Human Epigenetics
Linking early development and late life health

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LU
MC

The genetic code is not enough

AGTGCCGGGAAGTGGGGCTTGGC
CCAGGGCCCCCAAGACACACAGA
CGGCACAGCAGGGCTGGTTCAAG
GGCTTTATTCCATCTCTCTCGGT
GCAGGAGGCGGCGGGTGTGGGGC
TGCCTGCGGGCTGCGTCTAGTTG
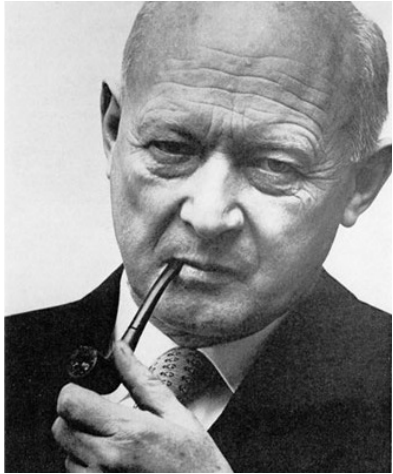CAGTAGTTCTCCAGCTGGTAGAG

Epigenetics

- Instructing the DNA where, when and how much to express a gene.
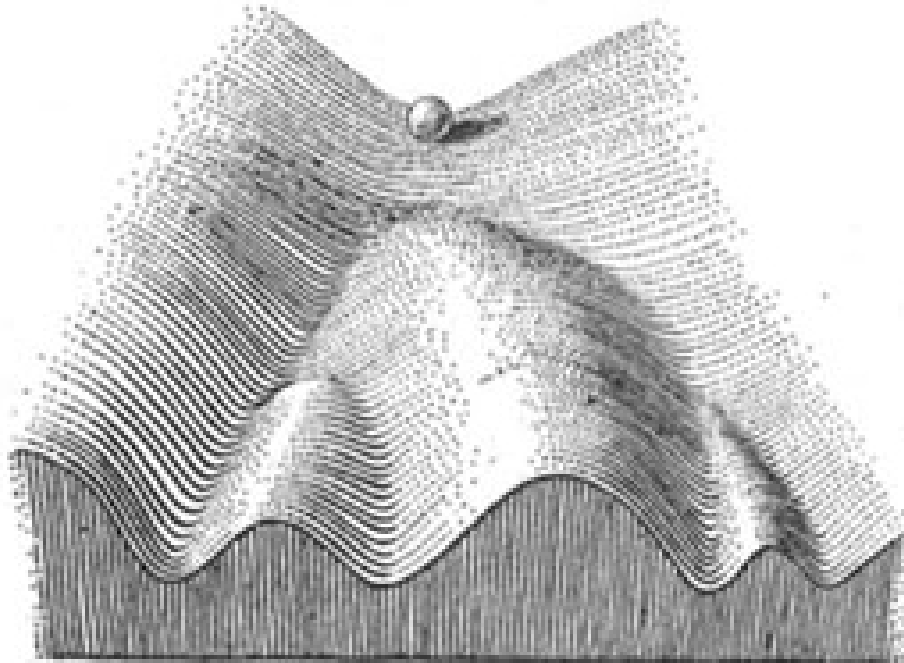
Focus on DNA methylation
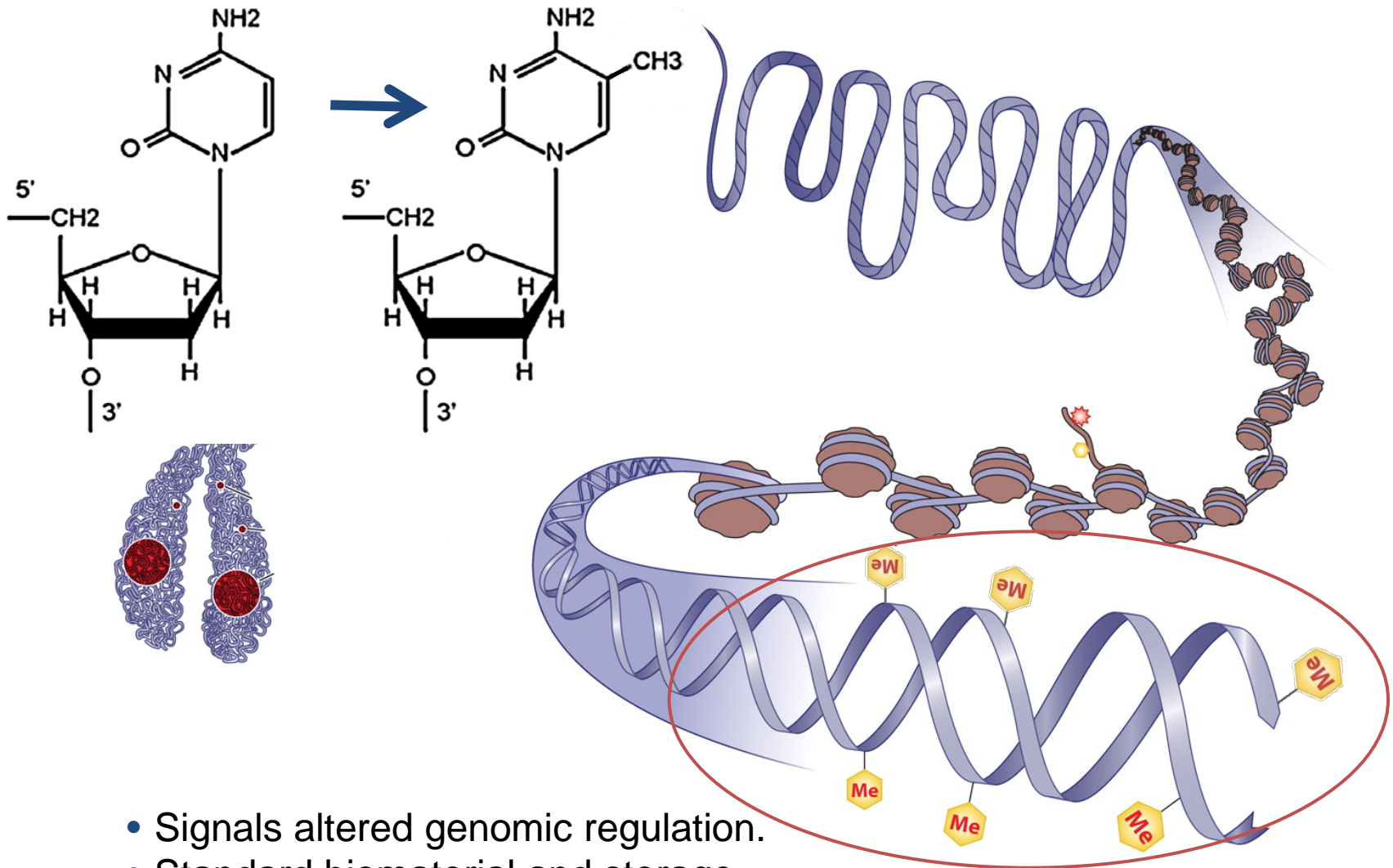
- Signals altered genomic regulation.
- Standard biomaterial and storage.
- High-throughput profiling.

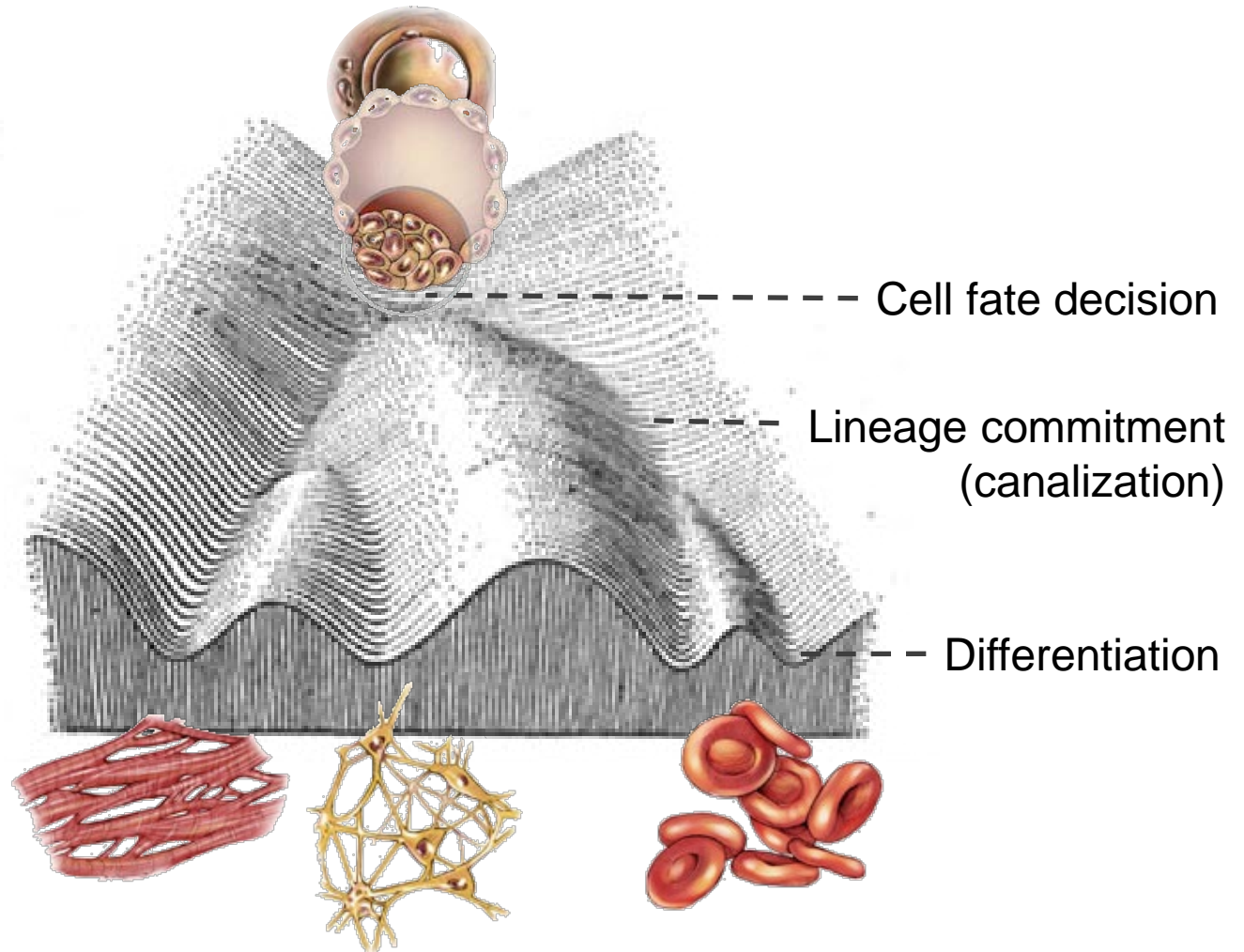# Waddington's epigenetic landscape

1905-1975

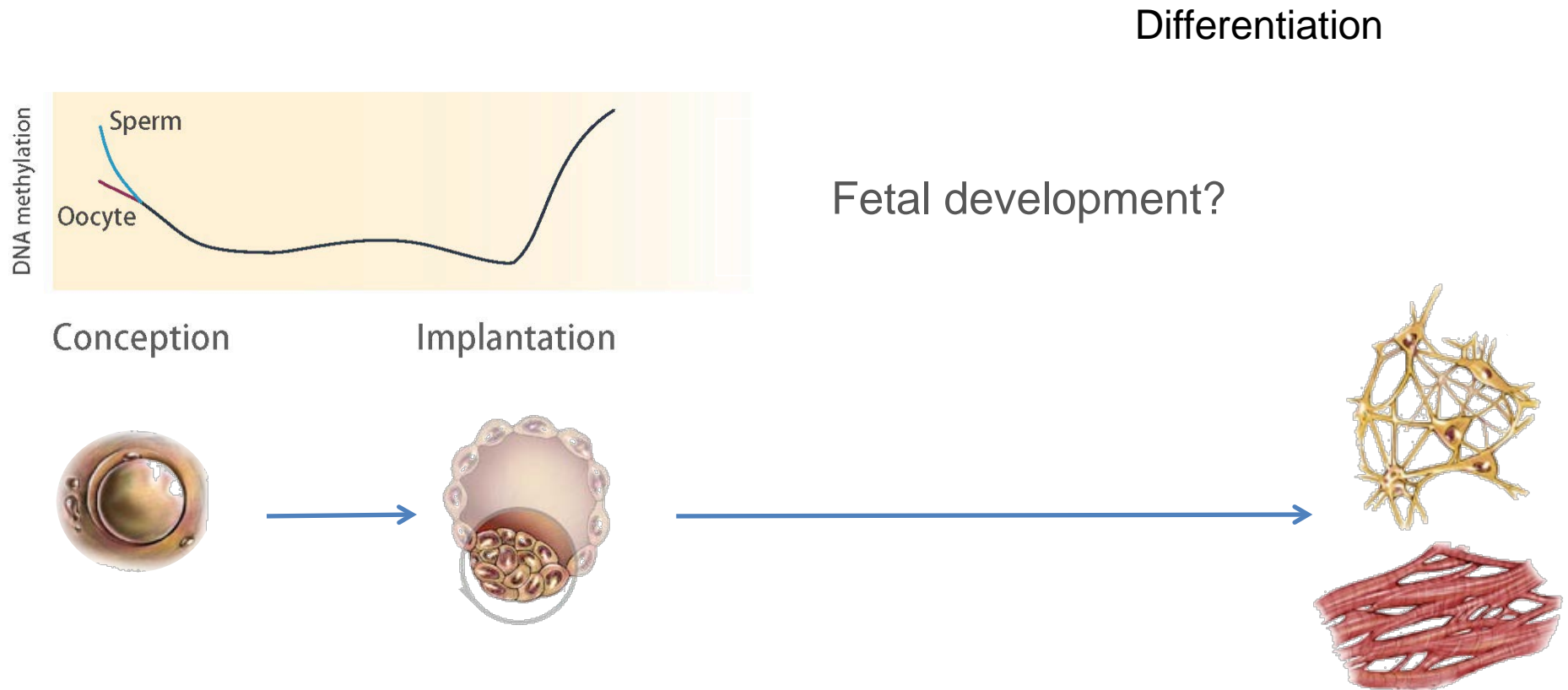From: The strategy of genes, 1957

Waddington's epigenetic landscape

Cell fate decision

Lineage commitment
(canalization)

