences DNA methylation of the glucocorticoid receptor (GR) gene in the hypothalamus of the pup and results in persistent behavioral responses to stress

Low care behavior

– Results in increased methylation of GR promoter region in the hippocampus, decreased expression of GR, heightened HPA stress response in pup

http://learngenetics.utah.edu/content/epigenetics/rats/; Meany, M.J. Annu Rev NeuroSci (2001);

Are similar alterations seen in humans?

- Followed-up in humans
  - McGowan *et al* Nat Neurosci (2009)

Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse

Patrick O McGowan[1,2], Aya Sasaki[1,2], Ana C D'Alessio[3], Sergiy Dymov[3], Benoit Labonté[1,4], Moshe Szyf[2,3], Gustavo Turecki[1,4] & Michael J Meaney[1,2,5]

Examined postmortem brain tissue (hippocampal) in suicide vitcims.

Children with a history of childhood abuse were associated with increased methylation of the GR promoter and decreased GR expression

Are similar alterations seen in humans?

- Followed-up in humans
  - McGowan *et al* Nat Neurosci (2009)

Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse

Patrick O McGowan[1,2], Aya Sasaki[1,2], Ana C D'Alessio[3], Sergiy Dymov[3], Benoit Labonté[1,4], Moshe Szyf[2,3], Gustavo Turecki[1,4] & Michael J Meaney[1,2,5]

Psychological Medicine (2012), **00**, 1–11.
doi:10.1017/S0033291712002784

ORIGINAL ARTICLE

Increased serotonin transporter gene (*SERT*) DNA methylation is associated with bullying victimization and blunted cortisol response to stress in childhood: a longitudinal study of discordant monozygotic twins

I. Ouellet-Morin, C. C. Y. Wong, A. Danese, C.M. Pariante, A. S. Papadopoulos, J. Mill and L. Arseneault

Maternal smoking and DNA methylation changes present at birth

450K Epigenome-Wide Scan Identifies Differential DNA Methylation in Newborns Related to Maternal Smoking during Pregnancy

Bonnie R. Joubert,[1] Siri E. Håberg,[2] Roy M. Nilsen,[3] Xuting Wang,[1] Stein E. Vollset,[2,4] Susan K. Murphy,[5] Zhiqing Huang,[5] Cathrine Hoyo,[5] Øivind Midttun,[6] Lea A. Cupul-Uicab,[1] Per M. Ueland,[4] Michael C. Wu,[7] Wenche Nystad,[2] Douglas A. Bell,[1] Shyamal D. Peddada,[1] and Stephanie J. London[1]

- Correlated maternal plasma cotinine (biomarker of smoking) measured during pregnancy to the DNA methylome (473,844 CpGs) in 1,062 newborn cord blood samples
- In utero exposure to maternal smoking linked to DNA methylation changes at 26 CpG sites (in 10 genes; **p-value < 1.06 × 10–7**)
- Possible mechanism for later adverse health outcomes in children of smokers?

