HARVARD
SCHOOL OF PUBLIC HEALTH
Powerful ideas for a healthier world

Department of Environmental Health

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Laboratory of Environmental Epigenetics

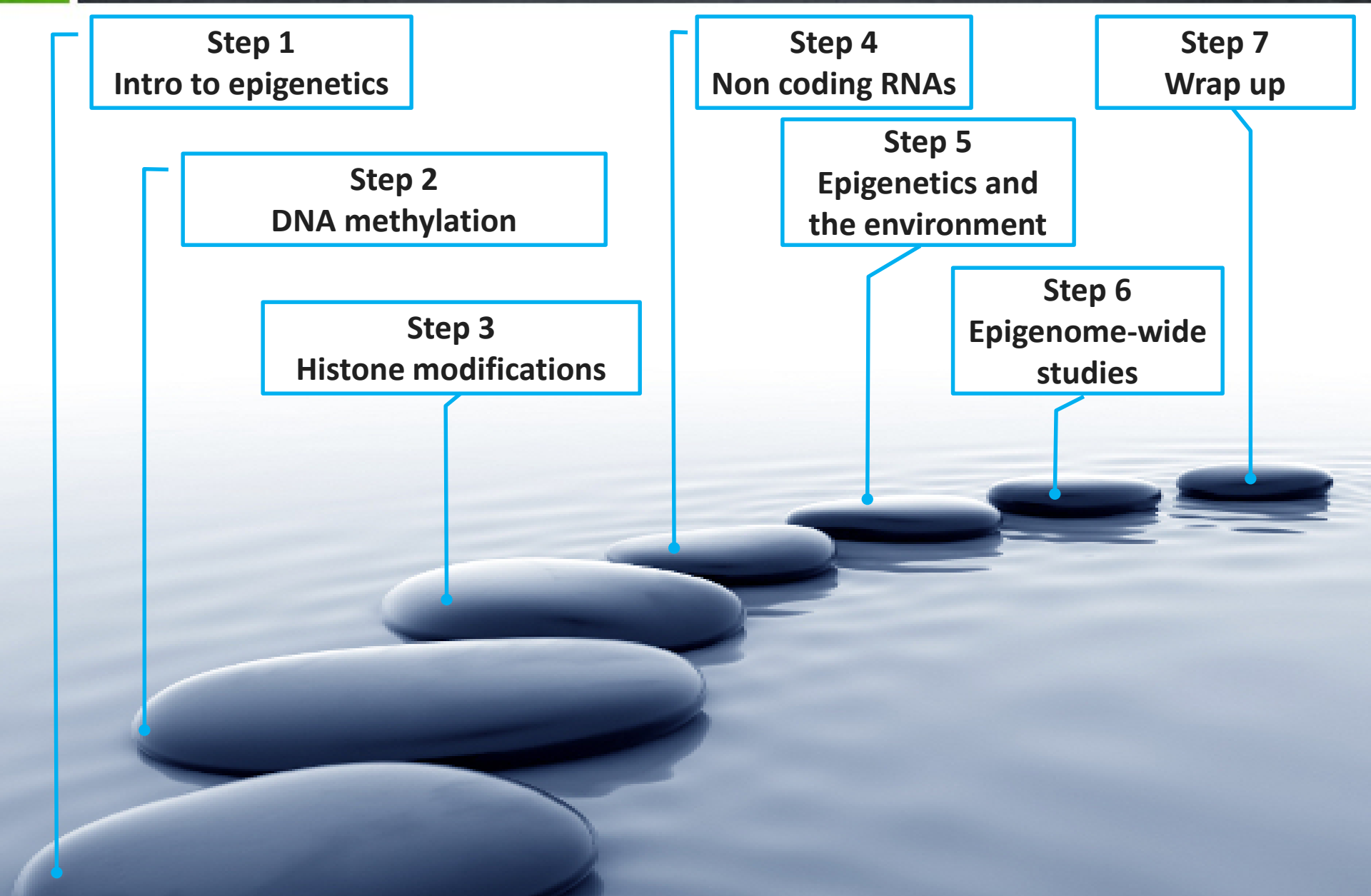
Epigenetics and Environmental Health

A Step-by-Step Tutorial

Objective of my presentation

- To review:
  - General epigenetic concepts
  - Why we may be interested in epigenetics
  - Environmental influences and epigenetics

Step by step

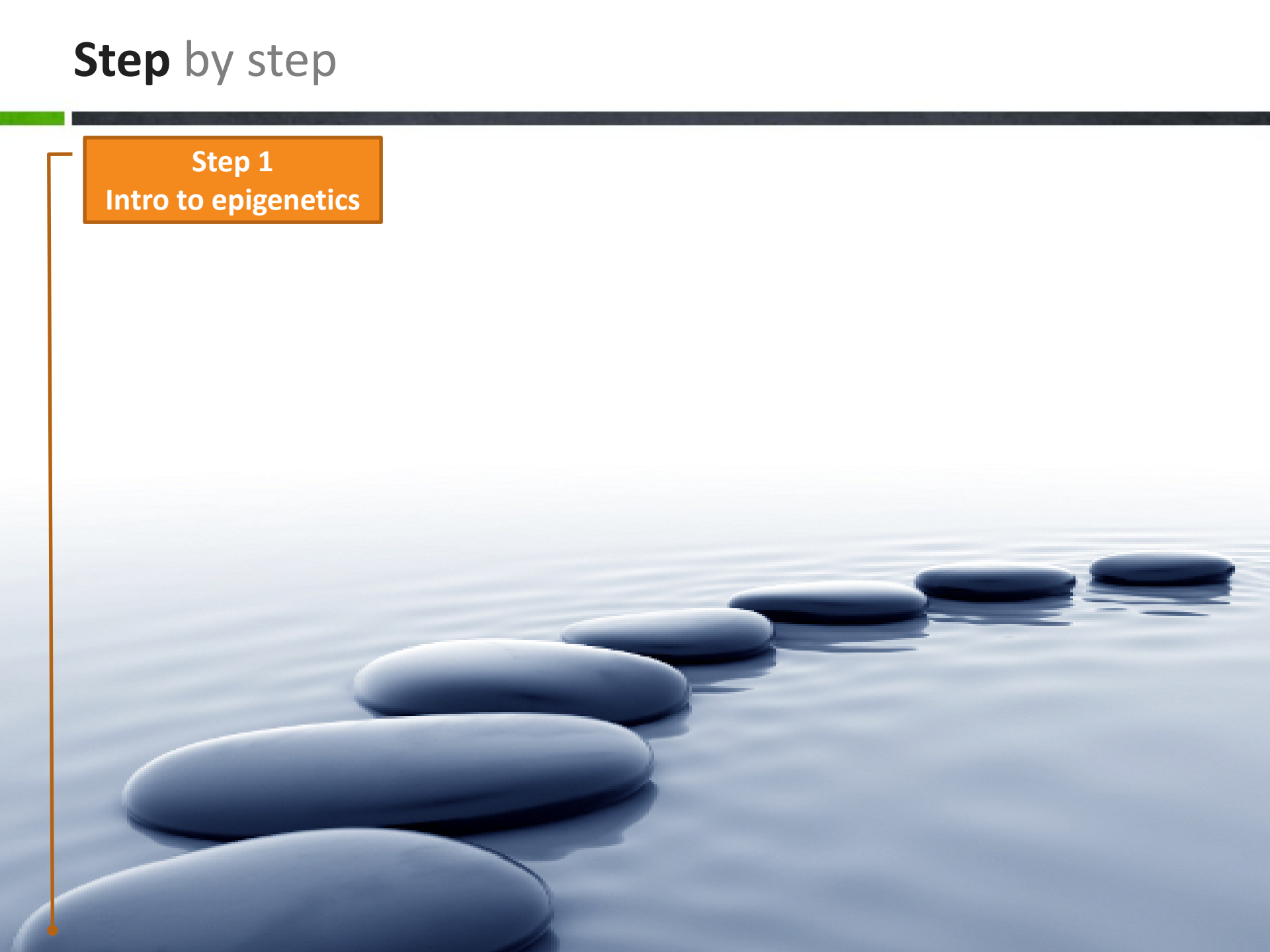

Step 1
Intro to epigenetics
Step 2
DNA methylation
Step 3
Histone modifications
Step 4
Non coding RNAs
Step 5
Epigenetics and the environment
Step 6
Epigenome-wide studies
Step 7
Wrap up

Epigenetics glossary

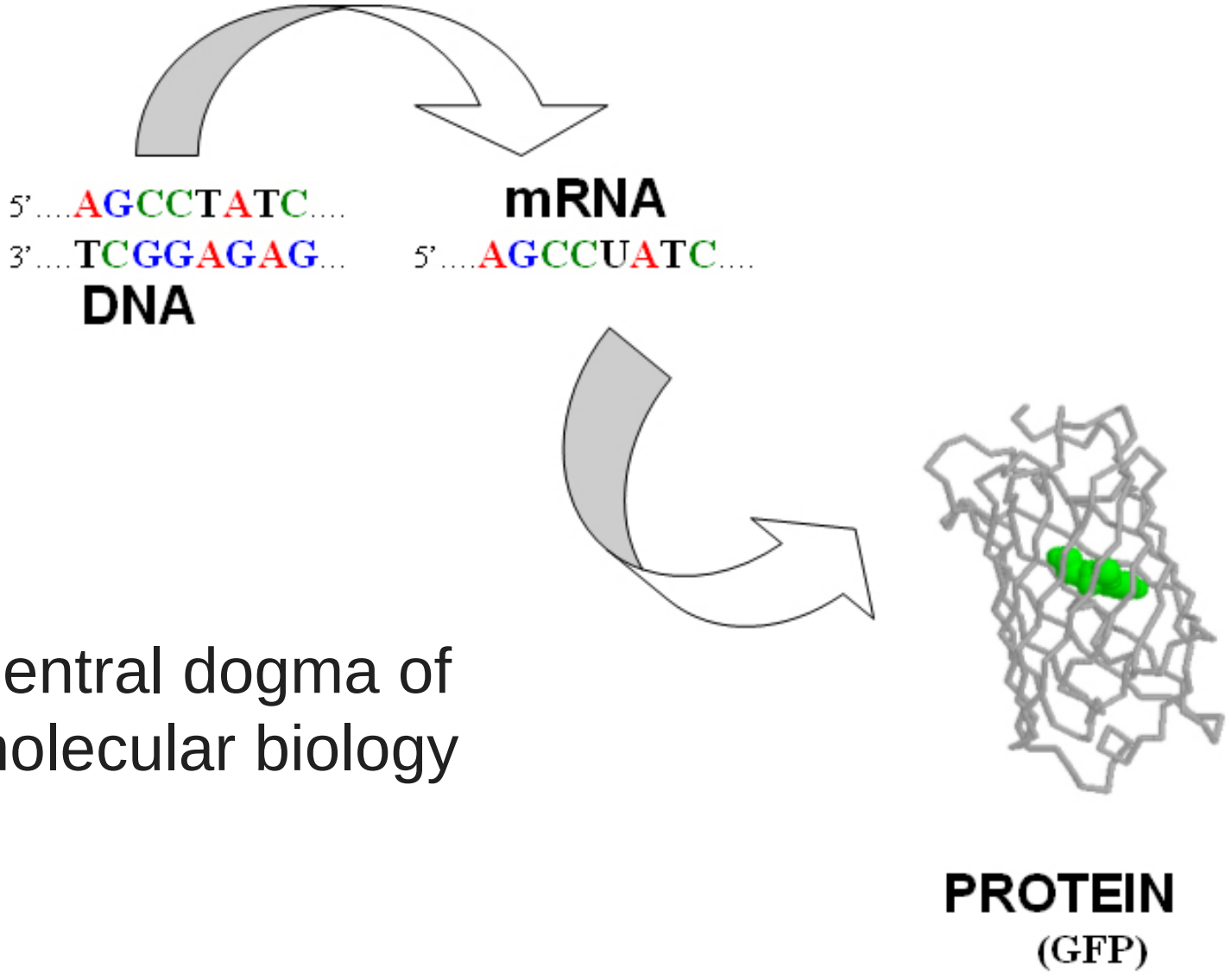
- Glossary enclosed with the seminar invitation
- Compiled from online sources
- General and technical definitions:
  - Epigenetics and the epigenome
  - General concepts
  - Epigenetic mechanisms
    - DNA methylation
    - Histone Modifications
    - Non coding RNAs

Step by step

Step 1
Intro to epigenetics



Gene expression



Central dogma of molecular biology

Greek "Epi"

Used as a prefix

- Above
- Over
- On
- Upon
- Besides
- In addition to
- Toward
- Among

Greek "Epi"

Prometheus

One of the Titans.
He stole fire from the gods and gave it to mankind, and was severely punished for it

Epimetheus

Prometheus' brother
He fell in love and married Pandora in spite of the warnings of his more intelligent brother

Greek "Epi"

Prometheus

PRO-metheus

He who thinks **in advance**

Epimetheus

EPI-metheus

He who thinks **afterwards**

Epigenetics intervenes afterwards,
i.e. on the DNA sequence, without modifying it

Epigenetics

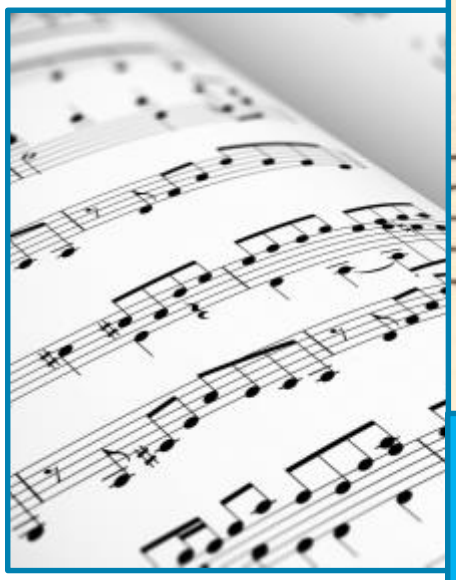
- Changes in gene expression that:
  -do not depend on the DNA sequence
  -can be stable
    - Through cell division (mitotically stable)
    - Transgenerational inheritance (limited evidence in humans)
  – may persist even in the absence of the conditions that established them (biological memory)