Differentiation

DNA Methylation is dynamic during development

Cell fate decision   Lineage commitment

Smith et al. *Nature* 2012
Seisenberger et al. *Mol Cell* 2012

DNA Methylation is dynamic during development

Differentiation
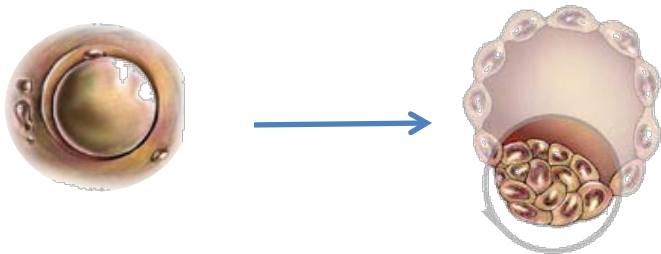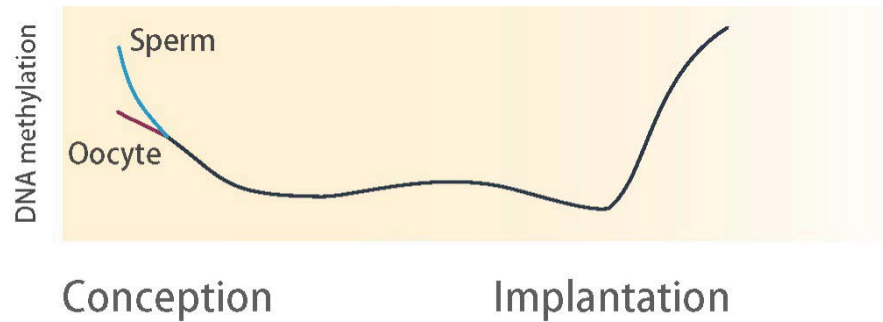
Fetal development?

- Tissues: amnion, muscle, adrenal, pancreas (elective abortions).
- Time point: 1st to 2nd trimester (week 9, 18, 22).
- Technology: Illumina 450k methylation array.

Slieker et al. *PLoS Genet* 2015

Principle methylation array

M?   M?   M?   **M?**   x 485,000 (out of 28M)

...CCTGGTA**CG**TCTAGC**CG**TAATTAGCT**CG**ATTC**CG**GT...

Bisulfite conversion

unmethylated   methylated

...UUTGGT**UG**TUTAG...   ...UUTGGT**CG**TUTAG...

unmeth signal   meth signal

β-value = meth / (meth + unmeth)

Infinium II bead type

T—●
C—●
A—●
G—●

C
GC
CpG locus   5'

Bisulfite converted DNA
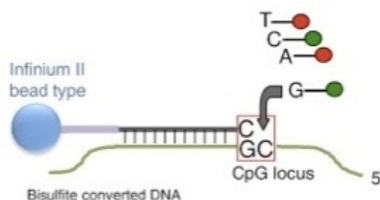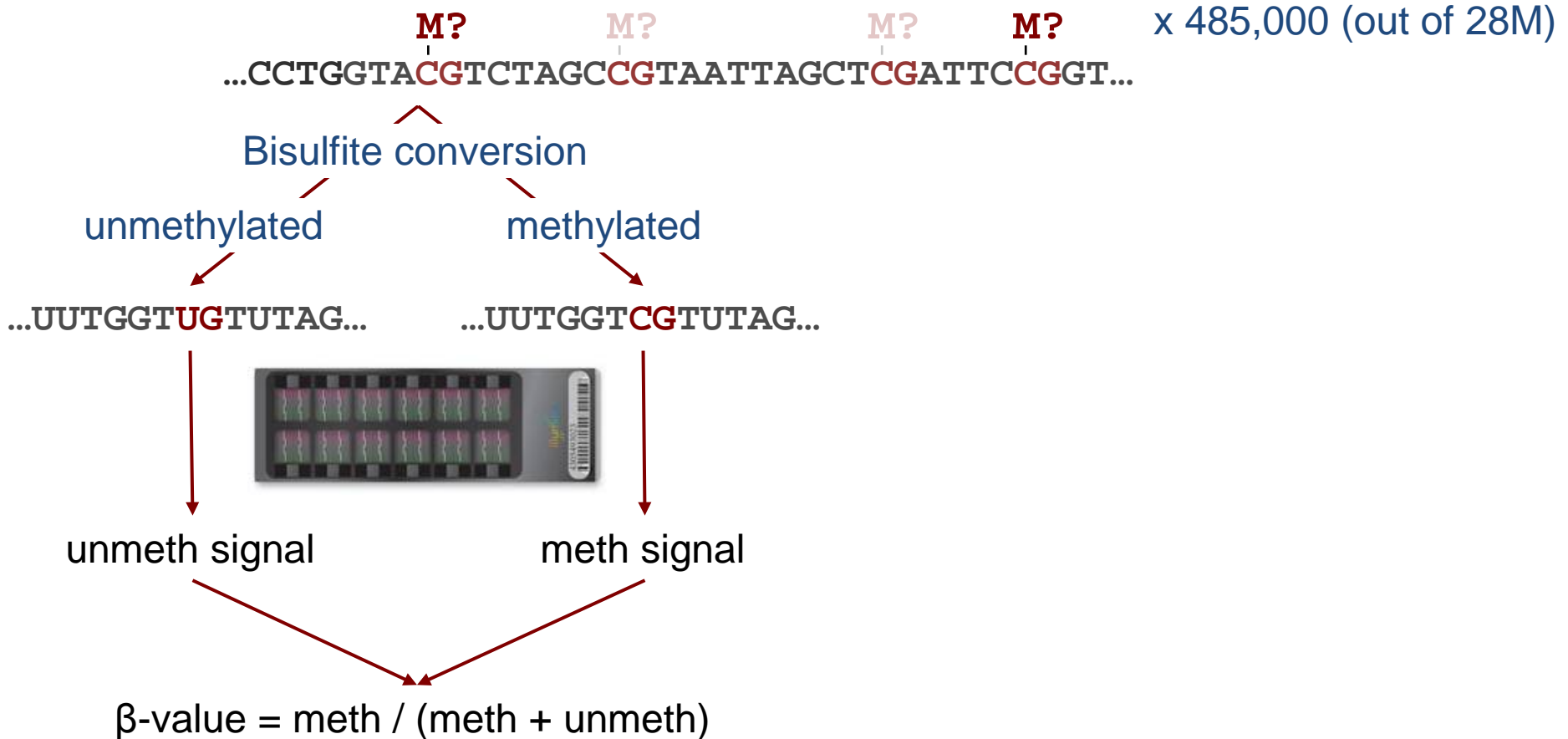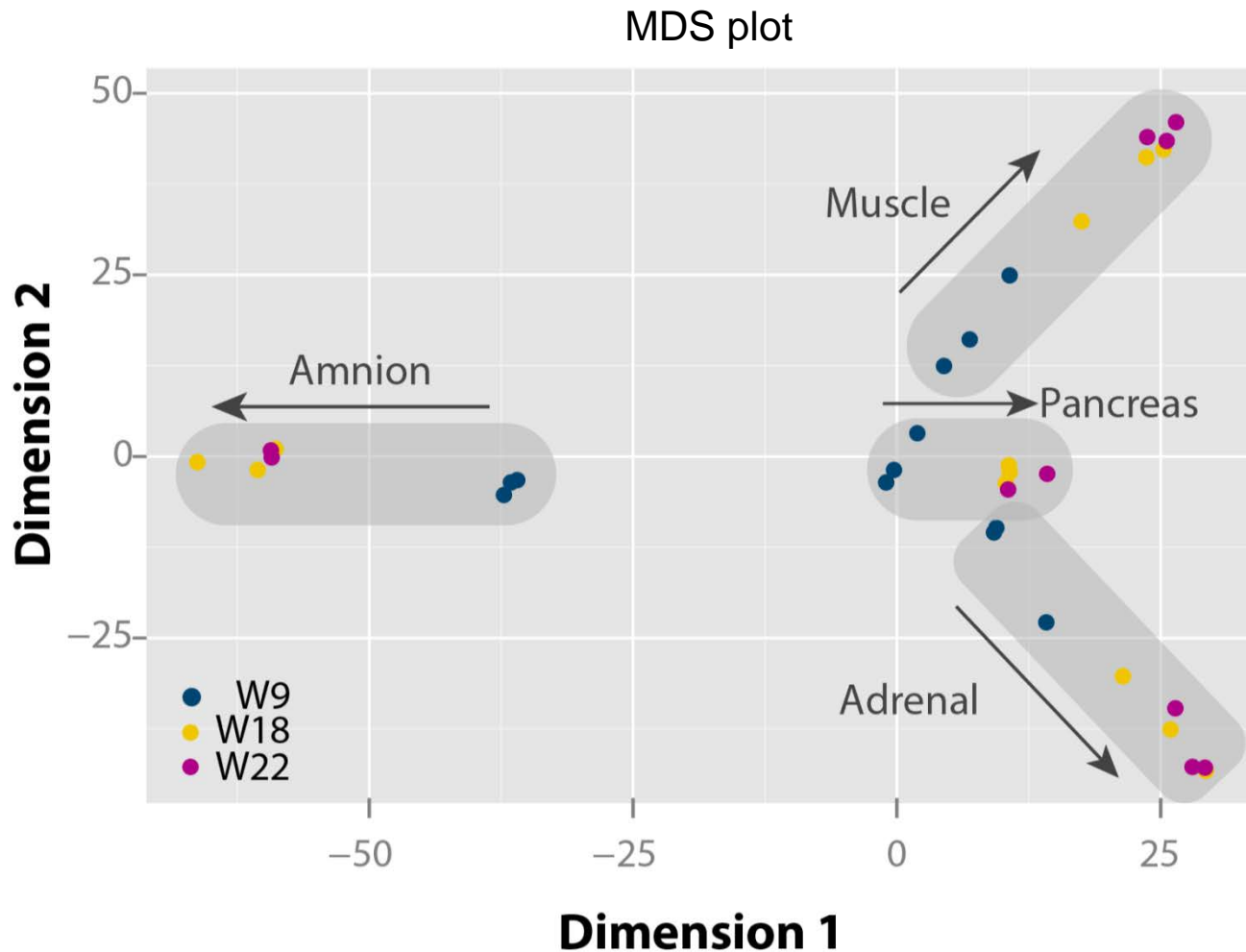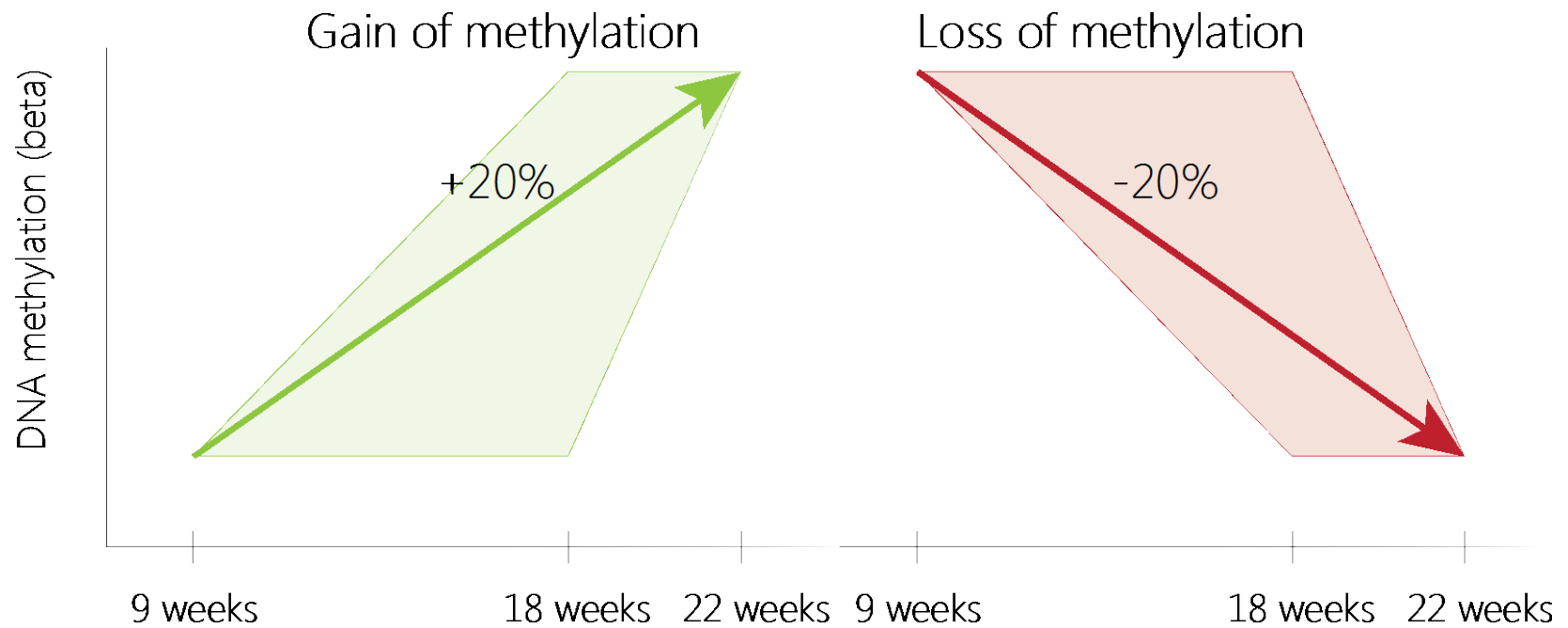
Global methylation patterns