ISSUES AND CONSIDERATIONS WHEN THINKING (AND READING ABOUT) ABOUT EPIGENETIC RESEARCH

Characterizing the epigenome is an active area of research

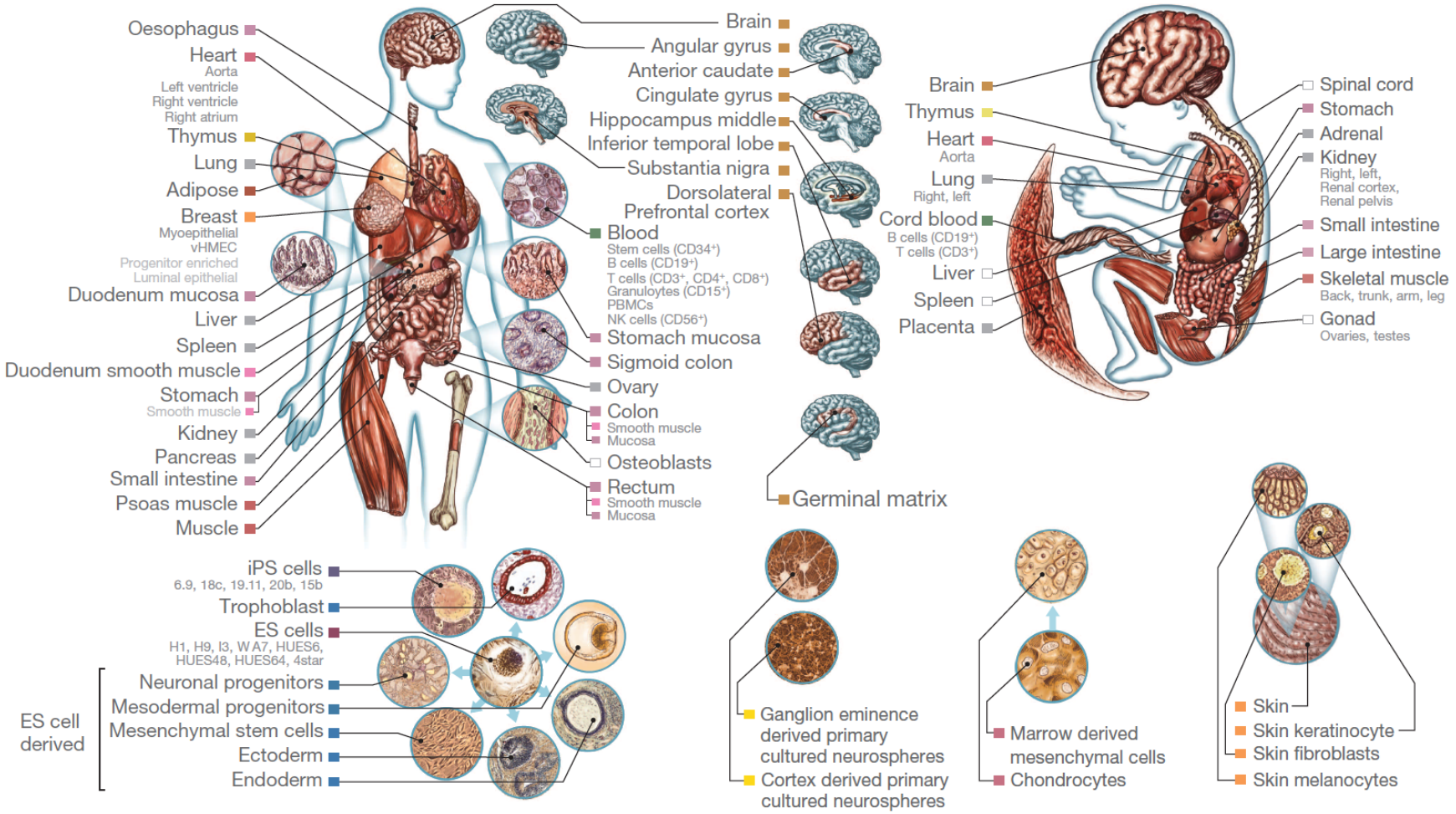
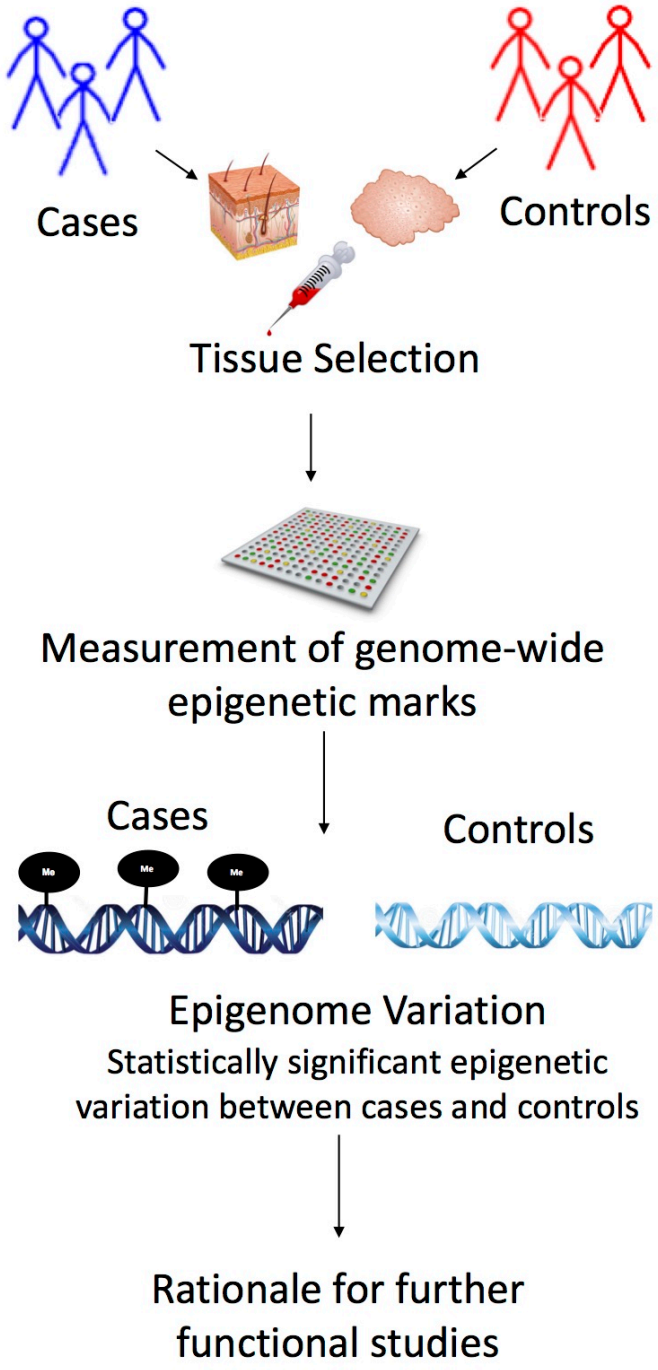