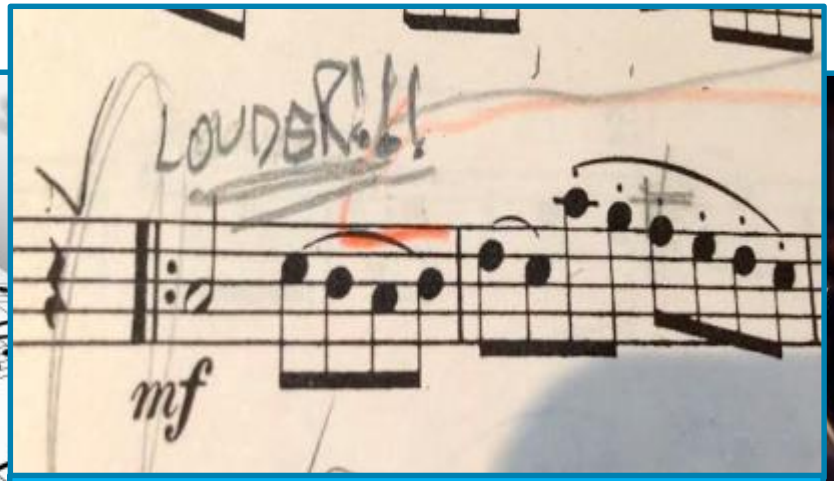
(adapted from Richards, Nat Gen 2006)

A symphonic example

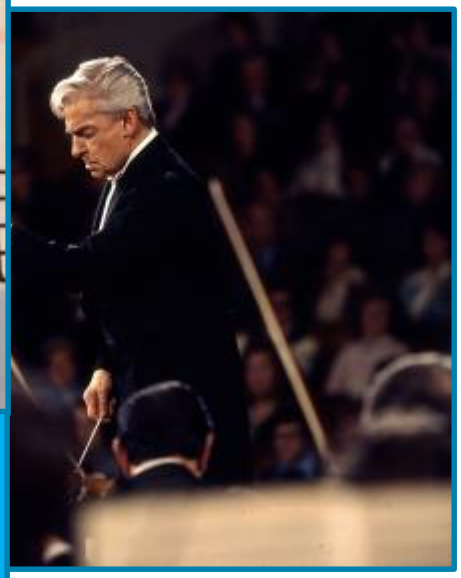
DNA

Phenotype

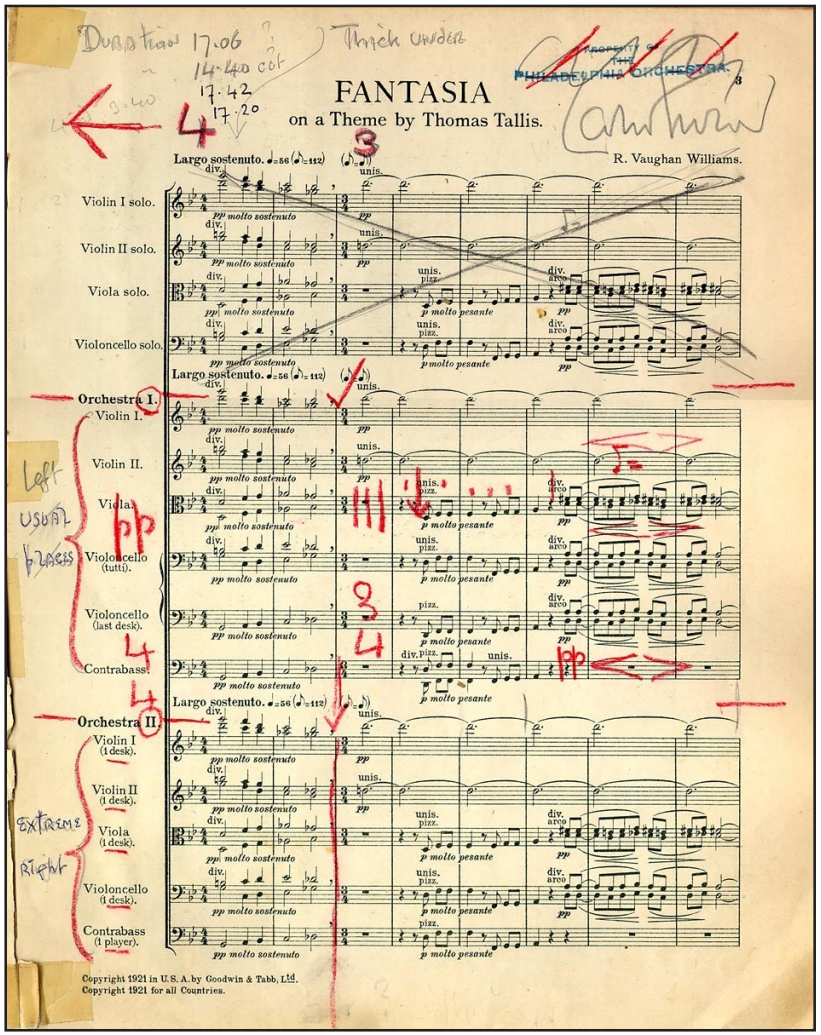




Epigenetics

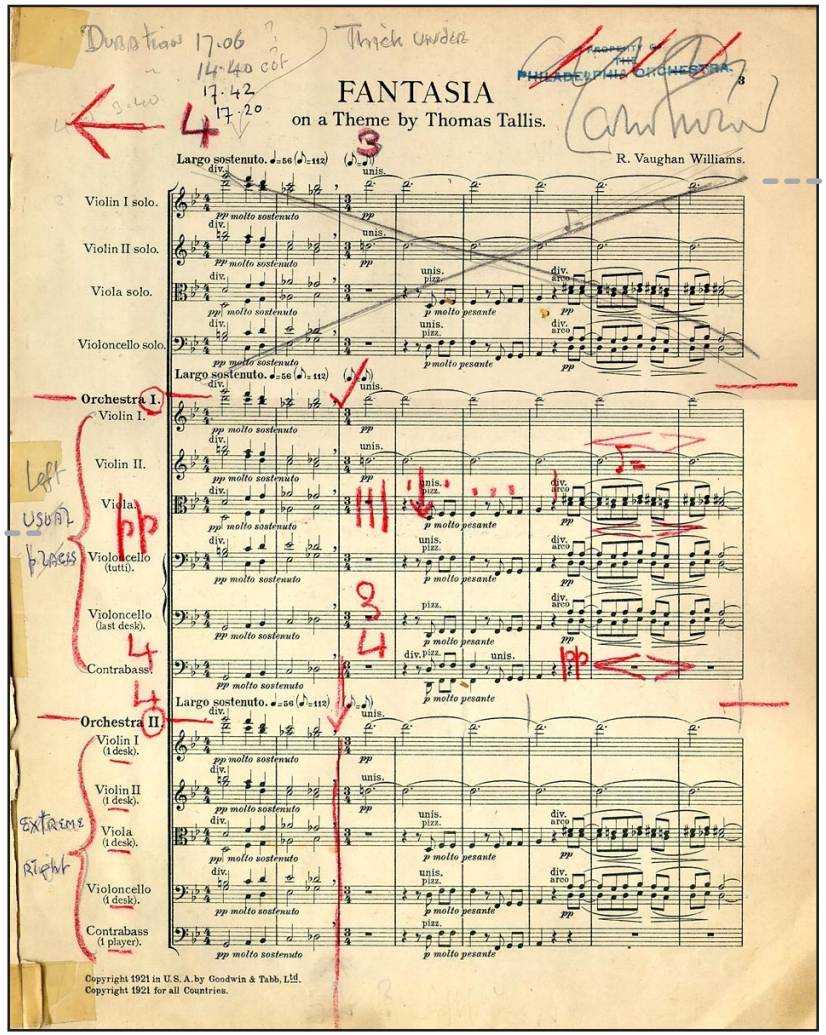


Epigenetics & Music Use the Same Markings



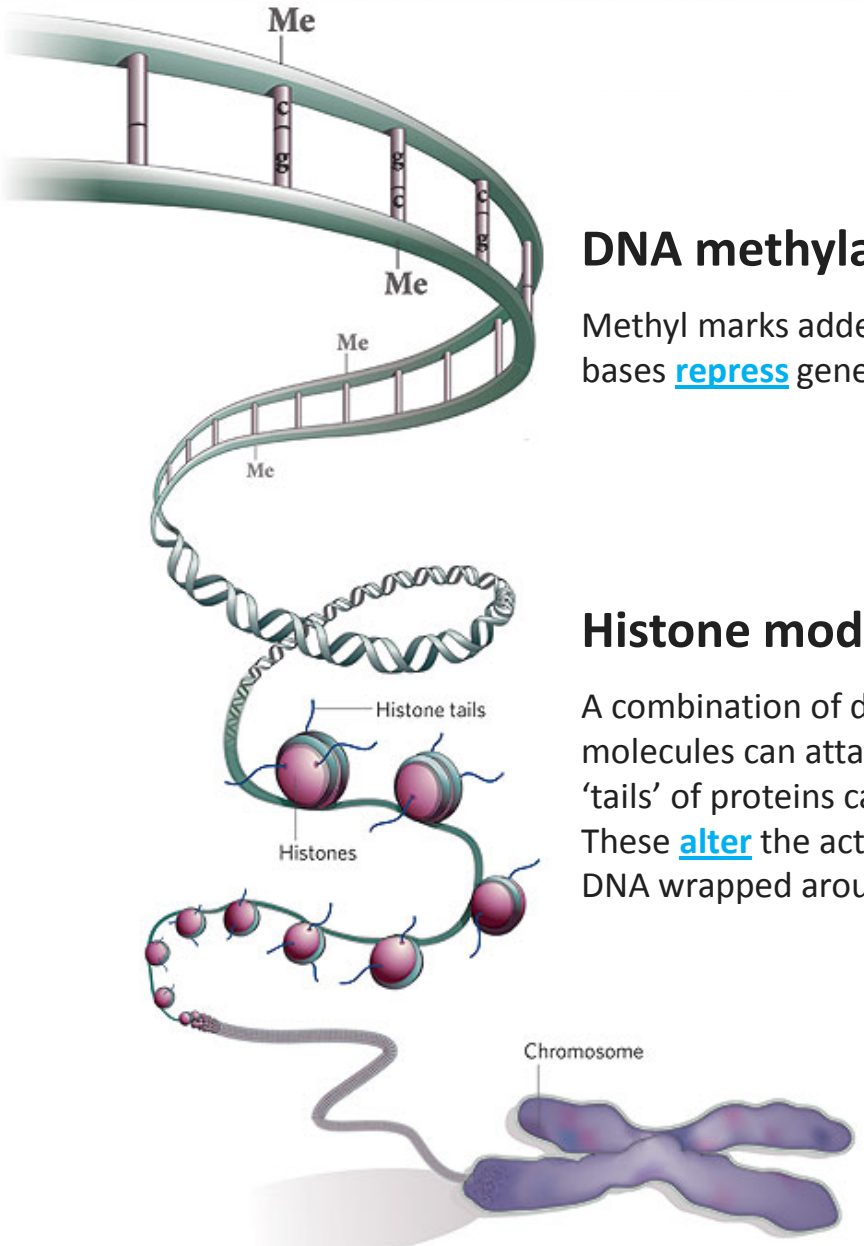
Epigenetics & Music Use the Same Markings

markings in **ink** (permanent)



pencil markings (can be erased)

Epigenetic markings

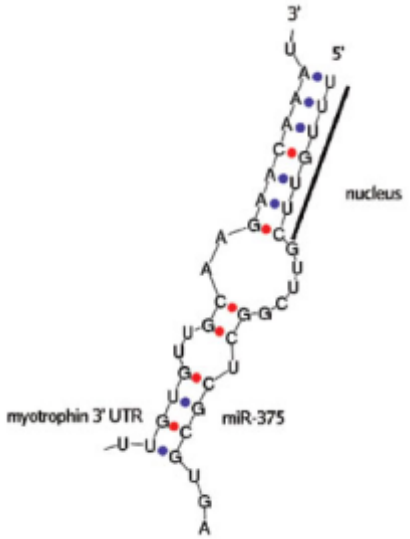


DNA methylation

Methyl marks added to certain DNA bases **repress** gene transcription

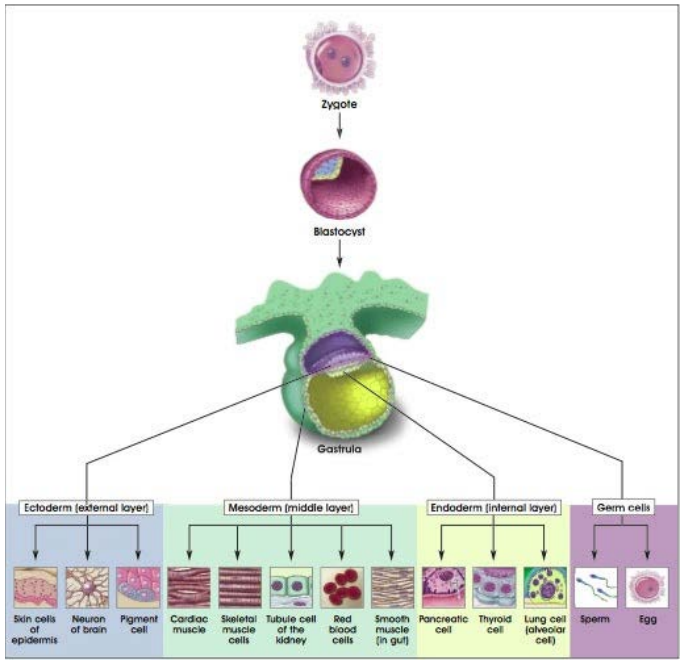
Histone modifications

A combination of different molecules can attach to the 'tails' of proteins called histones. These **alter** the activity of the DNA wrapped around them