Instructions to the DNA are gradually deposited
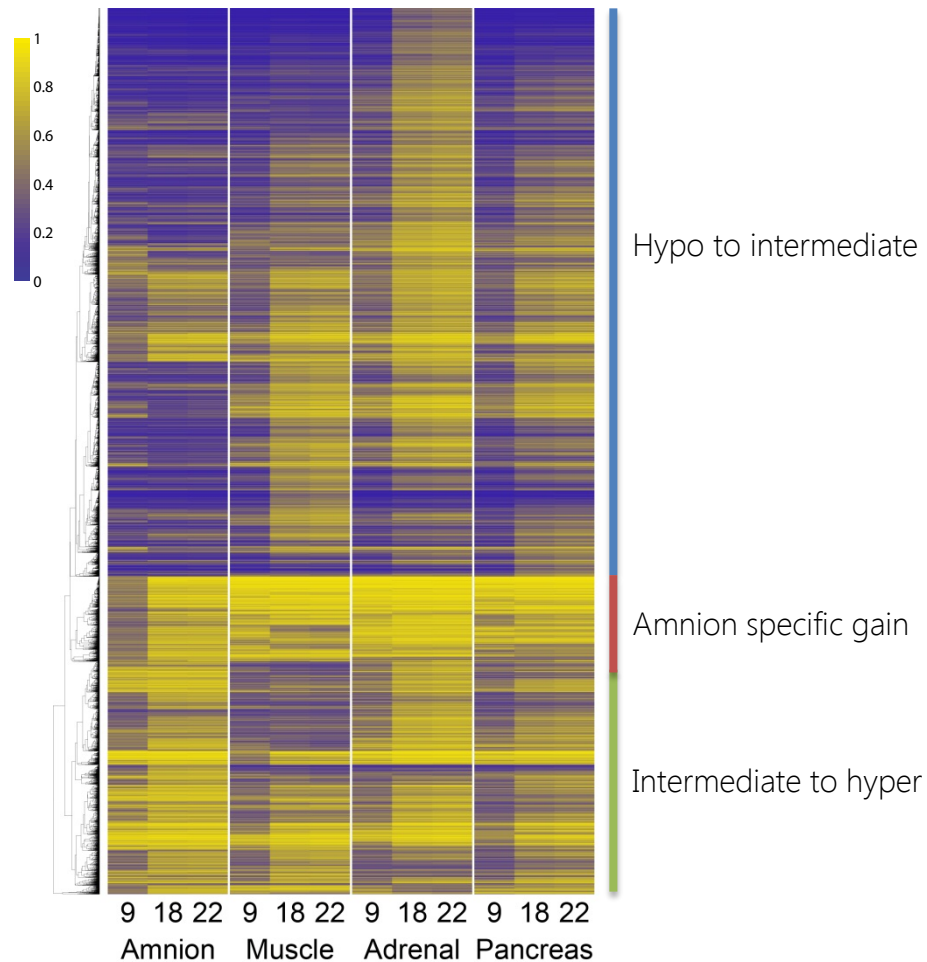

MDS plot

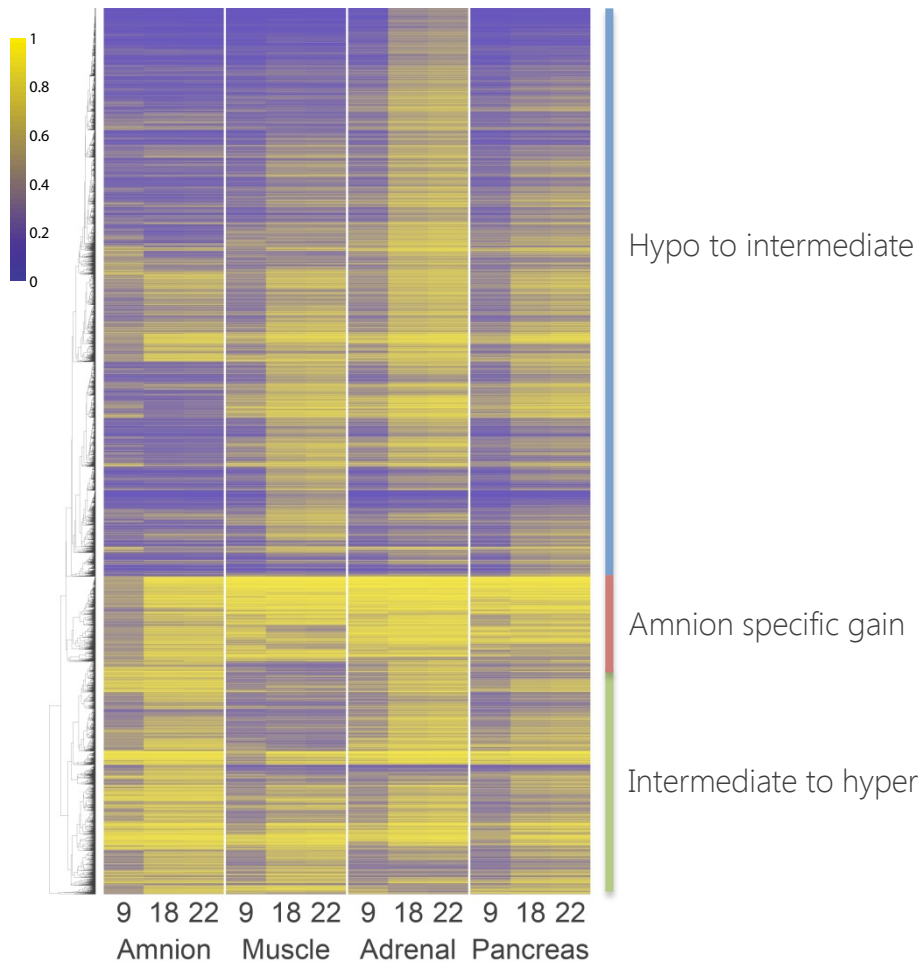# Locus-specific dynamics

Gain and loss of methylation

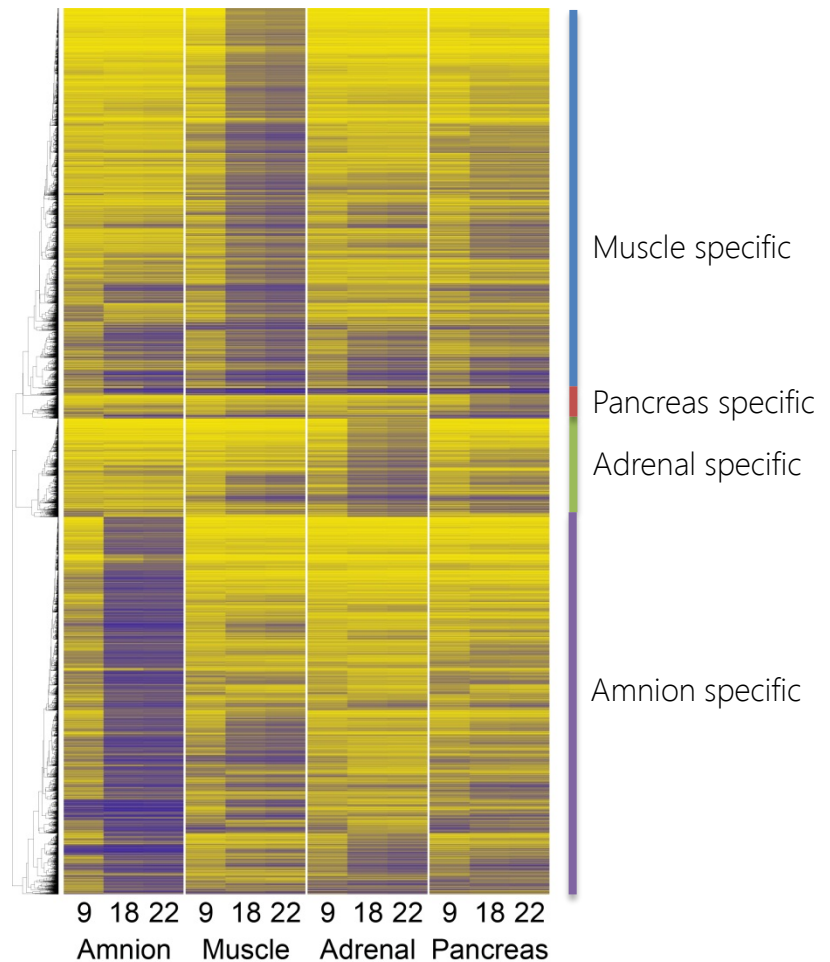Gain of methylation
(~25k CpGs)

Gain and loss of methylation


Gain of methylation
(~25k CpGs)

Loss of methylation
(~26k CpGs)

Developmental vs. tissue-specific

|	Gain of methylation	Loss of methylation
Amnion	Embryonic morphogenesis Regulation of transcription Intracellular signal transduction	Intracellular signal transduction Protein phosphorylation Chemical homeostasis
Muscle	Regulation of transcription Intracellular signal transduction Embryonic morphogenesis	Intracellular signal transduction Muscle contraction Regulation of metabolic process
Adrenal	Regulation of transcription Embryonic morphogenesis Intracellular signal transduction	Regulation of macromolecule metabolism Intracellular signal transduction
Pancreas	Regulation of transcription Embryonic morphogenesis	Intracellular signaling cascade Posttranscriptional regulation of gene expression

Effect methylation depends on genomic context
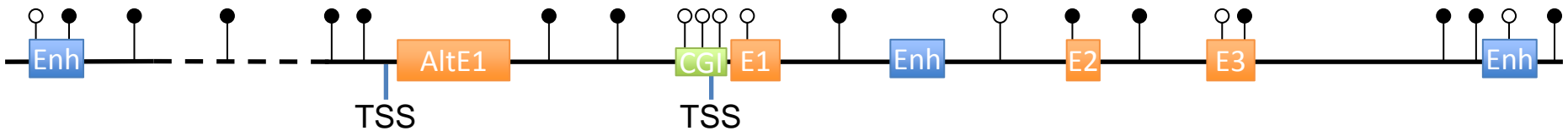
→ Genomic annotation is essential when interpreting DNA methylation data