Figure 1 | Tissues and cell types profiled in the Roadmap Epigenomics Consortium. Primary tissues and cell types representative of all major lineages in the human body were profiled, including multiple brain, heart, muscle, gastrointestinal tract, adipose, skin and reproductive samples, as well as immune lineages, ES cells and iPS cells, and differentiated lineages derived from ES cells. Box colours match groups shown in Fig. 2b. Epigenome identifiers (EIDs, Fig. 2c) for each sample are shown in Extended Data Fig. 1.

Human epigenomics is a relatively new branch of research

- Our basic understanding of what constitutes a ’normal’ epigenome and transcriptome is still in its infancy
- DNA methylation is the most studied; our understanding of histone modifications, ncRNA and chromatin structure is incomplete
- Extent and nature of cross-talk is not fully understood
- Yet to characterize
  - Epigenetic stability
  - Developmental plasticity of epigenetic marks
  - Population-level epigenetic variation

Epigenome-wide association studies: eWAS



Some general themes emerge

Differences are apparent and often novel

But….

- Sample sizes are frequently (too) small
- Effect sizes are small
- Loci identified are frequently novel
- Most studies fail to include a replication sample
- Comparisons across studies is difficult (methods)
- Environmental phenotyping often crude
- Consideration of mechanisms/pathways not given
- Reliance on peripheral tissues

Estimation of a significance threshold for Epigenome-Wide Association Studies

May 30, 2017

Ayden Saffari[1,2,3], Matt J. Silver[2], Patrizia Zavattari[4], Loredana Moi[4], Amedeo Columbano[4], Emma L. Meaburn[3], Frank Dudbridge[1,5]

[1]Department of Non-communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, United Kingdom., [2]MRC Unit The Gambia and MRC International Nutrition Group, London School of Hygiene and Tropical Medicine, London, [3]Department of Psychological Sciences, Birkbeck, University of London, London, UK, [4]Dept. Biomedical Sciences, University of Cagliari, Italy

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Sample sizes are frequently (too) small