microRNAs

Small non-coding RNAs that **block translation** of messenger RNAs into proteins



Epigenetics contribute to
tissue differentiation

Tissue specificity

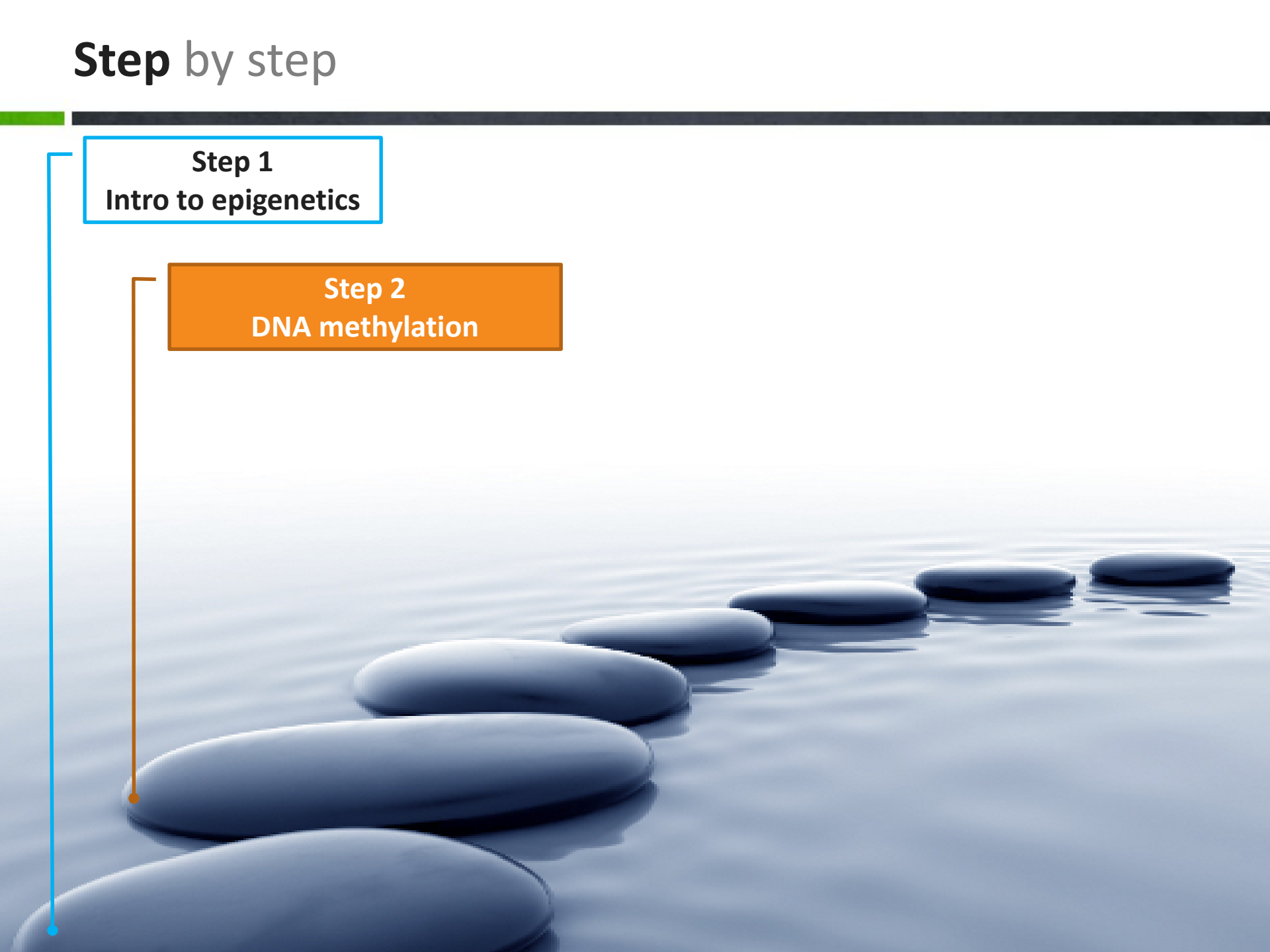
Epigenetic markings are **Tissue Specific.**

Potentially **each tissue or cell type** has a specific methylation profile.

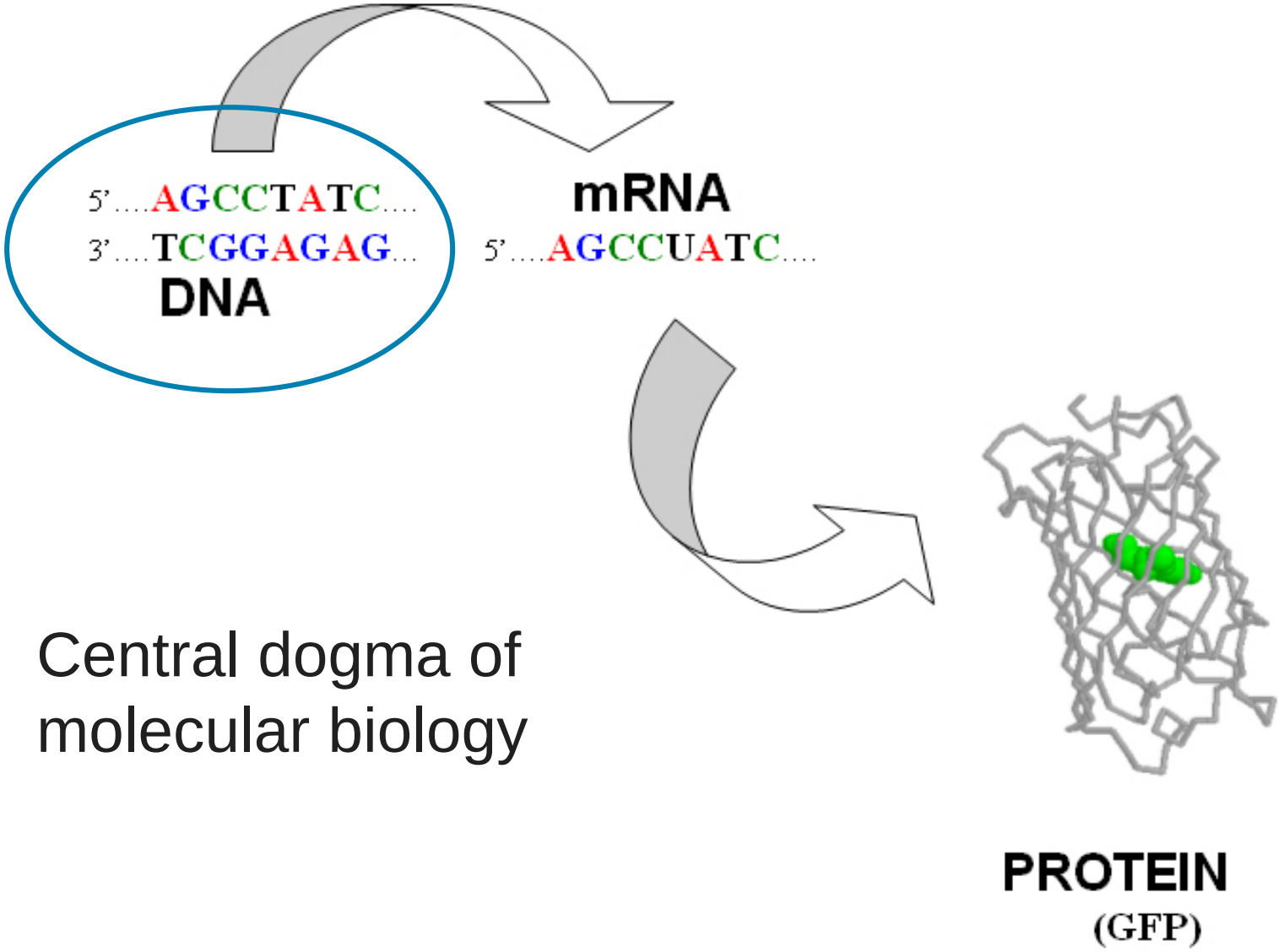
Step by step

Step 1
Intro to epigenetics

Step 2
DNA methylation



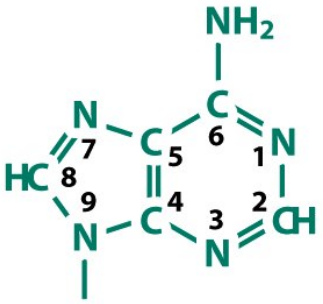
Gene expression



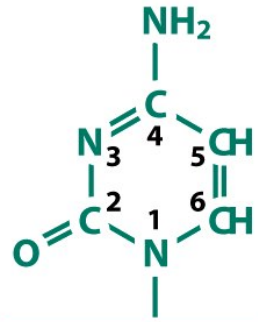
Central dogma of molecular biology

DNA methylation

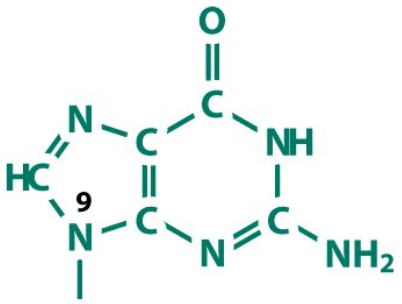
The four bases in DNA



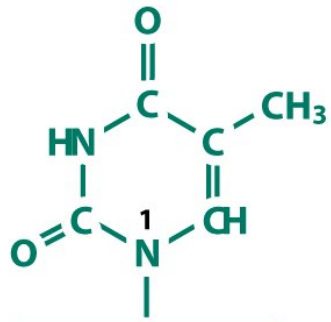
Adenine (A)



Cytosine (C)



Guanine (G)



Thymine (T)

Uracil

RNA

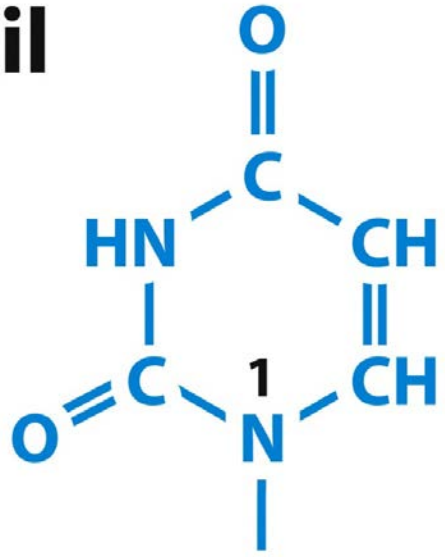


Figure 1.4b *Genomes 3* (© Garland Science 2007)

DNA methylation biochemistry

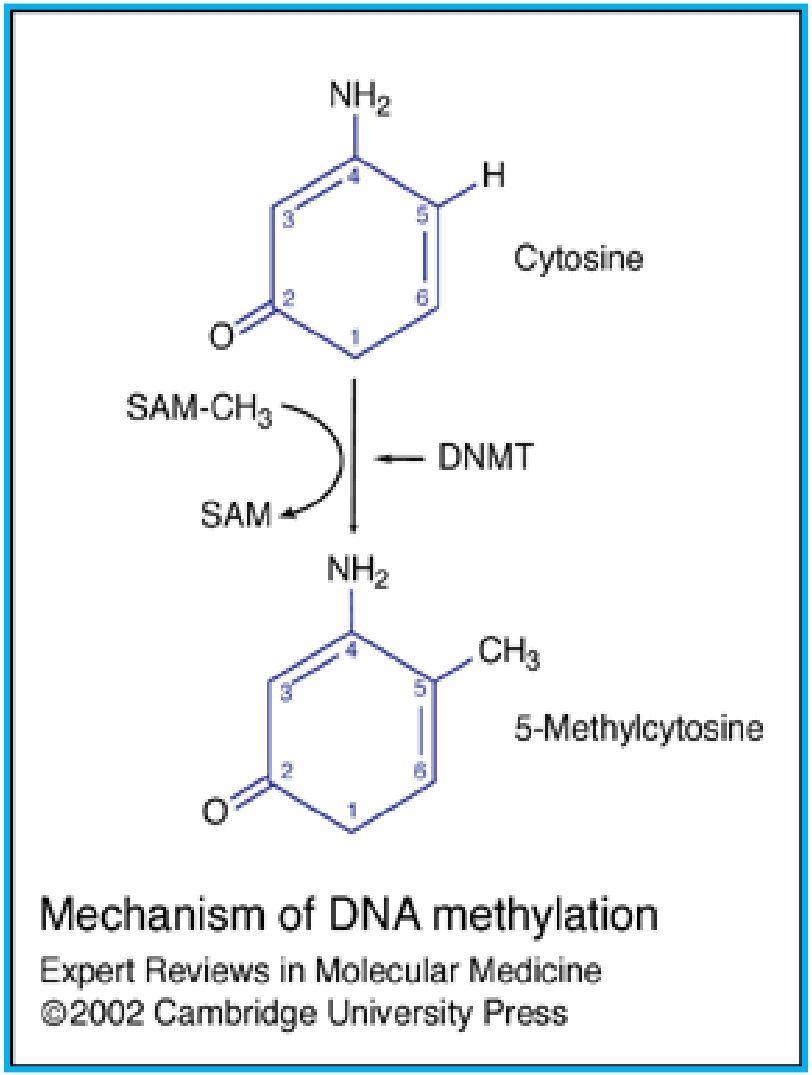
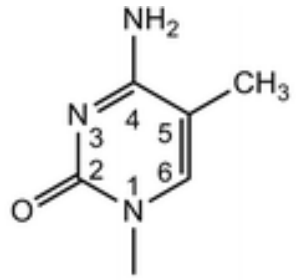


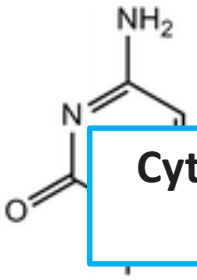
Figure 1.4b *Genomes 3* (© Garland Science 2007)

DNA methylation

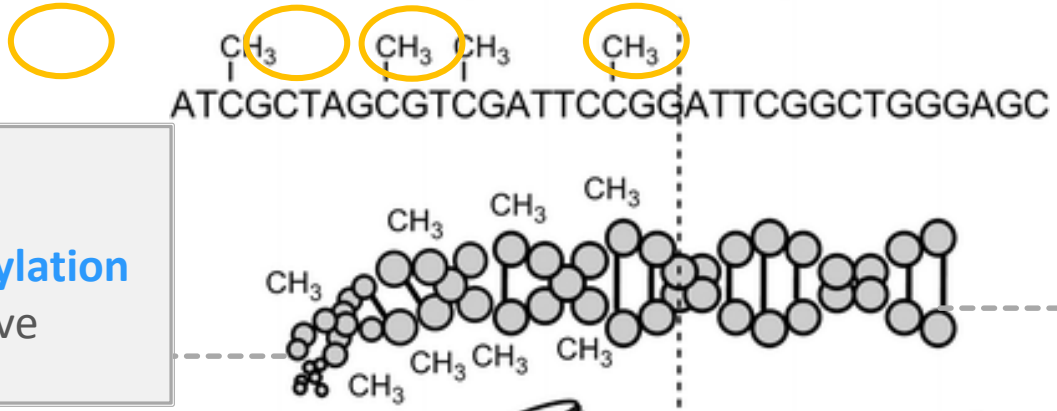
DNA methylation suppresses RNA expression