Reference epigenomes inform on biological function

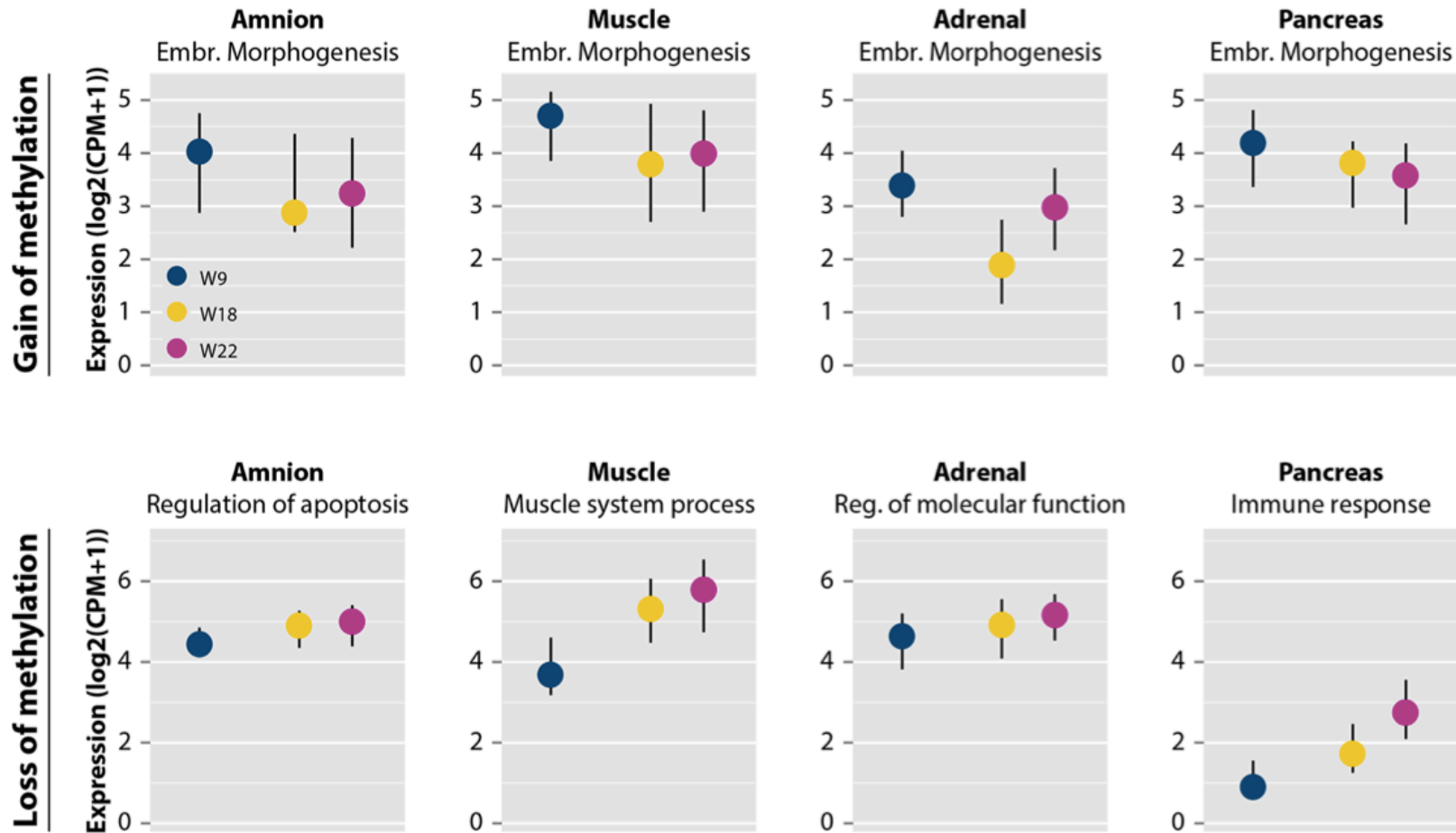## Chromatin states (or segmentation)

Roadmap Epigenomics Consortium. *Nature* 2015.
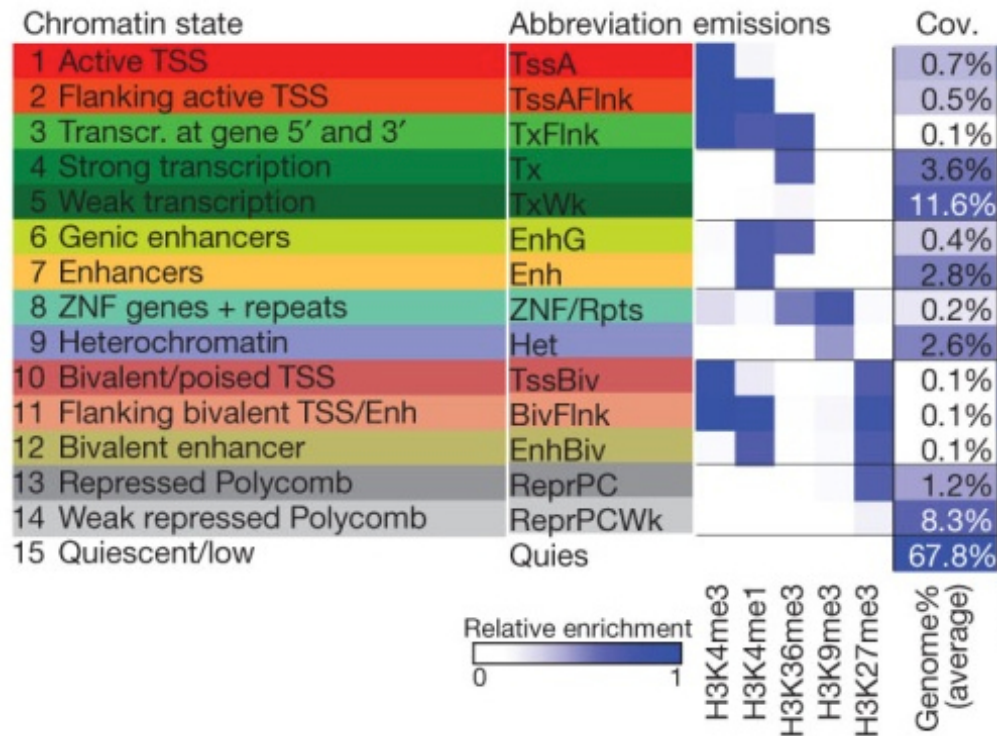
Dynamic methylation at enhancers

Chromatin segmentation
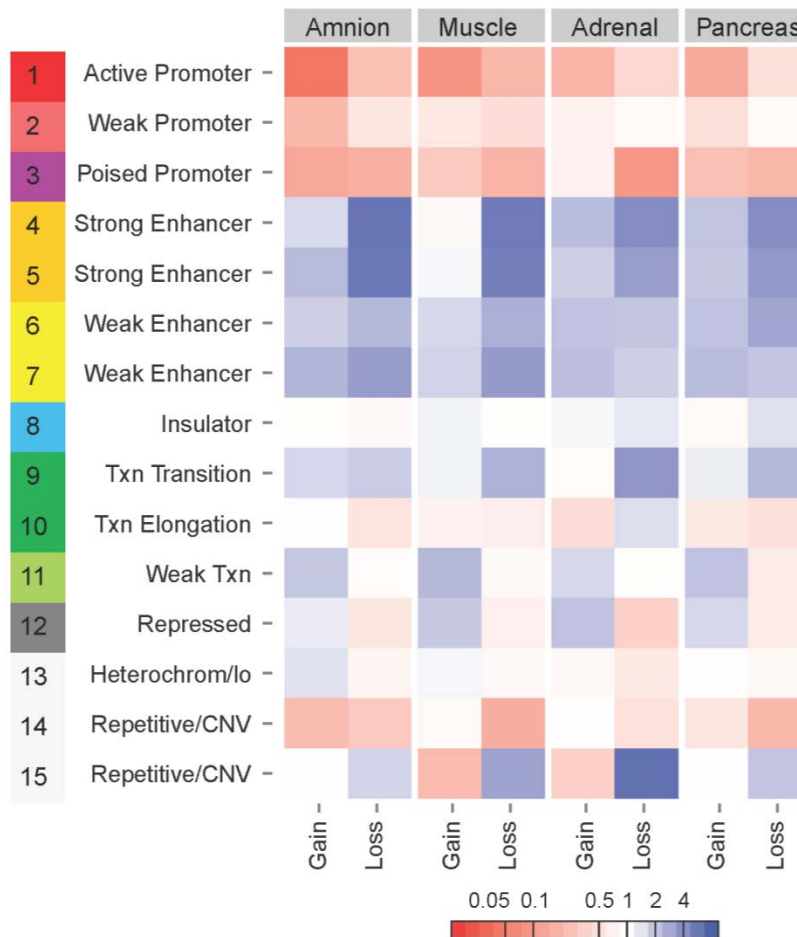
Methylation dynamics accompanied by expression changes

Establishment of epigenetic control in development
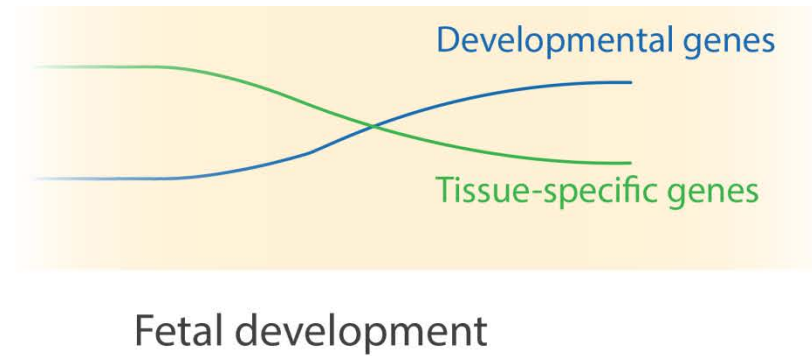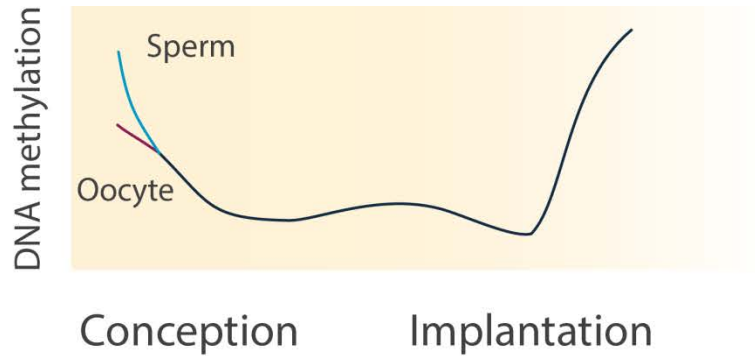
Quick test 1

1. The epigenome consists of multiple, inter-related layers of molecular information and is increasingly implicated in human disease.

a. Give a description (or definition) of epigenetics.

b. Mention 3 layers of information that make up the epigenome.

c. Mention 2 biological processes that are controlled by epigenetic regulation.

Waddington's epigenetic landscape
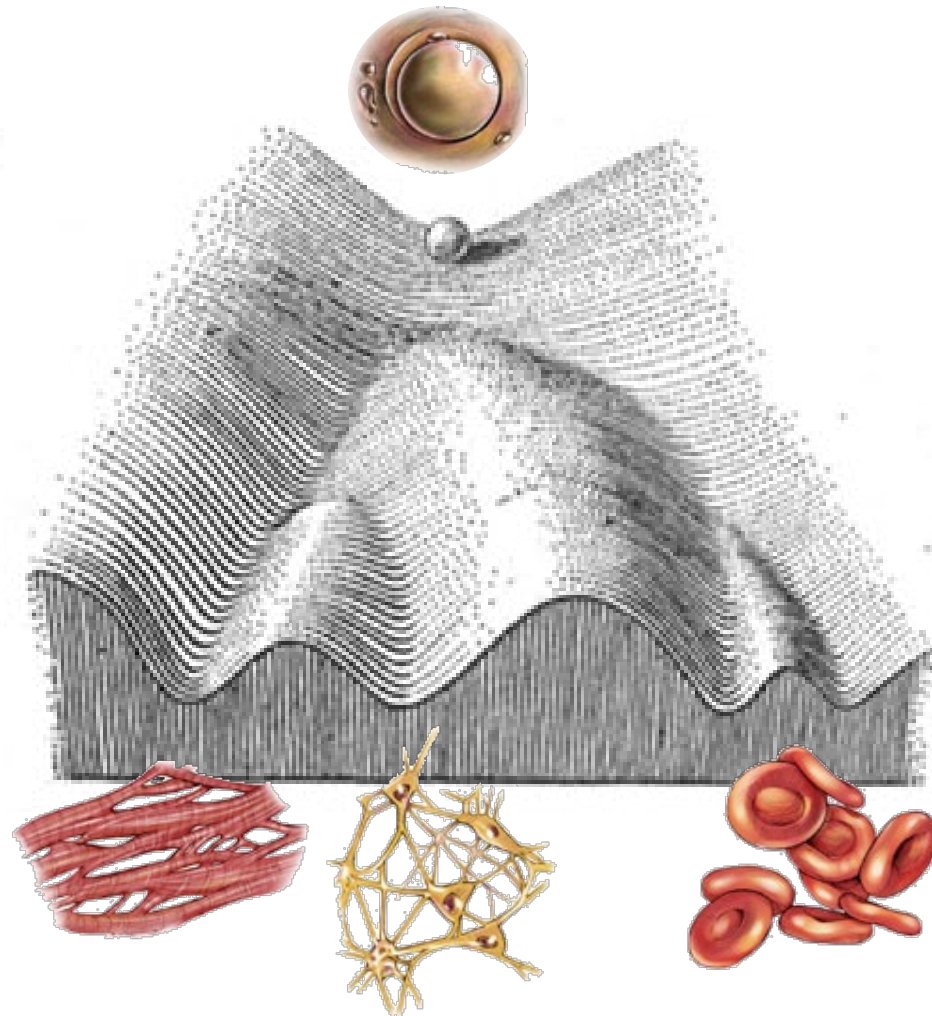
Waddington's epigenetic landscape
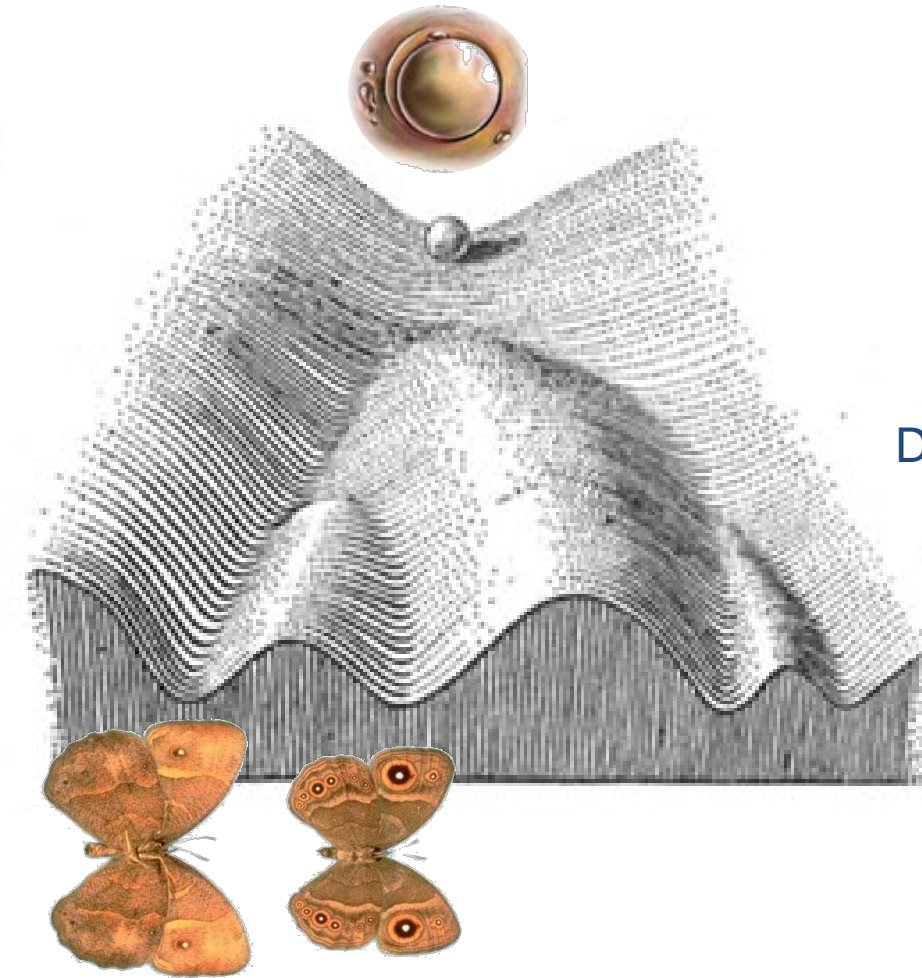
Developmental cue