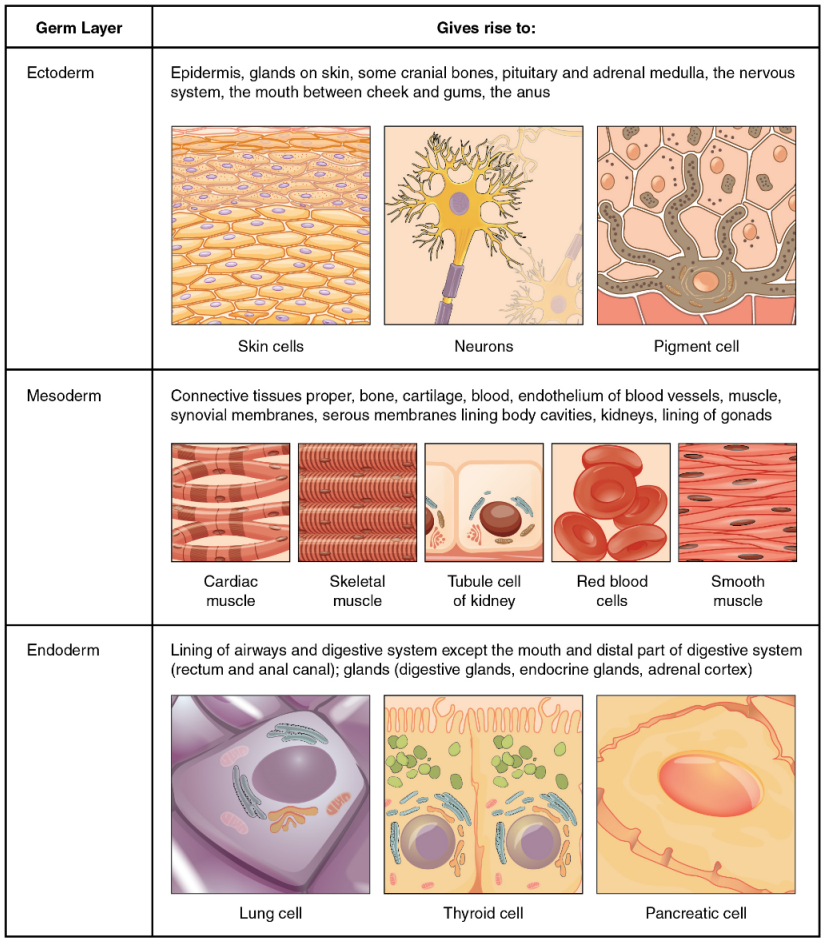
Abstract

Epigenome-wide association studies (EWAS) are designed to characterise population-level epigenetic differences across the genome and link them to disease. Most commonly, they assess DNA-methylation status at CpG sites, using platforms such as the Illumina 450k array which profile a subset of CpGs genome-wide. An important challenge in the context of EWAS is determining a significance threshold for declaring a CpG site as differentially methylated, taking multiple testing into account. We used a permutation method to estimate a significance threshold specifically for the 450k array and a simulation extrapolation approach to estimate a genome-wide threshold. These methods were applied to five different EWAS datasets derived from a variety of populations and tissue types. We obtained an estimate of $\alpha = 2.4 \times 10^{-7}$ for the 450k array, and a genome-wide estimate of $\alpha = 3.6 \times 10^{-8}$. We further demonstrate the importance of these results by showing that previously recommended sample sizes for EWAS should be adjusted upwards, requiring samples between ∼10% and ∼20% larger in order to maintain type-1 errors at the desired level.

Keywords: CpG, DNA methylation, epigenetic epidemiology, EWAS, FWER, GWAS, permutation, re-sampling, simulation extrapolation

The tissue issue (in Psychiatry and Psychology)

- Peripheral tissues such as saliva and blood (a heterogeneous tissue) are the most frequently studied
- Are peripheral tissue differences reflective of brain differences?
- Cross-tissues comparisons in the same sample are needed (but difficult to obtain)
- Arguable that saliva is a better choice than blood as it is derived from the ectoderm



Embryonic origin of tissues

The epigenome and transcriptome is not static

- Establishing causality is often difficult
- Epigenetic differences might arise prior to illness, and be secondary to disease processes and medications?
- Prospective longitudinal samples (with well characterized phenotypes and environmental exposures) are required to firmly establish causal effect – not feasible on a massive scale?

Thanks for listening

- Questions?