(more accurately: it is **usually** associated with suppressed RNA)



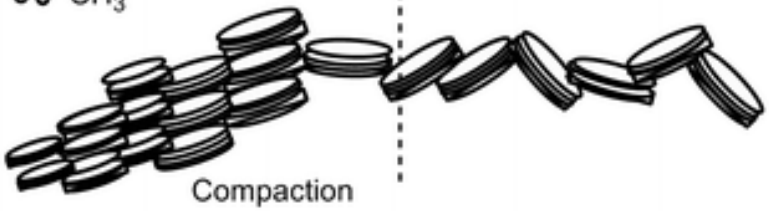


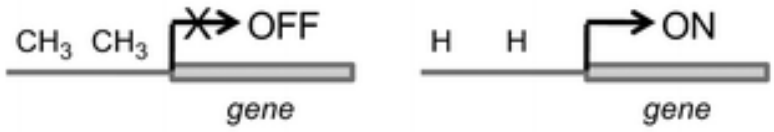
Cytosine-phosphate-Guanine
CpG sites



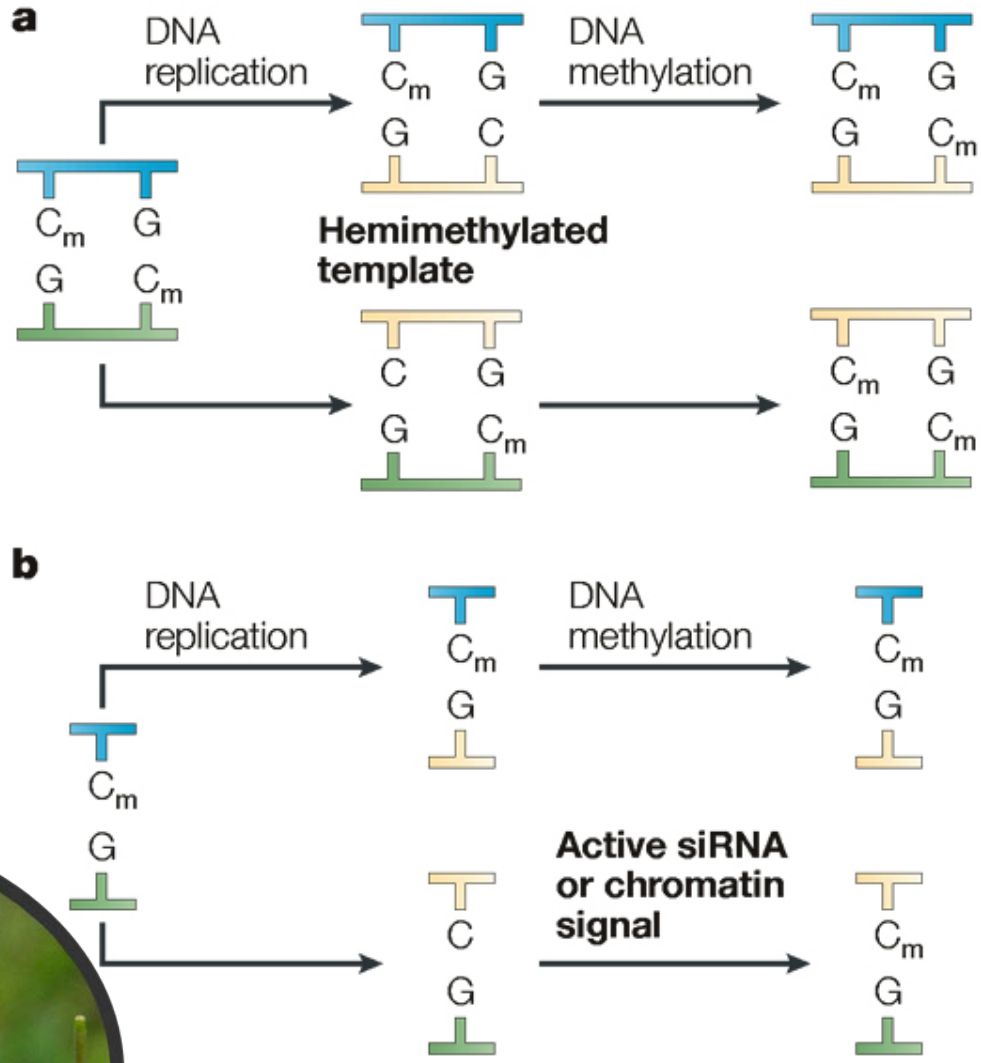
DNA methylation
inactive

DNA demethylation
active or poised to be activated

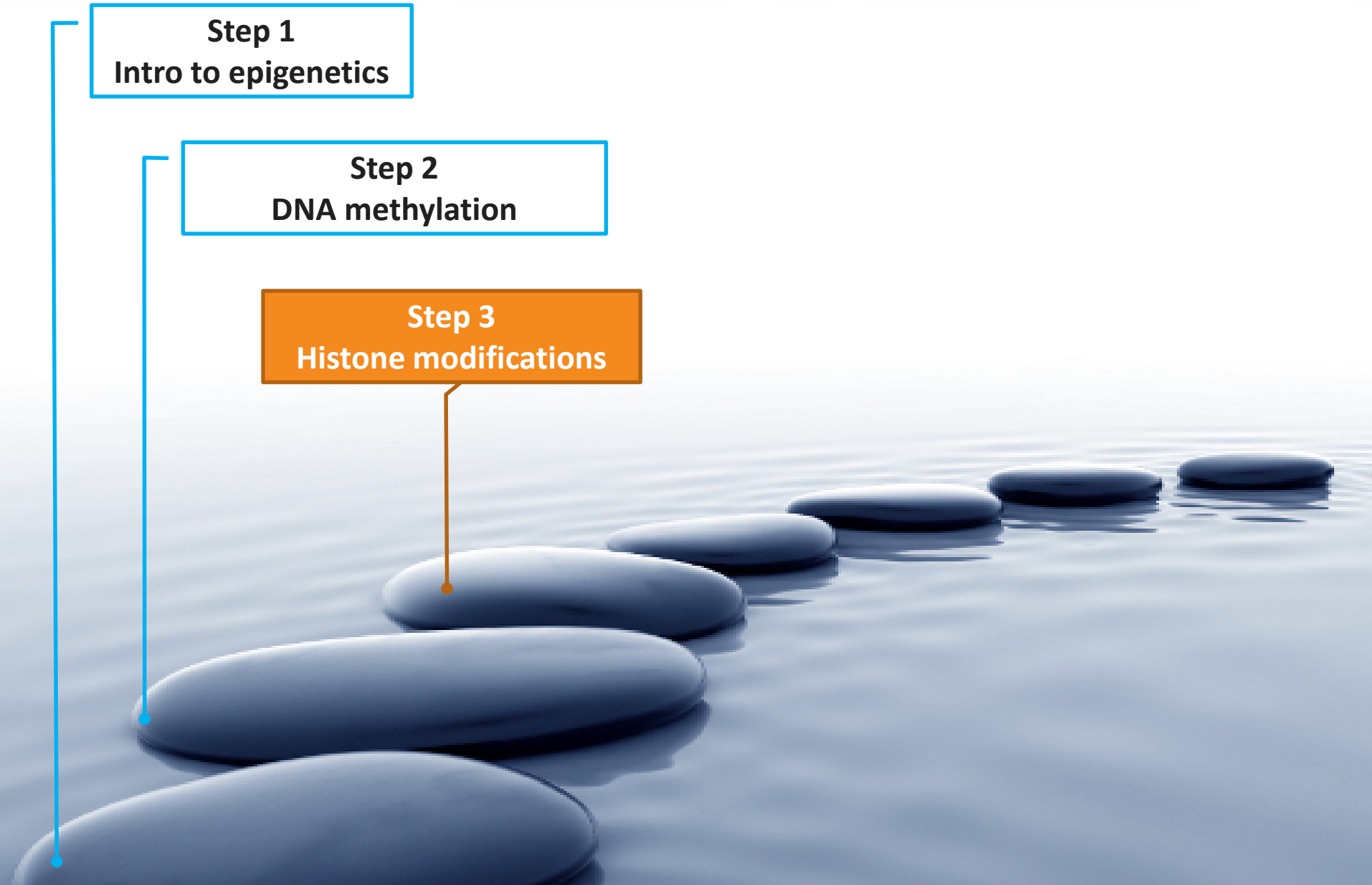




DNA Methylation maintenance in Arabidopsis Thaliana (thale cress)



Step by step


Step 1
Intro to epigenetics
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DNA methylation
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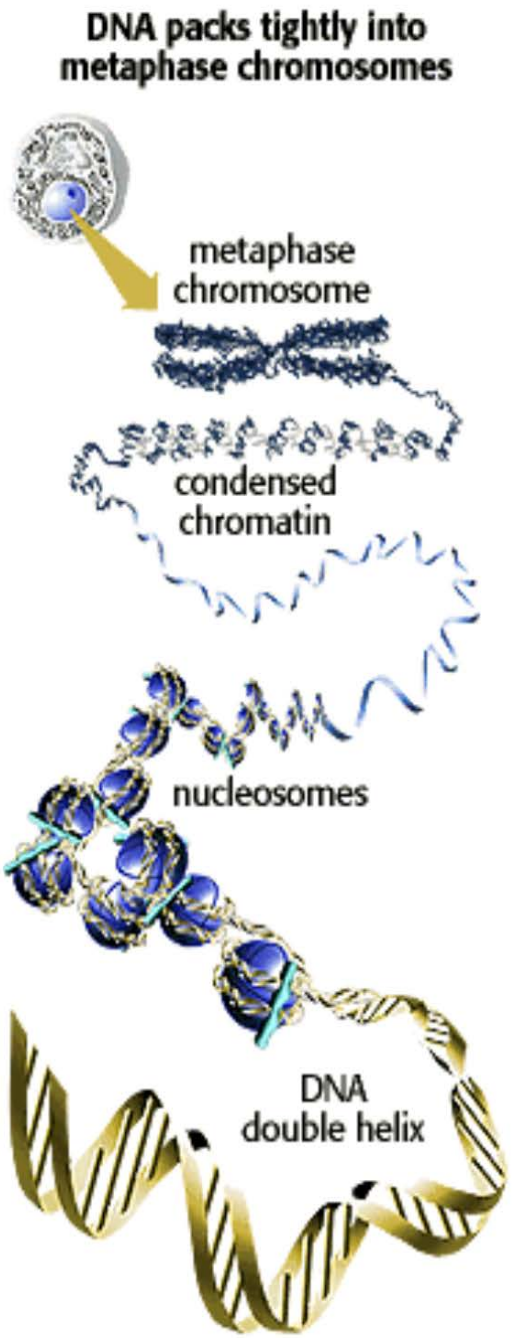
Histone modifications

A severe problem of packaging!

- Human cell has 2m of DNA
- Nucleus is 0.006 mm in diameter

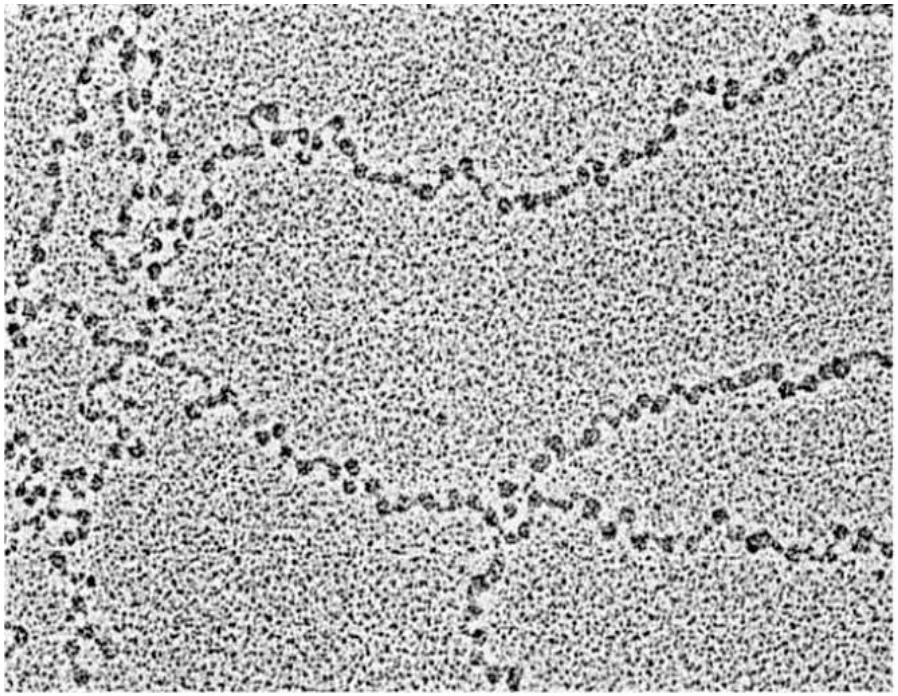
- Two opposing requirements:
  - 1. Compaction
  - 2. Access – Transcription
    Replication
    Repair



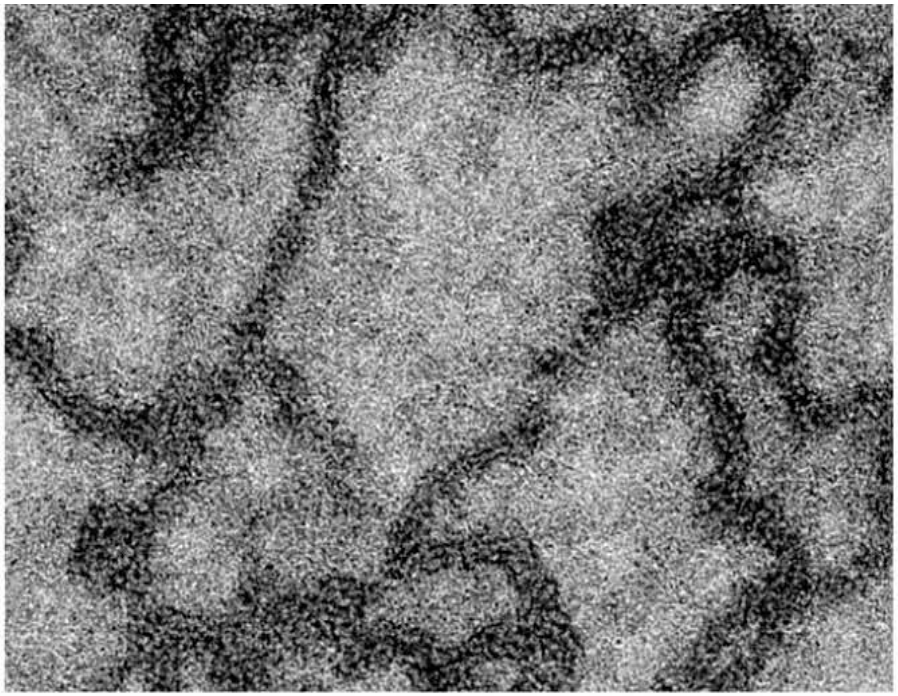
Chromatin

- Euchromatin –
  - Partially decondensed
  - Transcribed genes
- Heterochromatin –
  - Hypercondensed in interphase
  - Transcriptionally inert
  - Formation of chromosomal structures
    - Centromeres, telomeres