Developmental plasticity

Phenotypic plasticity

Waddington's epigenetic landscape
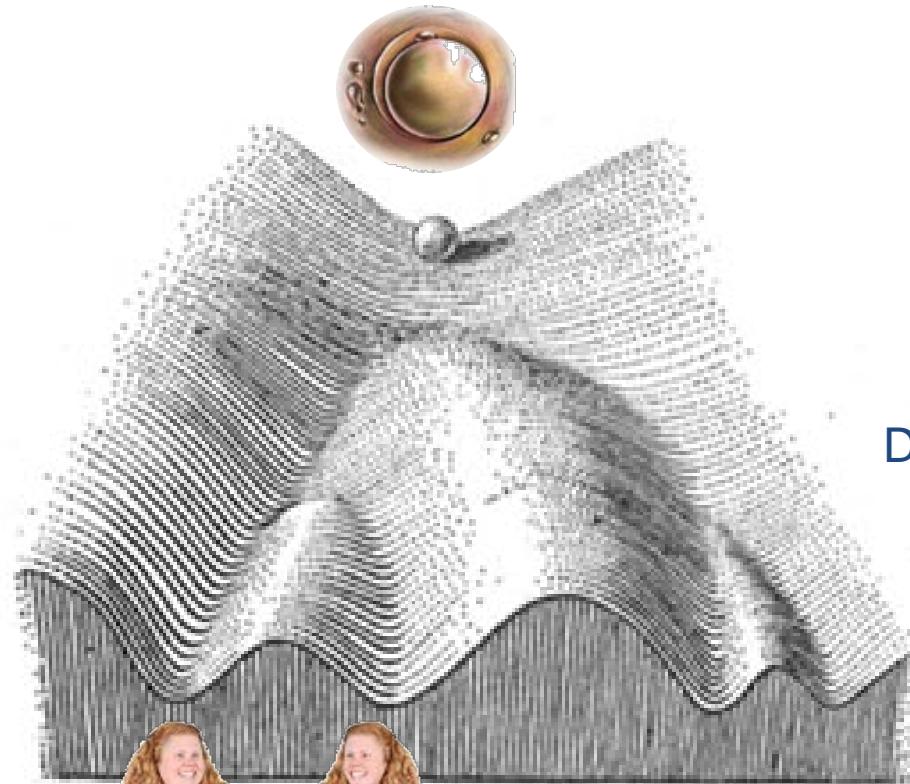
Developmental cue

Developmental plasticity

Phenotypic plasticity

Epigenetics of coat colour

Agouti mouse model (inbred)

- Normal: temporal expression in hair follicle leading to yellow band on each black hair
- Cryptic promoter in transposon leads to expression in every cell if unmethylated
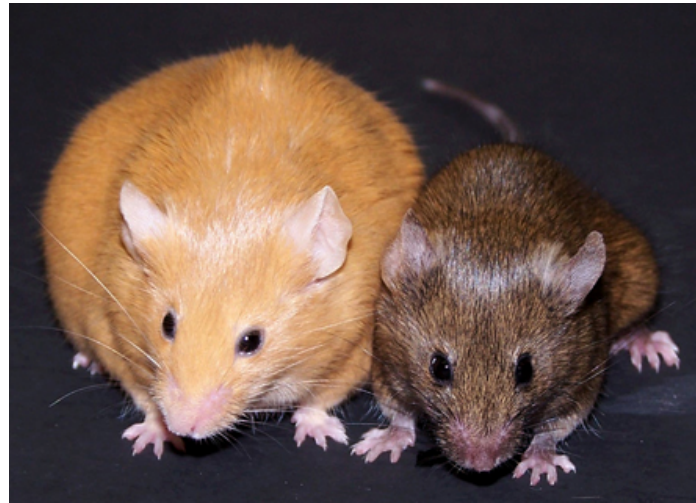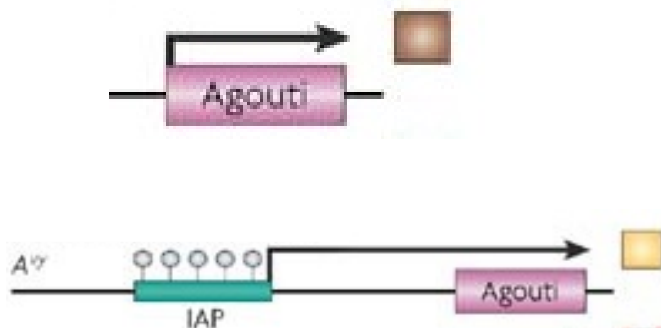
Jirtle & Skinner. *Nat Rev Genet* 2007

Epigenetics of coat colour

Agouti mouse model

- Methyl supplementation diet pregnant females
- Recorded as higher methylation of transposon in offspring that is expressed as a change in coat colour.

Jirtle & Skinner. *Nat Rev Genet* 2007
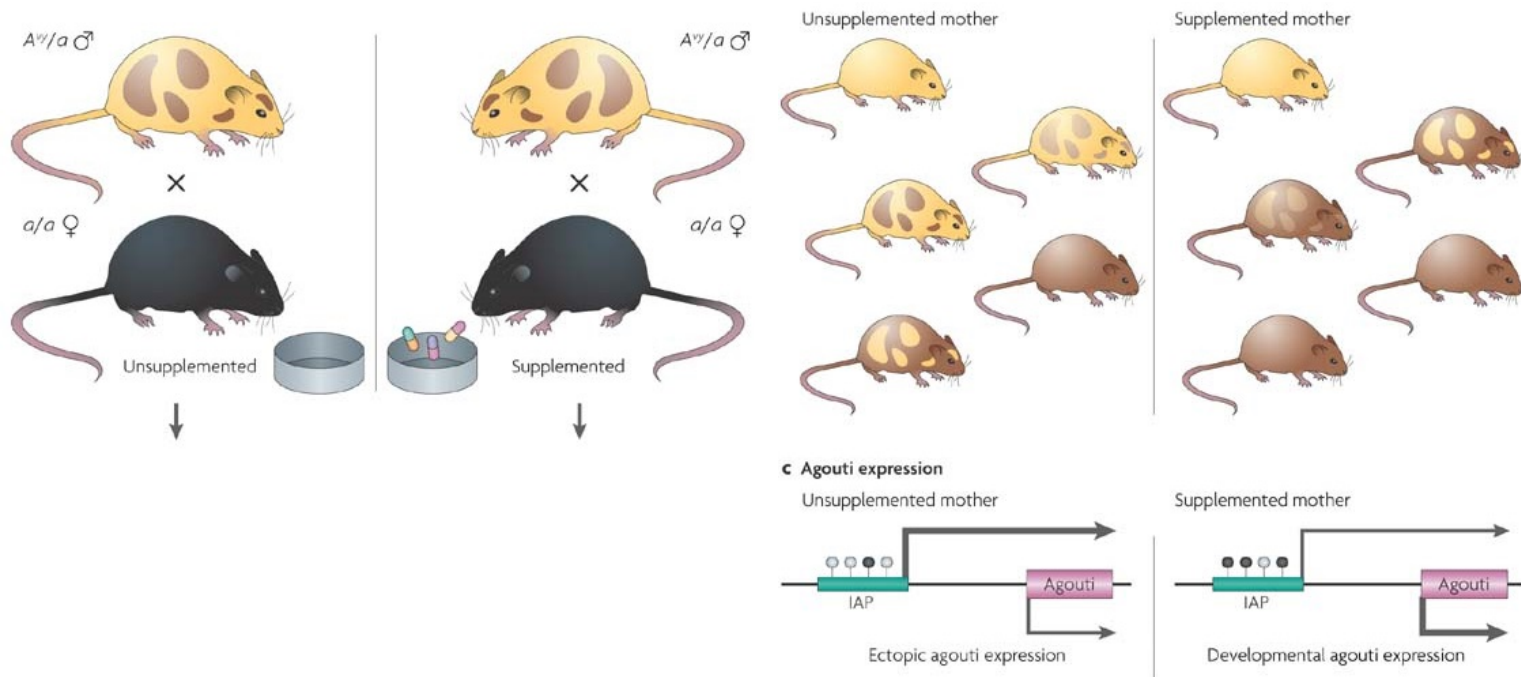
Epigenetics of coat colour

Agouti mouse model
- Methyl supplementation diet pregnant females
- Recorded as higher methylation of transposon in offspring that is expressed as a change in coat colour.
- Propagated during cell division and retained into adulthood: stable, soma-wide methylation of the transposon

Waterland and Jirtle. Mol Cell Biol 2003 / Heijmans et al. *Epigenetics* 2009.
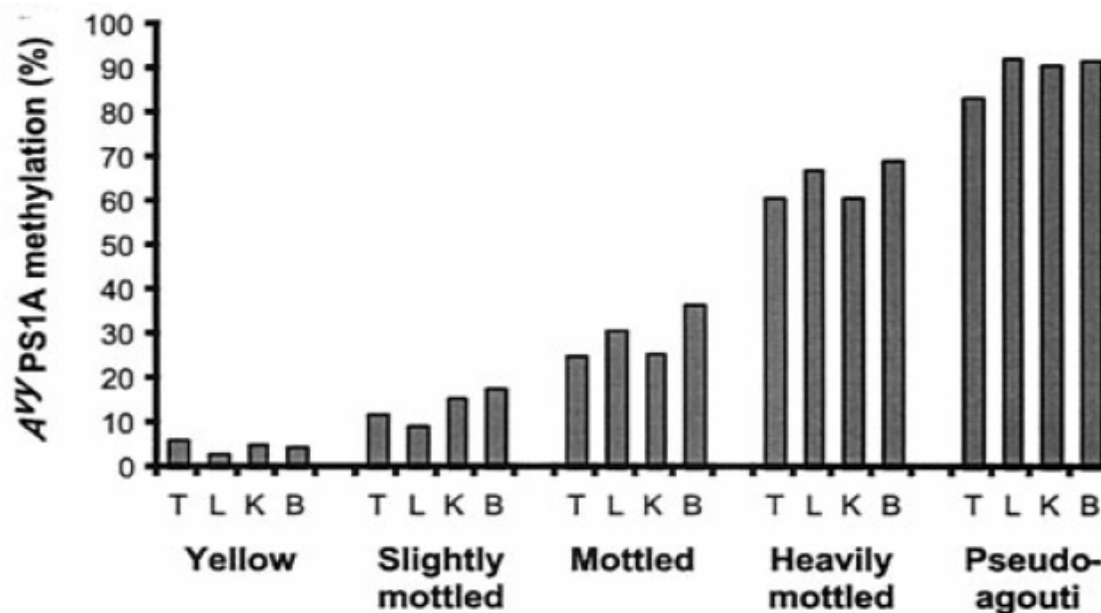
Epigenetics: the memory of the DNA

messy
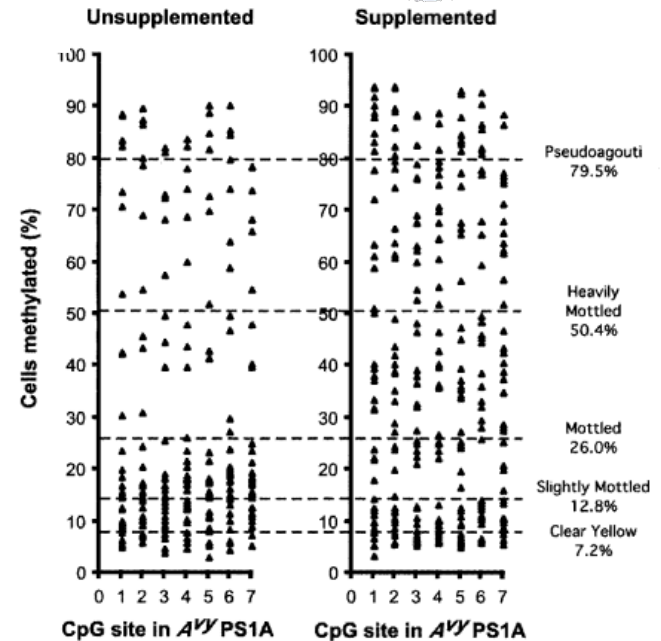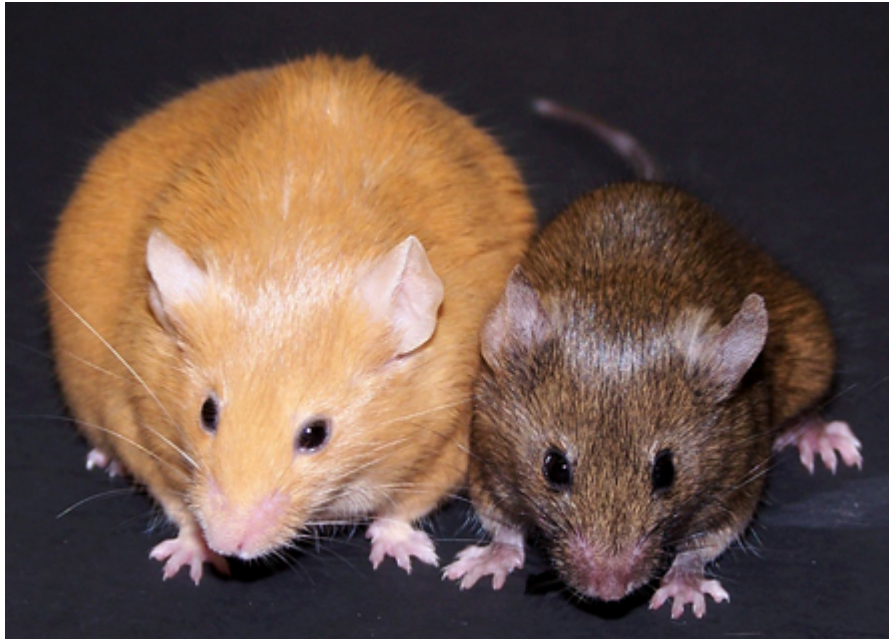
Waterland and Jirtle. *Mol Cell Biol* 2003

From mouse to man

Gametogenesis   Conception   Birth   Growth   Adulthood   Ageing

Epigenetic Mechanisms? → *Health*

=
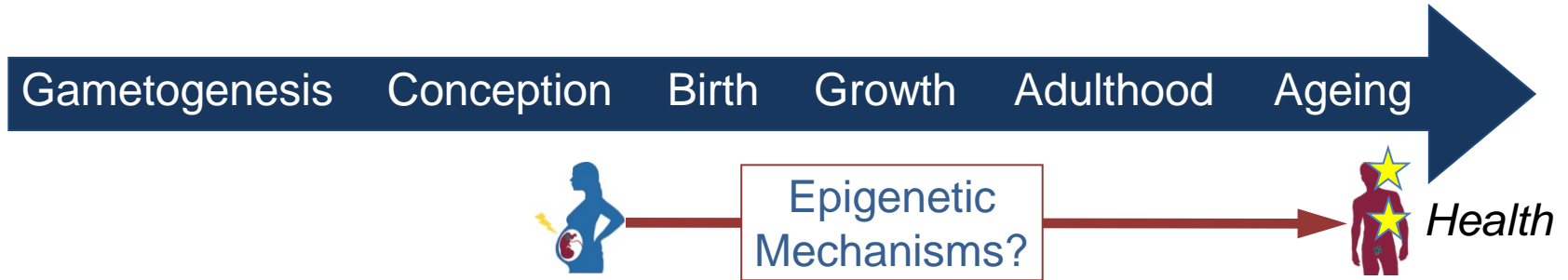
Dutch Hunger Winter at the end of WW2 '44-'45

LUMC

Dutch Hunger Winter

Gametogenesis	Conception	Birth	Growth	Adulthood	Ageing
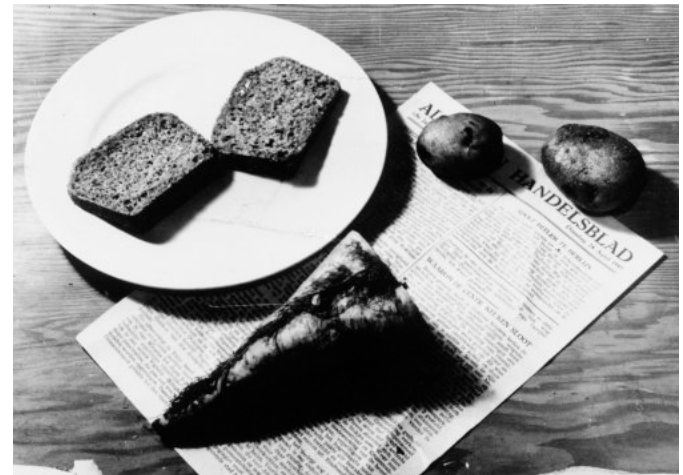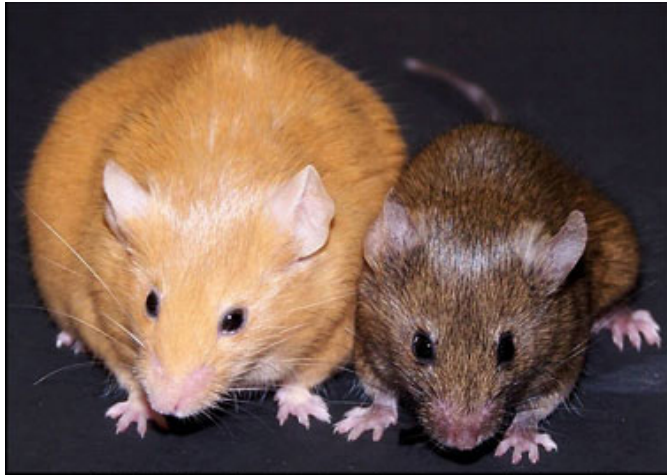
Health

- Severe famine during the winter of 1944-45 in WW2.
- Exposure during intra-uterine life associated with cardiometabolic health and schizophrenia.

Study design

- Quasi-experimental: daily rations <700 kcal/day set for whole population.
- Prospective: traced back exposed individuals at age 60y from records at institutions in affected cities; timing known.
- Best possible controls: unexposed, same-sex siblings.

Hypothesis

Gametogenesis	Conception	Birth	Growth	Adulthood	Ageing

Epigenetic Mechanisms?

Health
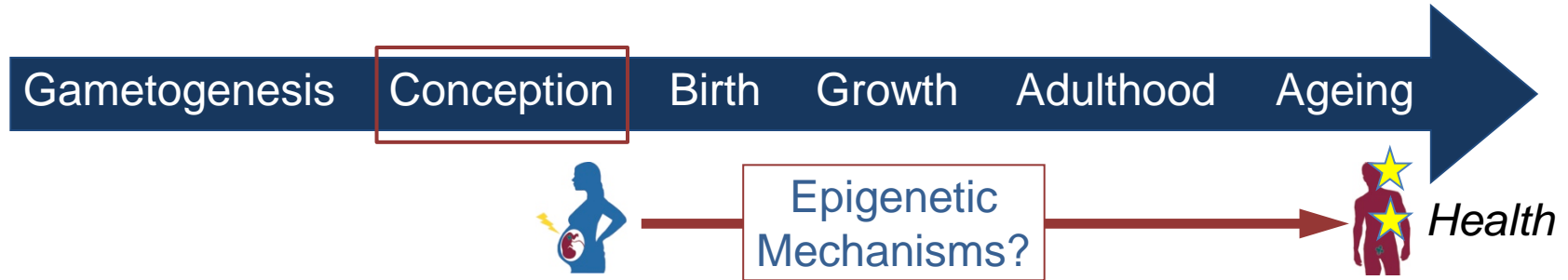
Focus on early gestation (ie. conception during Famine)
- Sensitive window? Critical stage in establishing and maintaining epigenetic marks.
- Soma-wide occurrence? Mitotic inheritance resulting in cross-tissue epigenetic differences (incl. peripheral tissues).
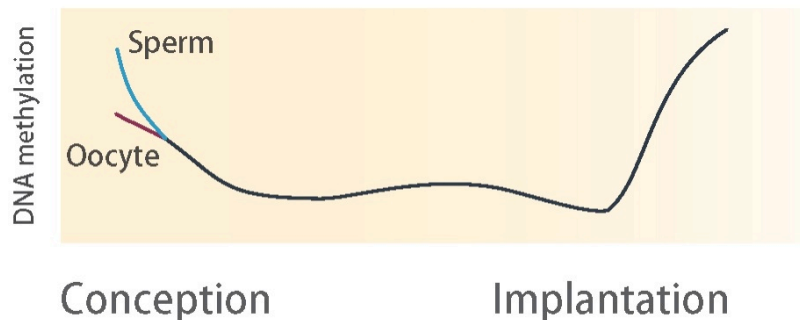
DNA methylation — Sperm / Oocyte — Conception / Implantation

Methylation of a growth gene

- DNA from blood of 60 individuals who were periconceptionally exposed to the Famine 6 decades ago.
- *IGF2*: Prenatal growth factor, also implicated in metabolic regulation and memory; epigenetically controlled.

2000
1500
1000
500
0

Daily ration
(kcal/day)

Dec 1944   Jan 1945   Feb 1945   Mar 1945   Apr 1945   May 1945

Estimated conception
(Last menstrual period)

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Methylation of a growth gene

- DNA from blood of 60 individuals who were periconceptionally exposed to the Famine 6 decades ago.
- *IGF2*: Prenatal growth factor, also implicated in metabolic regulation and memory; epigenetically controlled.

Heijmans et al. *PNAS* 2008
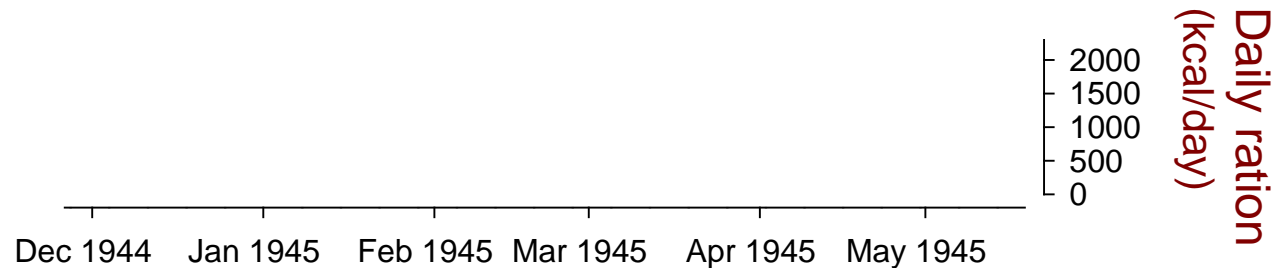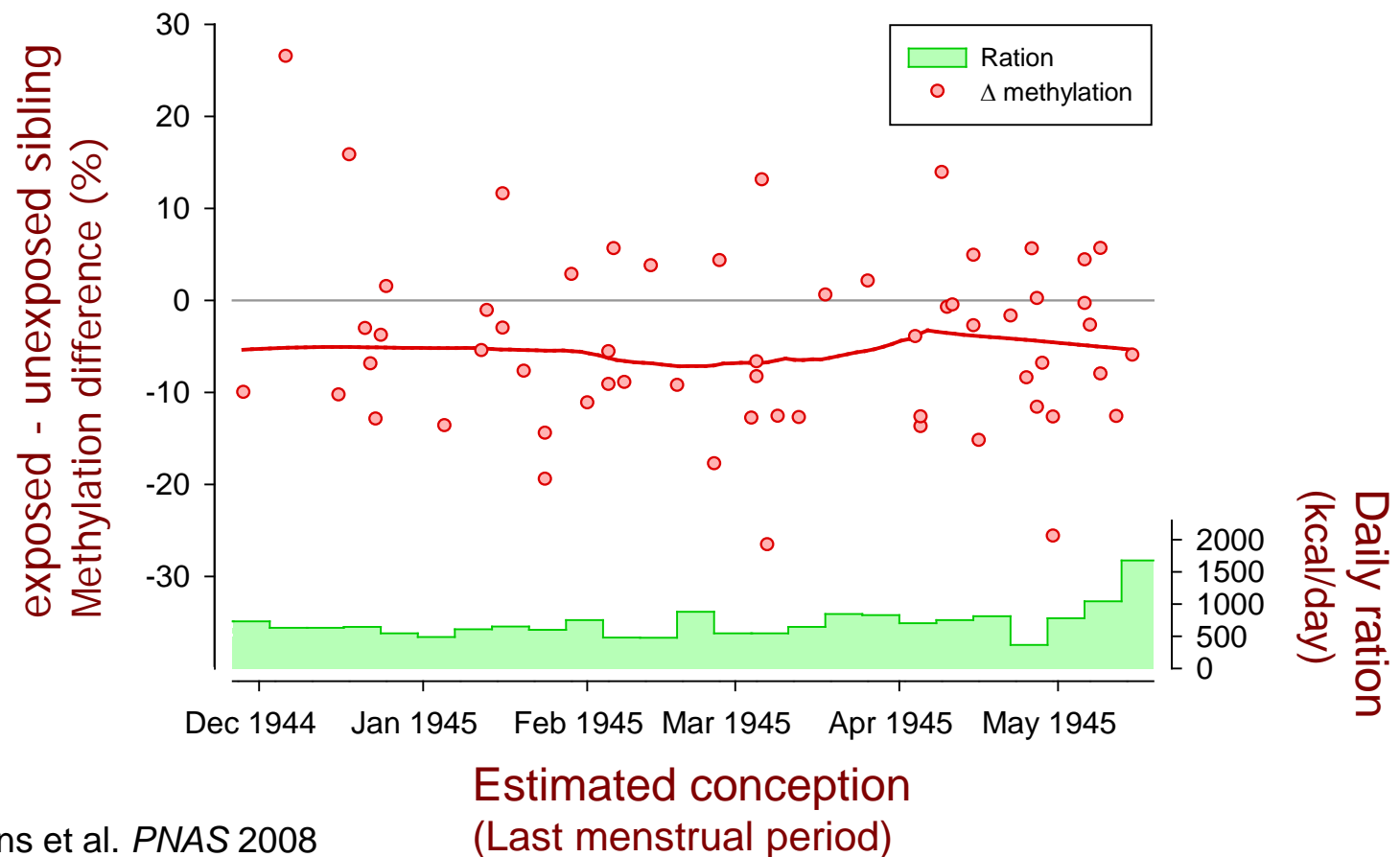
Looking across 16 genes