Electron micrographs of “chromatin preparations”



Beads on a string

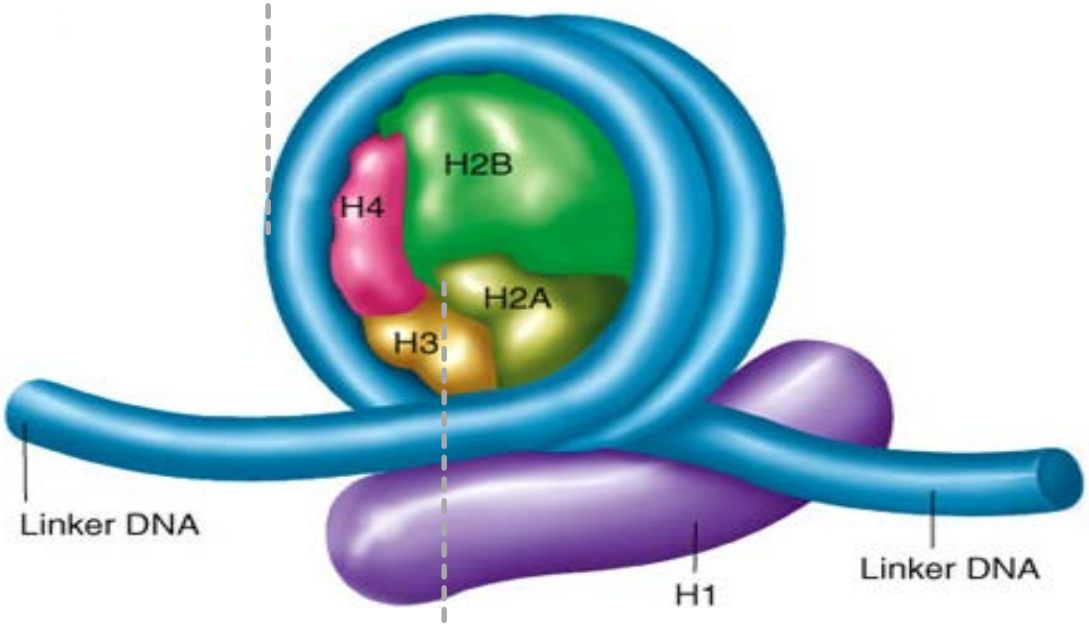


30-nm fibers

Chromosomal structure

Nucleosome – fundamental unit of chromatin

147 bp **DNA** wound 1.75 turns around histones



histone **octamer**:
2 x (H2A, H2B, H3, H4)

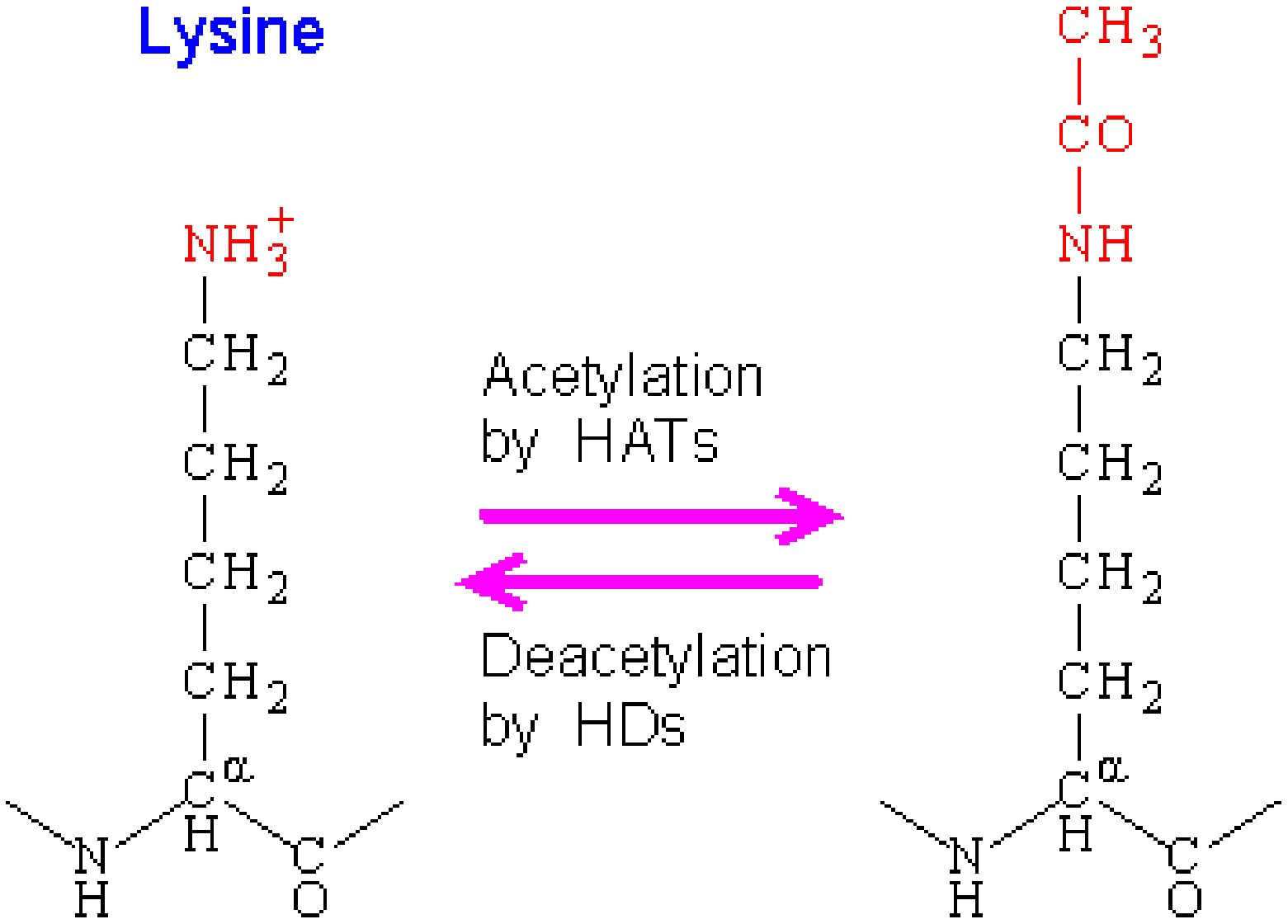
Histone Modifications

- **Histones**
  - Globular core domain
  - Unstructured N- and C-terminal tails

- **Post-translational modifications**:
  - *Acetylation* – Lys
  - *Methylation* (mono-, di- and tri-) – Lys and Arg
  - *Phosphorylation* – Ser and Thr
  - *Ubiquitination* (mono- and poly-) – Lys
  - *Sumoylation* (Lys); *ADP-ribosylation; glycosylation*; *biotinylation*; *carbonylation*

An Example: Histone acetylation

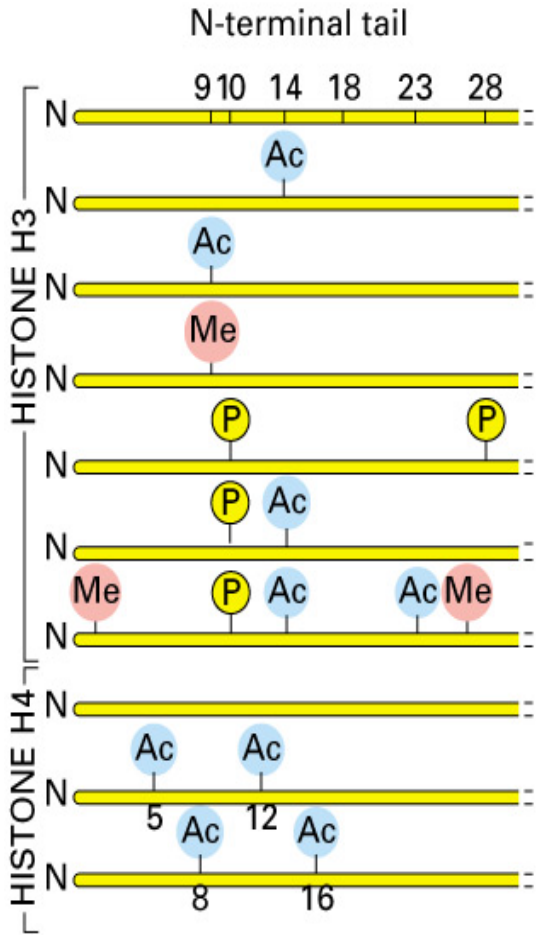
Lysine



Histone modifications

Histone modifications

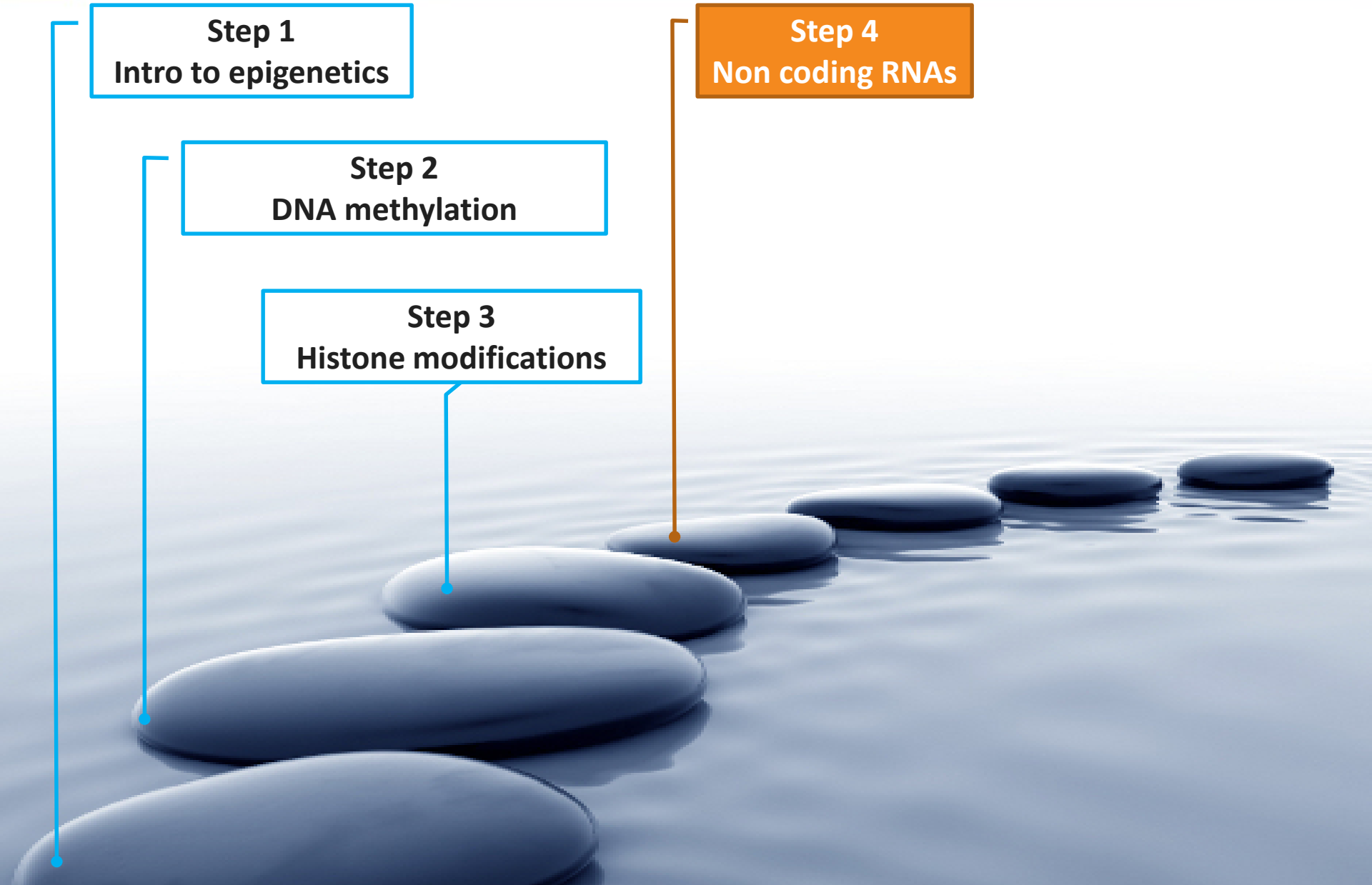
types and functions



modification state	"meaning"
unmodified	gene silencing?
acetylated	gene expression
acetylated	histone deposition
methylated	gene silencing/ heterochromatin
phosphorylated	mitosis/meiosis
phosphorylated/ acetylated	gene expression
higher-order combinations	?
unmodified	gene silencing?
acetylated	histone deposition
acetylated	gene expression

Ac - acetyl (lysine), **Me** - methyl (lysine), **P** - phosphoryl (Ser or Thr)