Gene	Early	Late
IGF2	↓	
GNAS	↑	↓
INSIGF	↓	
IL10	↑	
LEP	↑	↑
ABCA1	↑	

Tobi et al. *Hum Mol Genet* 2009
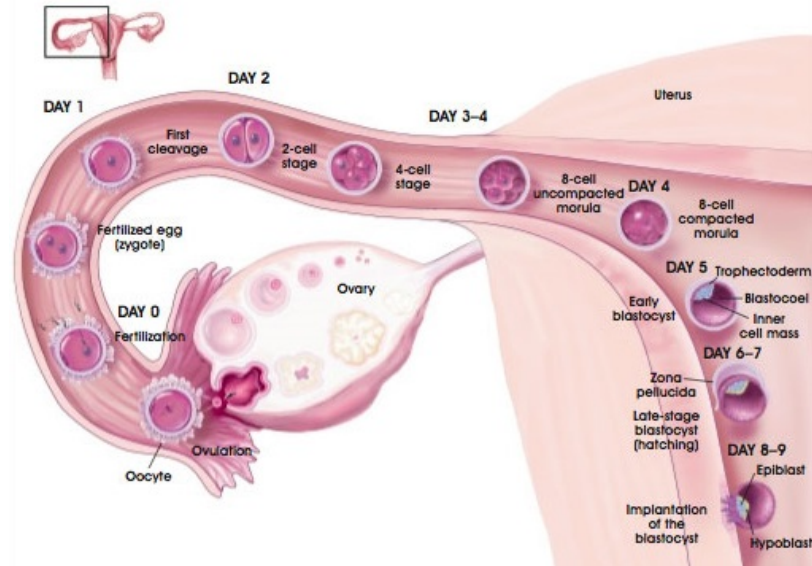
Picture: Terese Winslow, 2001

Genome-scale studies

Genes ⟶ Genome

Bisulfite sequencing

M?
'
...CCTGGTA**CG**TCTAG...

Unmethylated   Methylated   Bisulfite conversion

...UUTGGT**UG**TUTAG...   ...UUTGGT**CG**TUTAG...

HT sequencing

TTGGT**TG**TT

GGT**CG**TTTAG   TGGT**TG**TT

TTTGGT**TG**T

TTTGGT**TG**TTTAG   GT**TG**TTTAG

GT**CG**TTTAG   GGT**CG**TTTAG

TTTGGT**CG**TT   TTGGT**TG**TTT

Bisulfite sequencing

M?

...CCTGGTA**CG**TCTAG...

Unmethylated   Methylated   Bisulfite conversion

...UUTGGT**UG**TUTAG...   ...UUTGGT**CG**TUTAG...
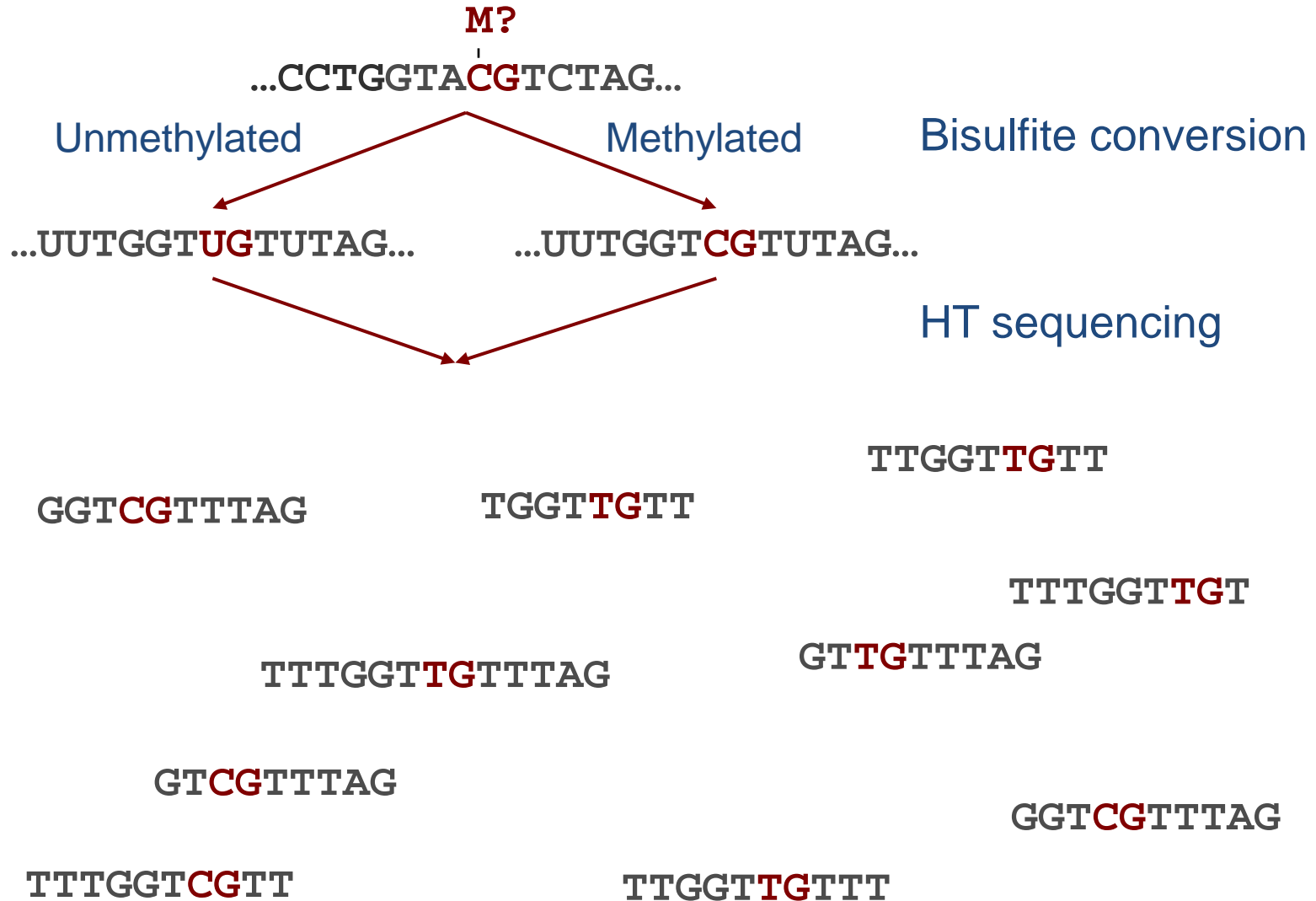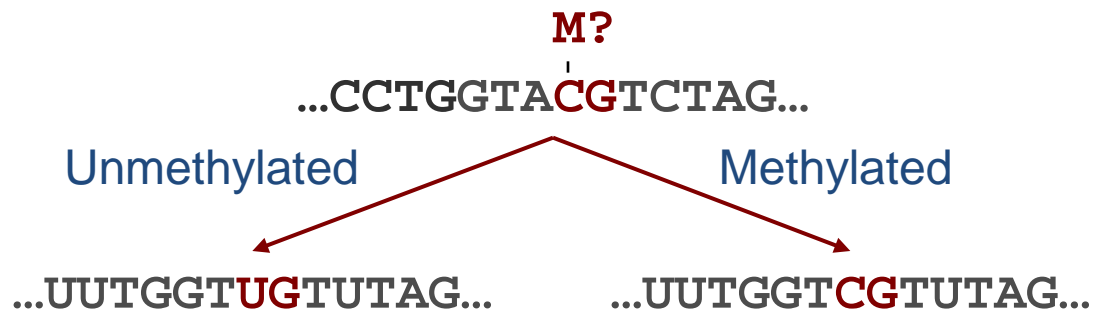
HT sequencing &
Alignment

TTGGT**TG**TT
TTTGGT**TG**T
   GT**TG**TTTAG
TGGT**TG**TT
TTTGGT**TG**TTTAG
TTGGT**TG**TTT
   GT**CG**TTTAG
TGGT**CG**TTTA
TTTGGT**CG**TT
   GGT**CG**TTTAG

This fake example
10x coverage, 4/10=40%
(95% CI, 17-70%)
methylated

Genome-scale study of prenatal famine exposure

- Focus on periconceptional exposure: 24 exposed + 24 sibling controls
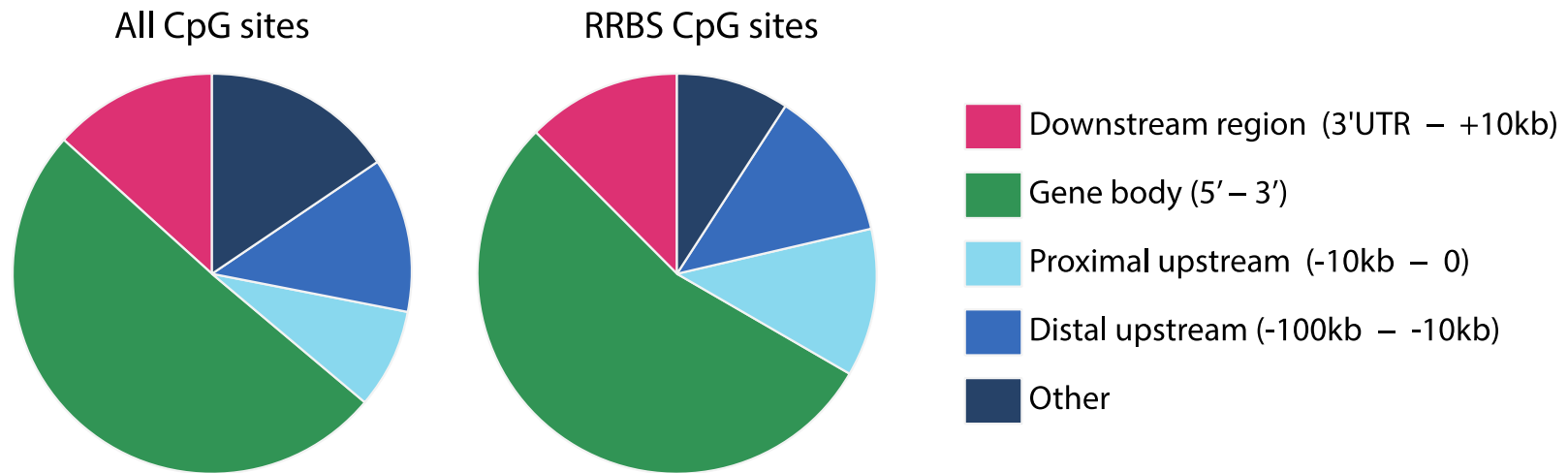- Reduced-Representation Bisulfite Sequencing (RRBS): *Msp*I (-CCGG-)

All CpG sites   RRBS CpG sites

- Downstream region (3'UTR − +10kb)
- Gene body (5' − 3')
- Proximal upstream (-10kb − 0)
- Distal upstream (-100kb − -10kb)
- Other

Elmar Tobi
Tobi et al. *Nat Commun* 2014
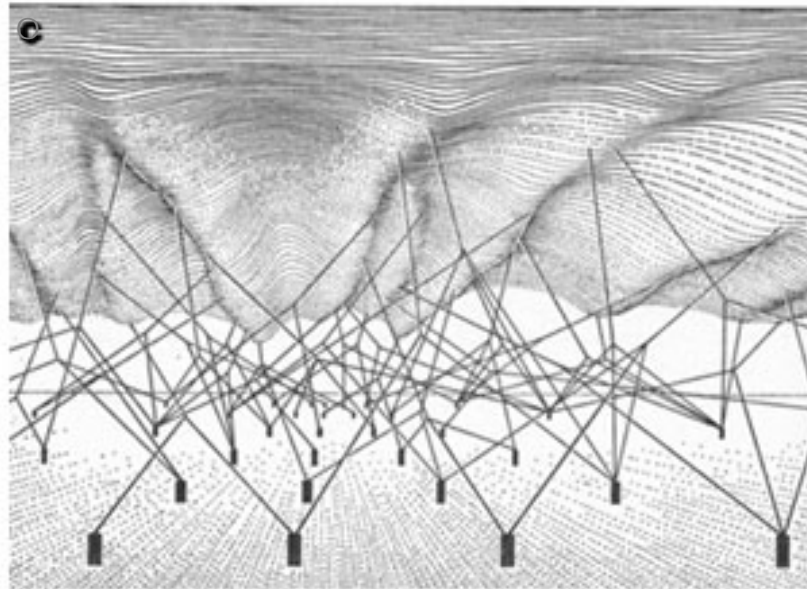
Genome-scale study of prenatal famine exposure

- Focus on periconceptional exposure: 24 exposed + 24 sibling controls
- Reduced-Representation Bisulfite Sequencing (RRBS)
- Methylation of 1.2M CpG sites after QC and exclusion uninformative sites (mean coverage 28x; call rate 0.998)
- Mapping to genomic features to decrease multiple testing, accumulate evidence over adjacent CpGs and increase interpretability.