Step by step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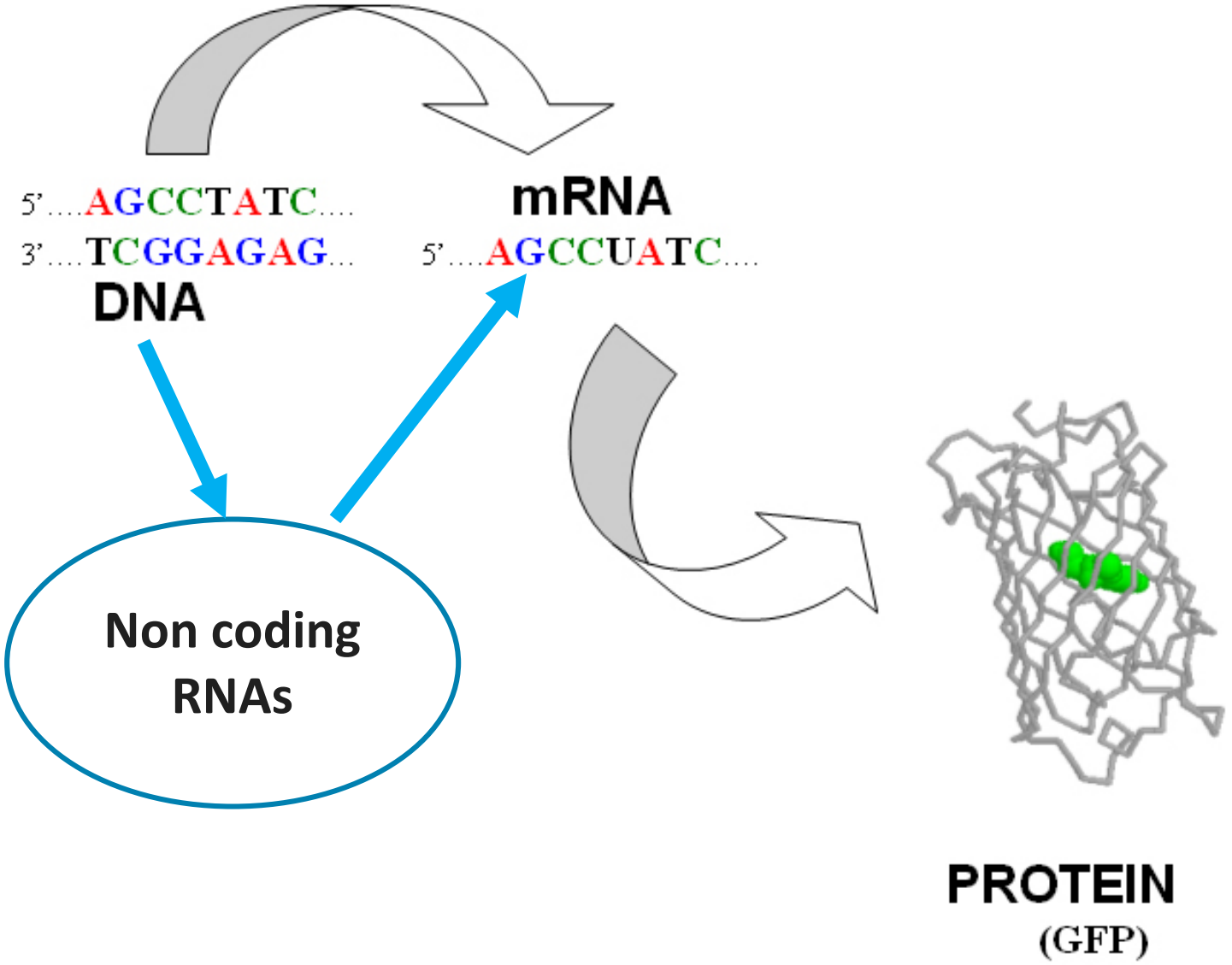
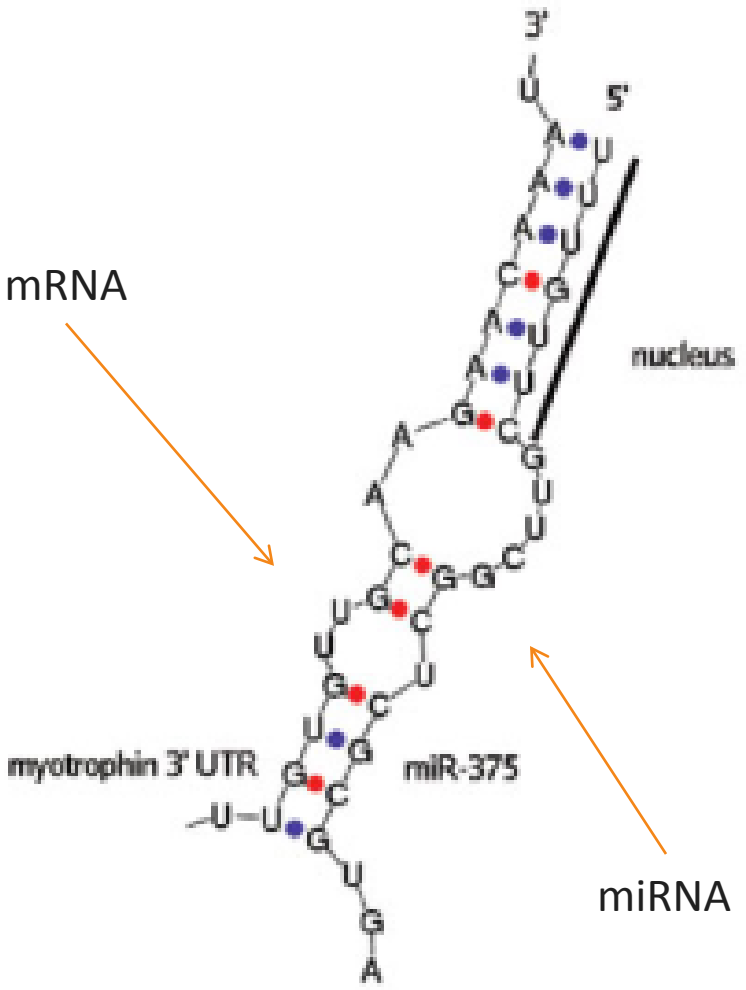
Gene expression



Meet the microRNAs (miRNAs)

- Small non-coding RNAs
  - 20-22 nt in length
- block the translation of messenger RNAs into proteins



miRNAs

- May regulate >30% of human genes
- miRBASE Release 21.0 (Sep 2014) has 28,645 entries
  - from 223 species
  - in humans: 1,881 precursors, 2,588 mature miRNAs
- Discovery of new miRNAsis ongoing …

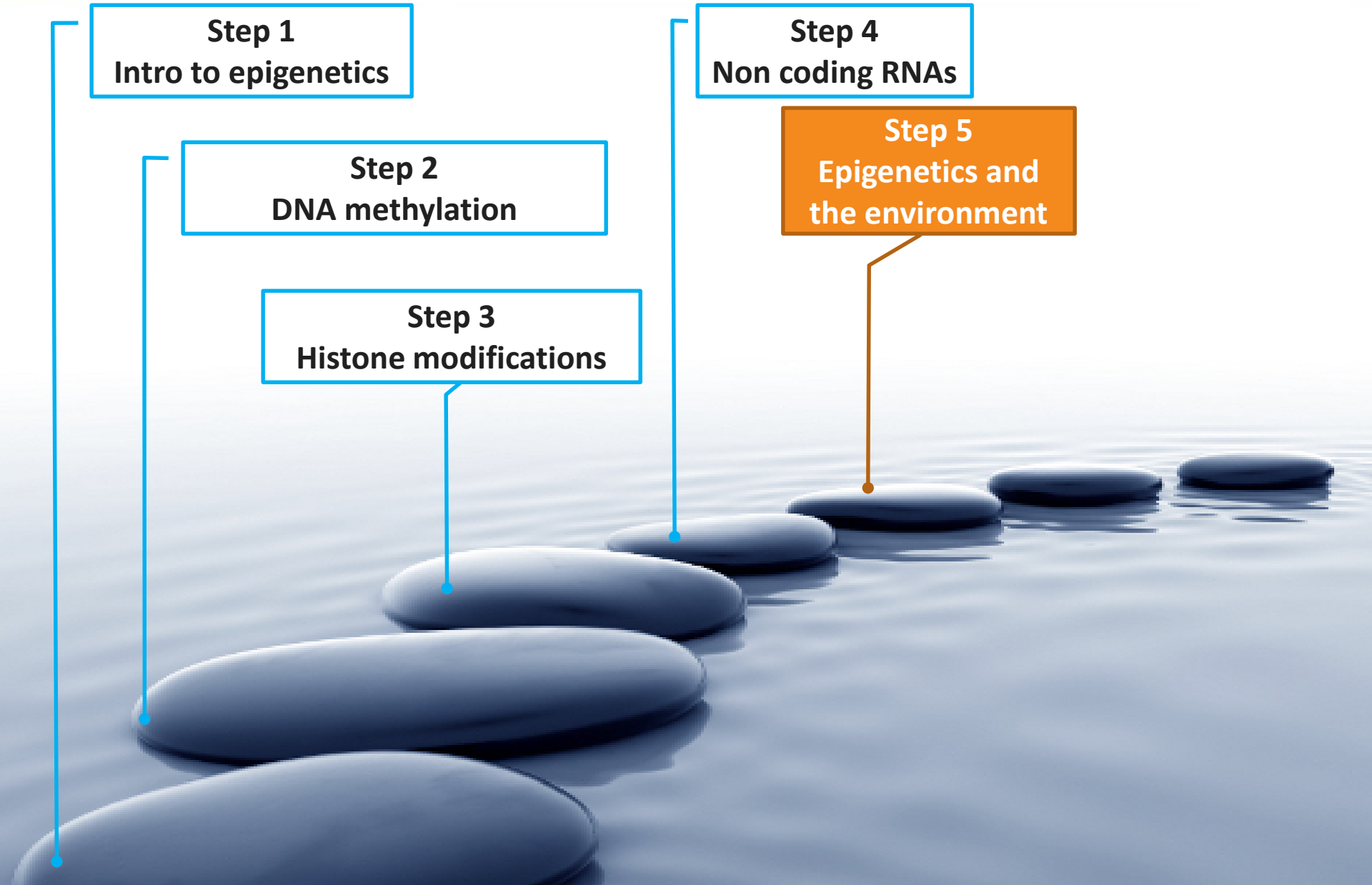
- Source: miRBASE database

  http://www.mirbase.org/cgi-bin/browse.pl?org=hsa

Other non-coding RNA types

- Non-coding RNA = miRNAs?
- Other types of non-coding RNAs
  - PIWI-interacting RNAs (piRNAs)
  - small nucleolar RNAs (snoRNAs)
  - promoter-associated small RNAs (PASRs)
  - transcriptional start sites associated (TSSa-RNA)
  - transcribed ultraconserved regions (T-UCRs)
  - promoter upstream transcripts (PROMPTS)
  - large intergenic non-coding RNAs (lincRNAs)

Step by step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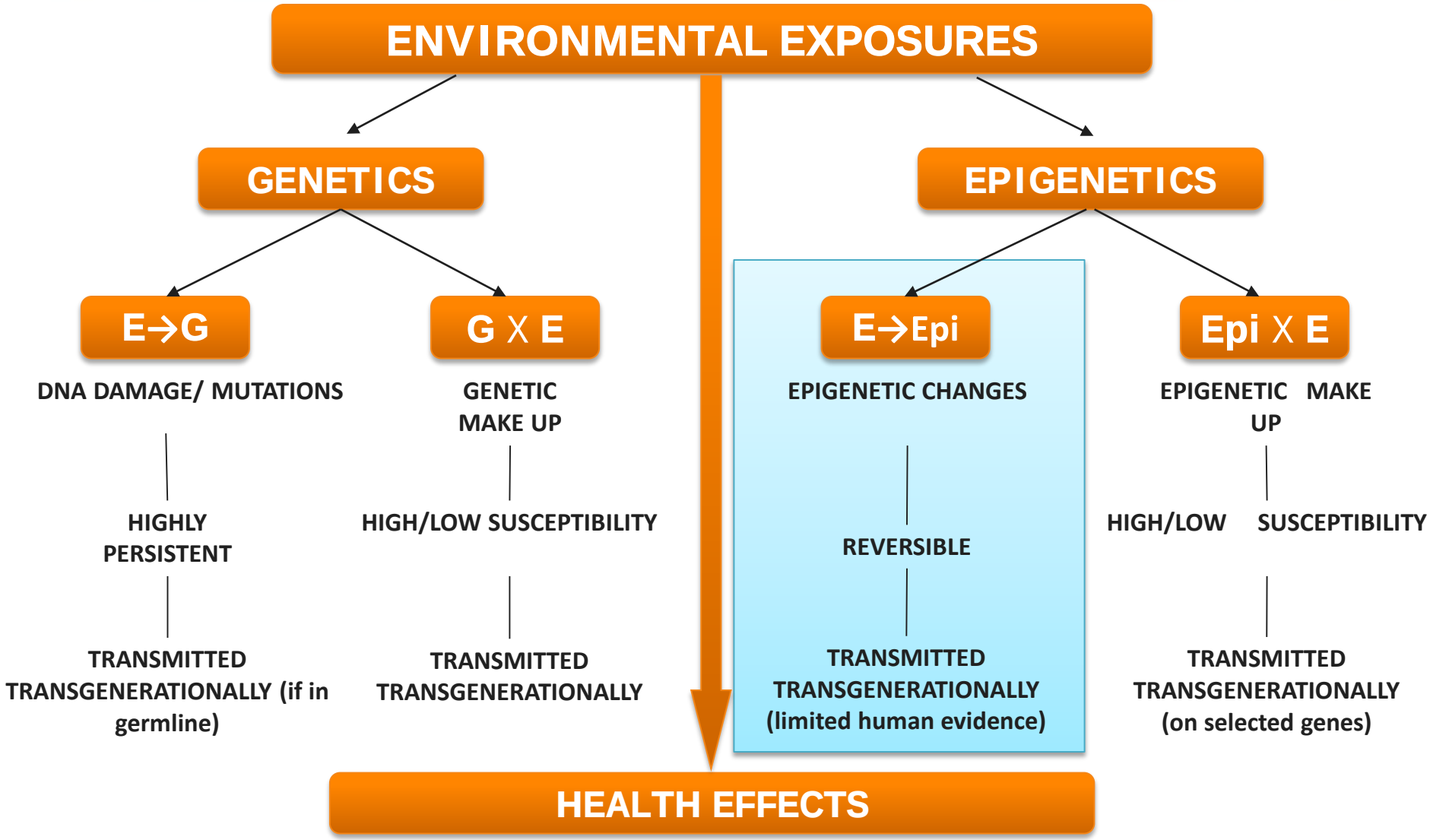
Epigenetics and
the environment

Environment, genetics, epigenetics



Adapted from Bollati & Baccarelli, Heredity 2010

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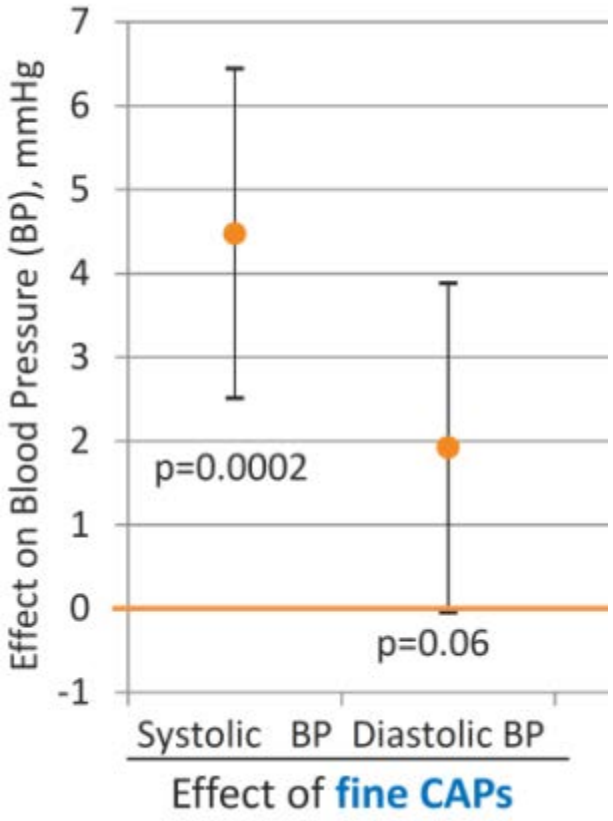
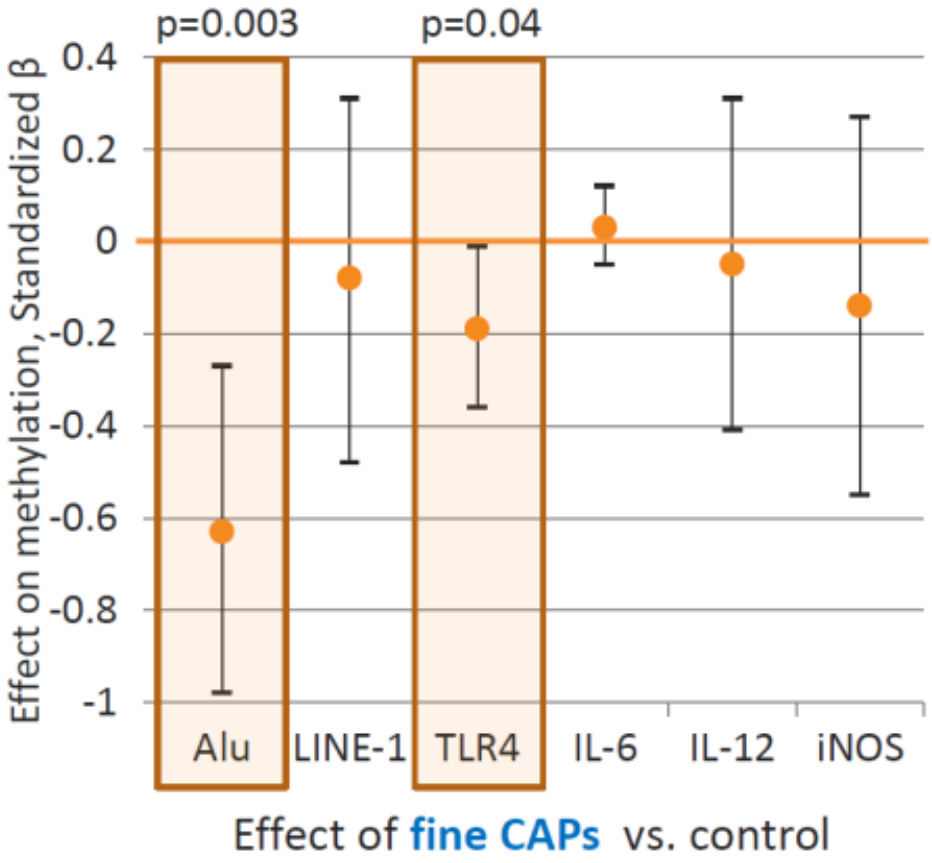
Concentrated Ambient Particle (CAP) exposure

Effects of fine CAPs
on Blood pressure and DNA methylation

Effect on **Blood Pressure**



Effect on **Blood DNA methylation**

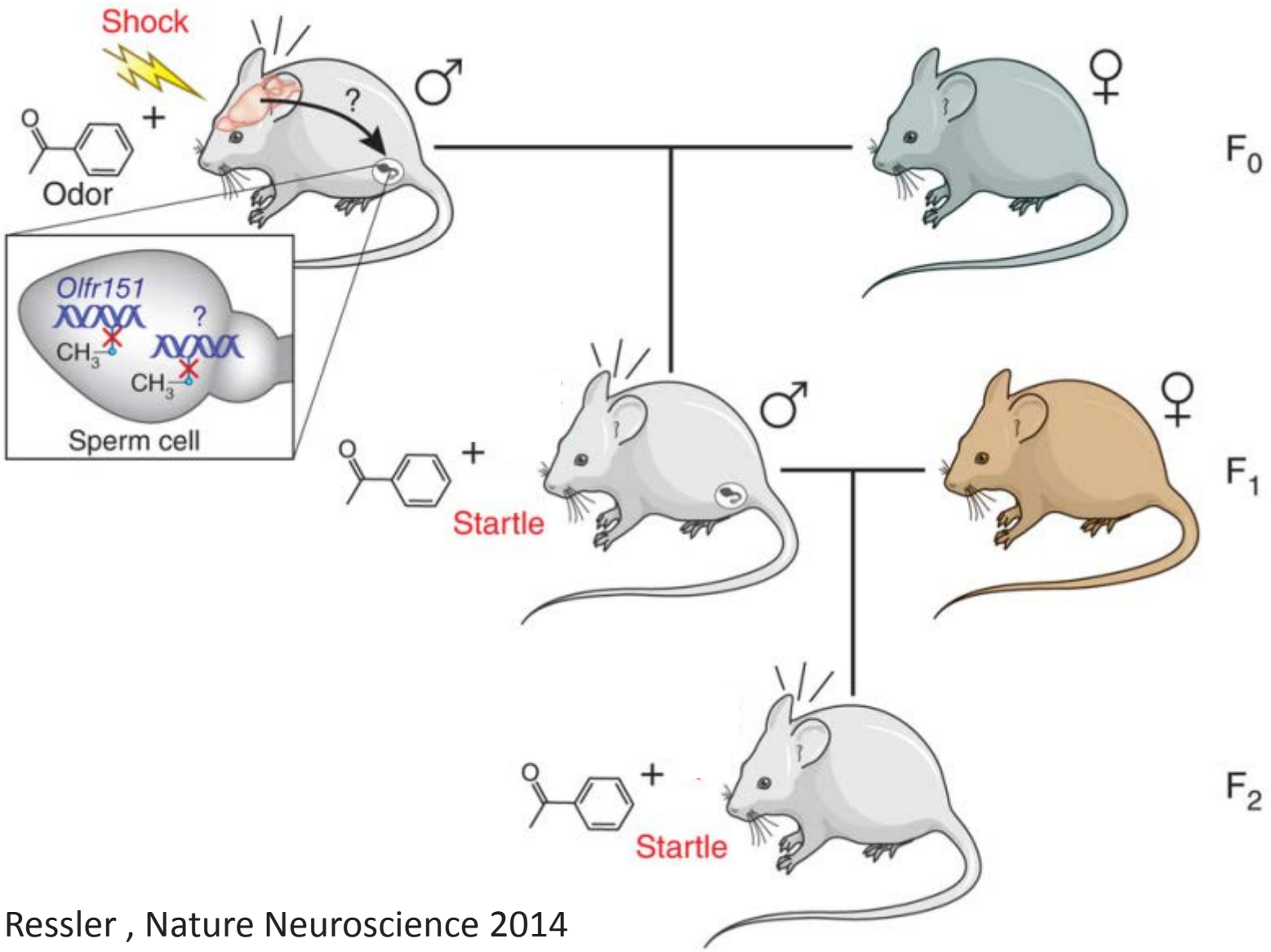


Differences of **fine CAP** exposure vs **control**

Bellavia et al. JAHA 2013

Parental olfactory experience influences behavior

and neural structure in subsequent generations



Dias & Ressler , Nature Neuroscience 2014
(Graphics adapted from Szyf Nature Neuroscience 2014)

Dias & Ressler's experiment

- Offspring mice inherited conditioned fear to acetone odor
  - The father mouse experienced odor in conjunction with electric shock (after repeated experience, the mouse was conditioned to get a fear reaction upon exposure to odor alone)
  - The offspring mouse experienced fear to the acetone odor although never exposed to electric shock
- Experiment repeated with IVF to exclude any behavioral transmission through mothers
- Altered DNA methylation in an odorant gene found in the mouse sperm

THE *SINS* OF THE FATHER

The roots of inheritance may extend beyond the genome, but the mechanisms remain a puzzle.

BY VIRGINIA HUGHES

When Brian Dias became a father last October, he was, like any new parent, mindful of the enormous responsibility that lay before him. From that moment on, every choice he made could affect his newborn son's physical and psychological development. But, unlike most new parents, Dias was also aware of the influence of his past experiences — not to mention those of his parents, his grandparents and beyond.

Where one's ancestors lived, or how much they valued education, can clearly have effects that pass down through the generations. But what about the legacy of their health: whether they smoked, endured famine or fought in a war?

As a postdoc in Kerry Ressler's laboratory at Emory University in Atlanta, Georgia, Dias had spent much of the two years before his son's birth studying these kinds of questions in mice. Specifically, he looked at how fear associated with a particular smell affects the animals and leaves an imprint on the brains of their descendants.

Dias had been exposing male mice to acetophenone — a chemical with a sweet, almond-like smell — and then giving them a mild foot shock. After being exposed to this treatment five times a day for three days, the mice became reliably fearful, freezing in the presence of acetophenone even when they received no shock.

Ten days later, Dias allowed the mice to mate with unexposed females. When their young grew up, many of the animals were more sensitive to acetophenone than to other odours, and more likely to be startled by an unexpected noise during exposure to the smell. Their offspring — the 'grandchildren' of the mice trained to fear the smell — were also jumpier in the presence of acetophenone. What's more, all three generations had larger-than-normal 'M71 glomeruli', structures where acetophenone-sensitive neurons in the nose connect with neurons in the olfactory bulb. In the January issue of *Nature Neuroscience*[1], Dias and Ressler suggested that this hereditary transmission of environmental information was the result of epigenetics — chemical changes to the genome that affect how DNA is packaged and expressed without altering its sequence.

Biologists first observed this 'transgenerational epigenetic inheritance' in plants. Tomatoes, for example, pass along chemical markings that control an important ripening

20TH CENTURY FOX/THE KOBAL COLLECTION

EPIGENETICS

A lingering smell?

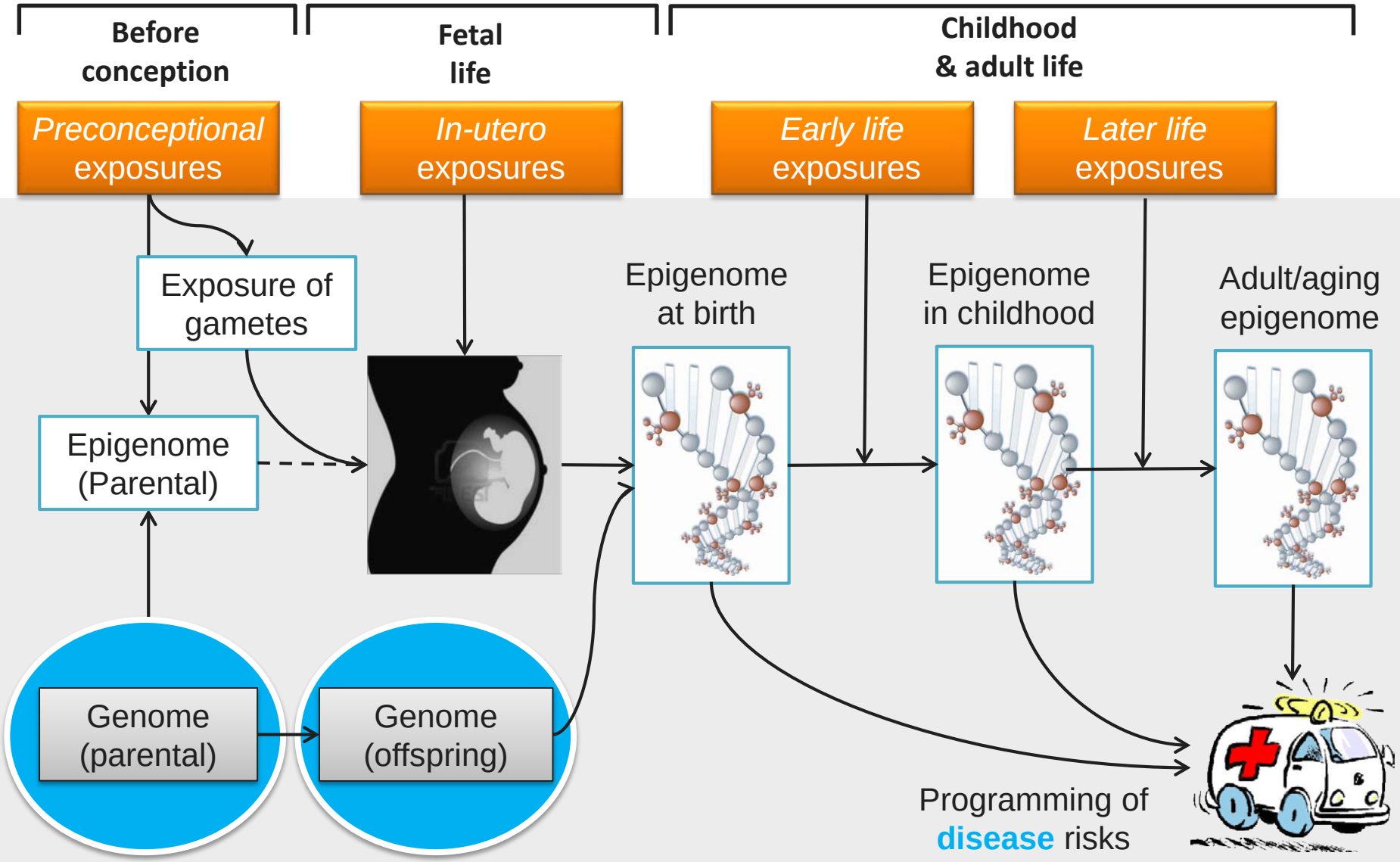
“ mice whose fathers had undergone a painful experience associated with a particular

Studies in animals have shown that stressful experiences can be passed onto offspring, often in the form of a general anxious or stress-sensitive phenotype. A new study now shows that highly specific experiences can also be inherited by subsequent generations, in terms of behaviour and anatomy, and that this transmission occurs through parental gametes.

Dias and Ressler trained male mice (F0 mice) to associate mild foot-shocks with one of two odours: ace- anatomical changes might underlie the behavioural effects; however, the two could not be directly correlated because they were assessed in different sets of animals.

Interestingly, the male offspring of F1-Ace males and F1-Prop males (that is, F2 males) also showed increased sensitivity to Ace and Prop, respectively. Moreover, like their fathers, F2-Ace males had larger M71-specific glomeruli. These findings suggest that a specific olfactory experience had sensitivity to Ace was not socially mediated and can also occur through the maternal line.

The authors reasoned that if F0-Ace mice transmit their olfactory experience through gametes, then DNA in sperm of F0-Ace males might show epigenetic changes in the gene encoding the M71 receptor (*Olfr151*). Indeed, *Olfr151* was hypomethylated in both F0-Ace sperm (compared with F0-Prop sperm) and F1-Ace sperm. Interestingly, however,



Disease programming throughout the lifecourse

Figure adapted from Fleisch, Wright & Baccarelli, J Mol Endocrinol, 2012

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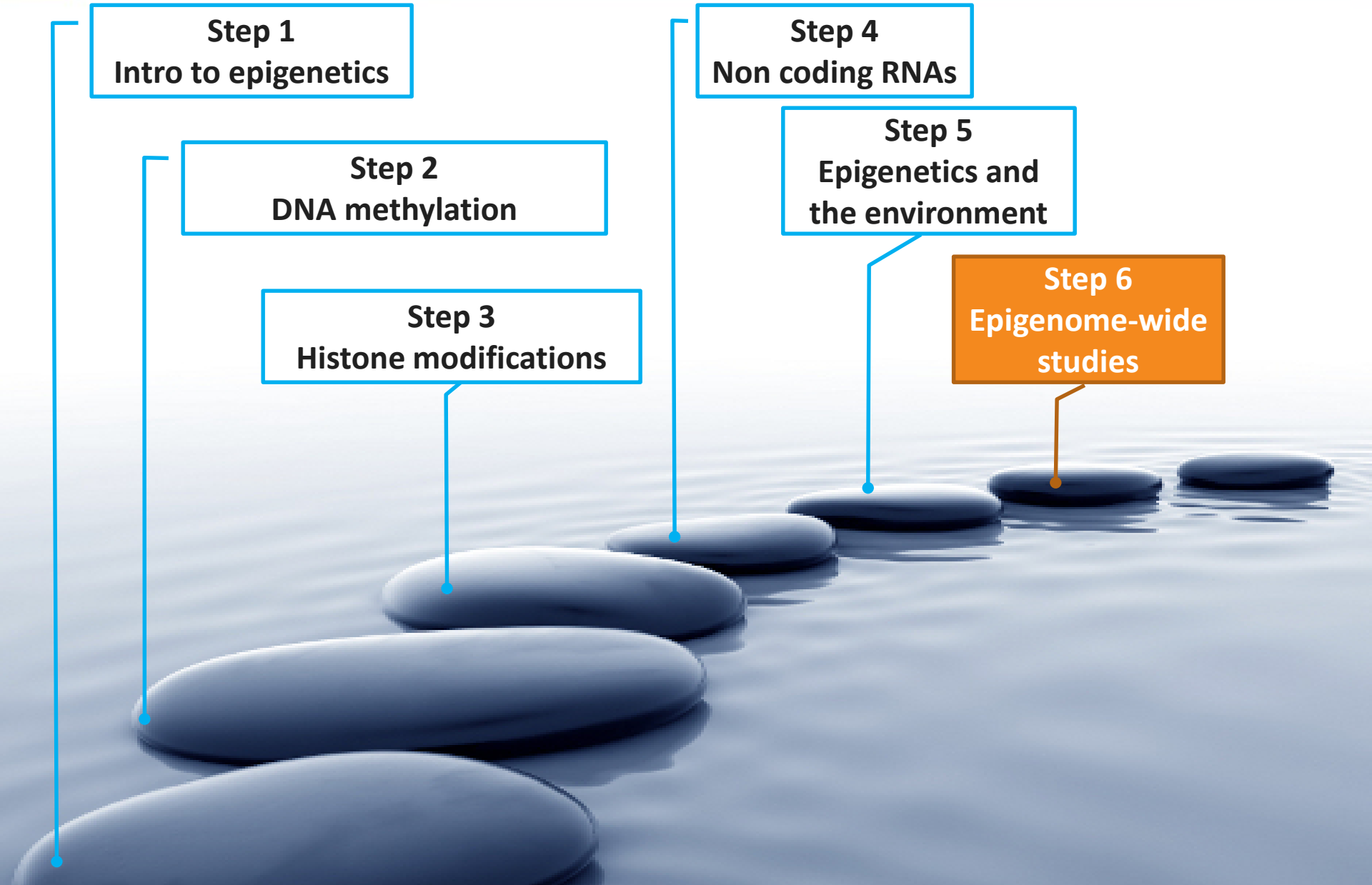
Step by step


Step 1
Intro to epigenetics
Step 2
DNA methylation
Step 3
Histone modifications
Step 4
Non coding RNAs
Step 5
Epigenetics and the environment
Step 6
Epigenome-wide studies

Some nomenclature

(DNA methylation used as example)

- Candidate gene (gene-specific) approach
  - A priori knowledge → candidate genes
  - test for association with exposure/risk factor
  - test for association with disease/phenotype
- Global (average) level of methylation (5mC content)
  - Average methylation of all CpG sites across the genome
  - test for association with exposure/risk factor
  - test for association with disease/phenotype
- Epigenome-wide approach (EWAS)
  - Agnostic approach → entire genome
  - test for association with exposure/risk factor
  - test for association with disease/phenotype

Examples (DNA methylation)

- Candidate gene approach
  - Participant #1's blood has 26% methylation in the IL6 promoter (N.B.: any other region of interest can be targeted, e.g., CpGi shore, shelf, etc.)
- Global methylation approach
  - Participant #1's blood has 4.5% methylation (i.e., 4.5% of all cytosines found in blood are methylated; no information on where the methylated cytosines are located)
- Genome-wide approach
  - Methylation in Participant #1's blood is measured at a high number of CpG sites (e.g, if we use Illumina Infinium 450K beadchip → we will get ≈486,000 numbers [one for each CpG site] for Participant #1's blood)

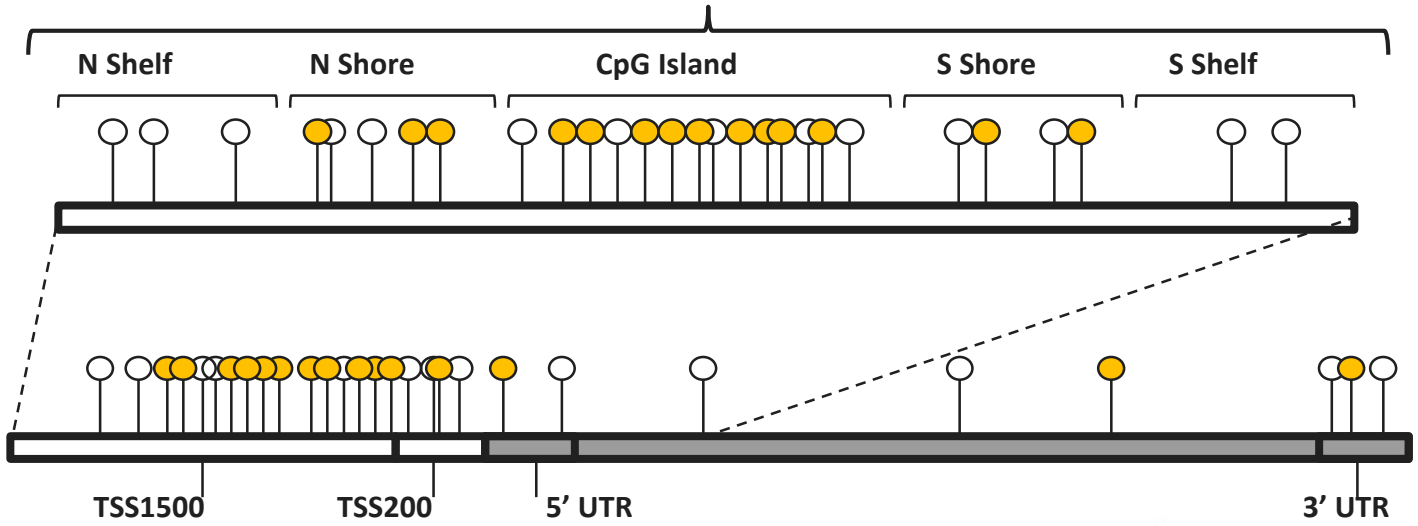
GWAS/EWAS

- Genetics
  - Genome wide association studies
  - We study single nucleotide polymorphisms (SNPs) or other differences (e.g., insertion, deletion, copy number variations)
- Epigenetics
  - Epigenome wide association studies
  - We study DNA methylation, histone modifications, etc.
  - Screen for 100Ks to millions of individual features (e.g., CpG sites)

Features covered in the 450k Infinium BeadChip

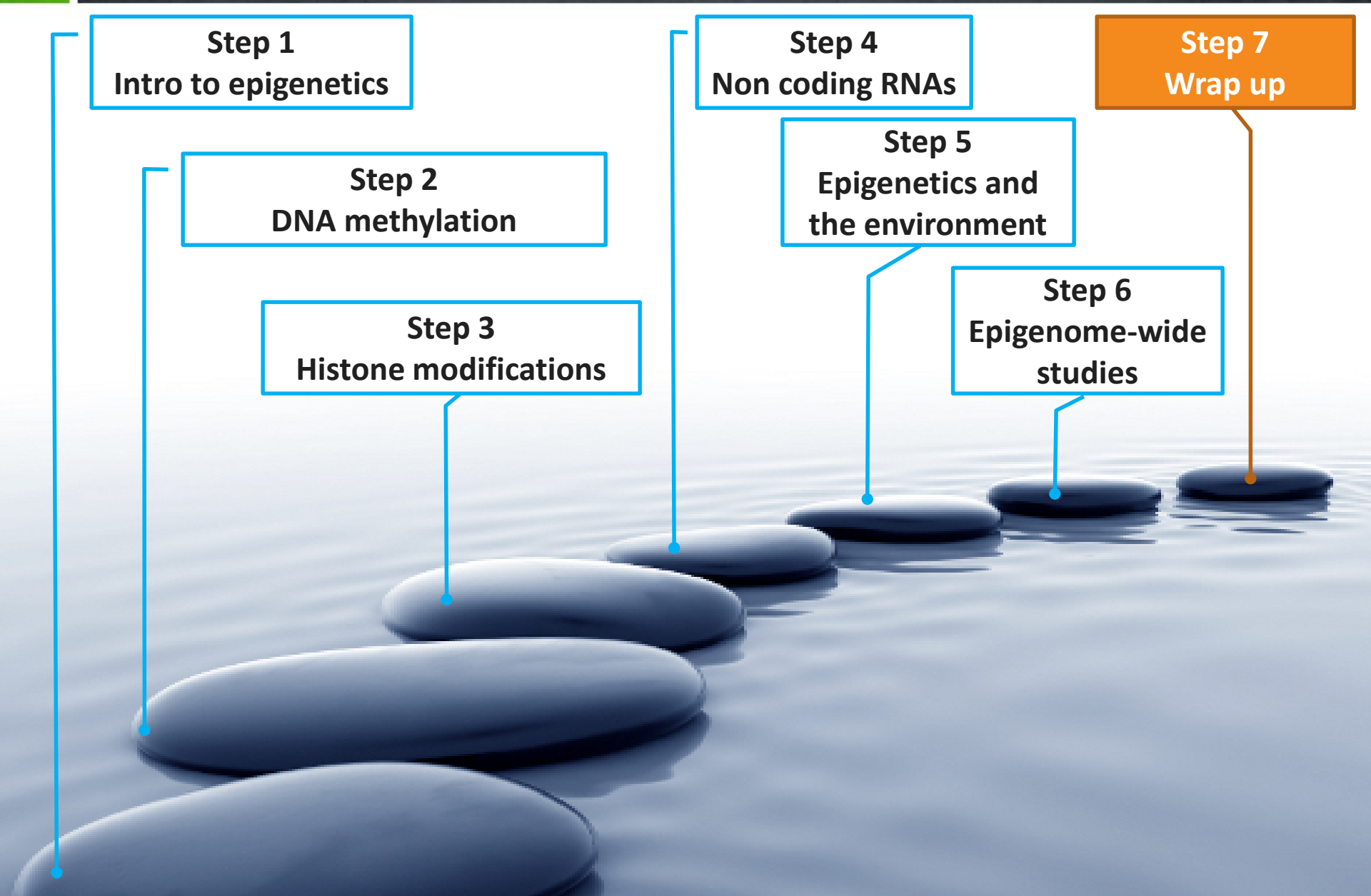
The 450K BeadChip covers a total of 77,537 CpG Islands and CpG Shores (N+S)

Region Type	Regions	CpG sites covered on 450K BeadChip array	Average # of CpG sites per region
CpG Island	26,153	139,265	5.08
N Shore	25,770	73,508	2.74
S Shore	25,614	71,119	2.66
N Shelf	23,896	49,093	1.97
S Shelf	23,968	48,524	1.94
Remote/Unassigned	-	104,926	-
Total		485,553	



The 450K BeadChip covers a total of 20,617 genes

Step by step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Wrap up

Conclusions

- Epigenetics is all about control of gene expression
  - Relatively stable
  - Biological programming
  - Influenced by the environment
- Epigenetics investigates different mechanisms
  - Not limited to those presented here
- Expanding research on how environmental toxicants may reprogram the epigenome and affect human health

Some readings

- Michels K. *Epigenetic Epidemiology*. Springer, 2012
- Relton CL & Davey Smith G. *Is epidemiology ready for epigenetics?* Int J Epidemiol. 2012 Feb;41(1):5-9.
- Hou L, Zhang X, Wang D, Baccarelli A. *Environmental chemical exposures and human epigenetics.* Int J Epidemiol. 2012 Feb;41(1):79-105.

Thanks!