Promoter, enhancer etc.

11/28

3/16   3/8   6/14   2/6   0/11   0/5

Tobi et al. *Nat Commun* 2014

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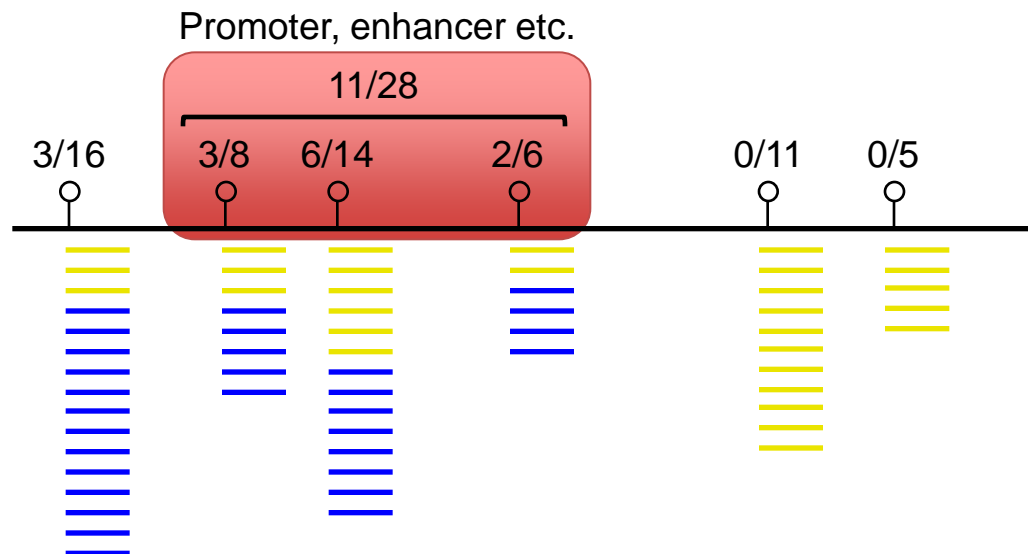
Validated top-hits

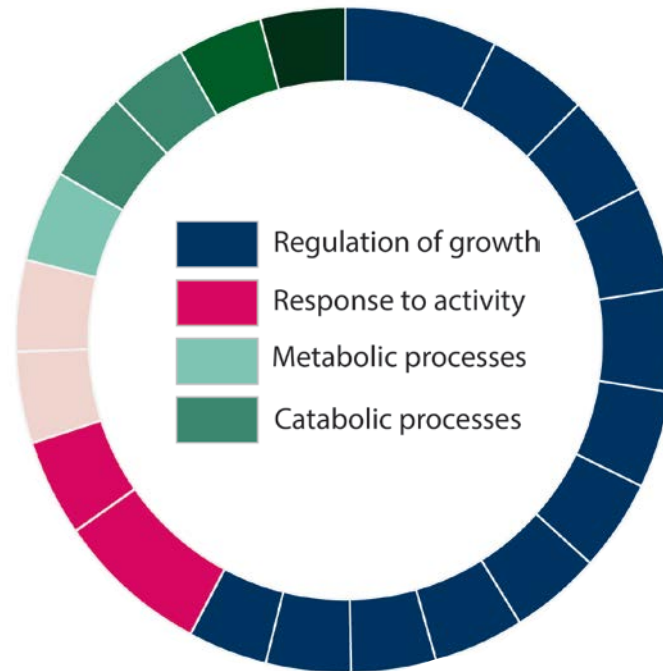| Gene	Location	Function
SMAD7 SMAD family member 7	25kb downstream	TGFβ signaling, colorectal cancer, β-cell function & development
CDH23 cadherin-related 23	Intragenic	Inner ear development, hearing loss
INSR insulin receptor	Intragenic	Insulin signaling, growth, height
CPT1A carnitine palmitoyltransferase-1	Intragenic	Fatty acid β-oxidation, fatty acid-induced IR and inflammation in adipocytes
KLF13 Krüppel-like factor 13	Intragenic	LDLR regulation, schizophrenia
RFTN1 raftlin	Intragenic	Eye development, obesity

LU
MC

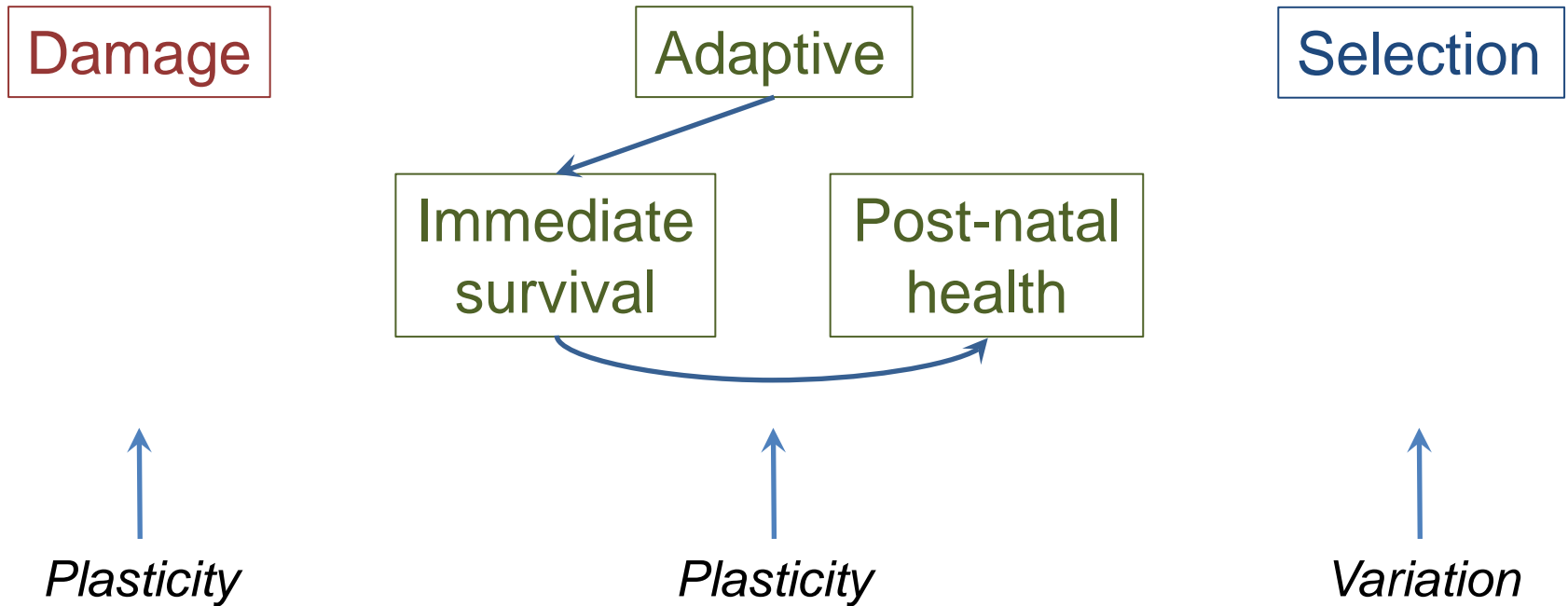# Waddington's epigenetic landscape
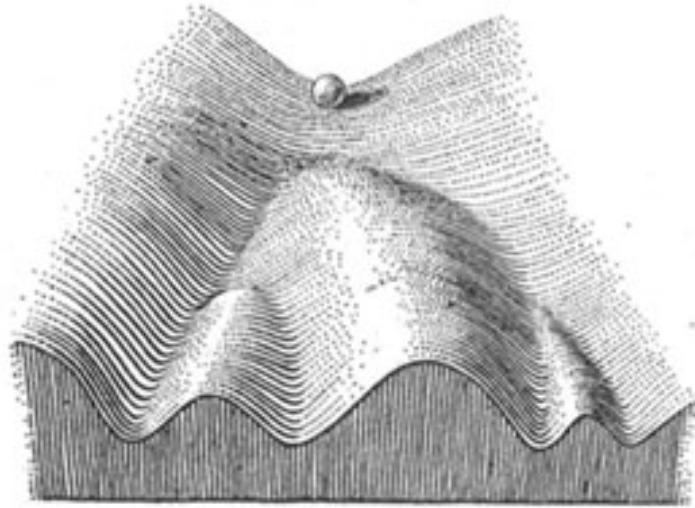
Genome-scale view of epigenetic differences after famine exposure

Regulation of growth
Response to activity
Metabolic processes
Catabolic processes

Tobi et al. *Nat Commun* 2014

Why? – some speculation

Damage

Adaptive

Immediate survival

Post-natal health

Selection

Plasticity

Plasticity

Variation
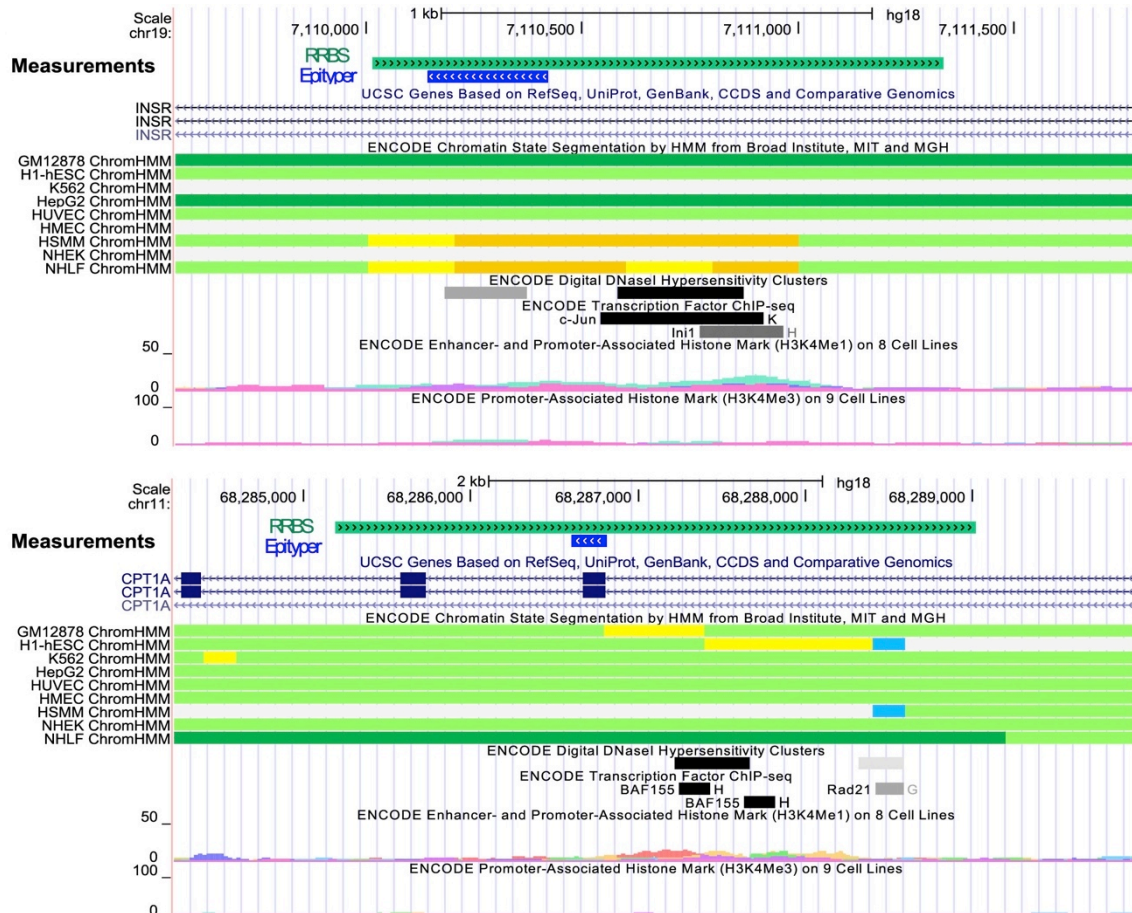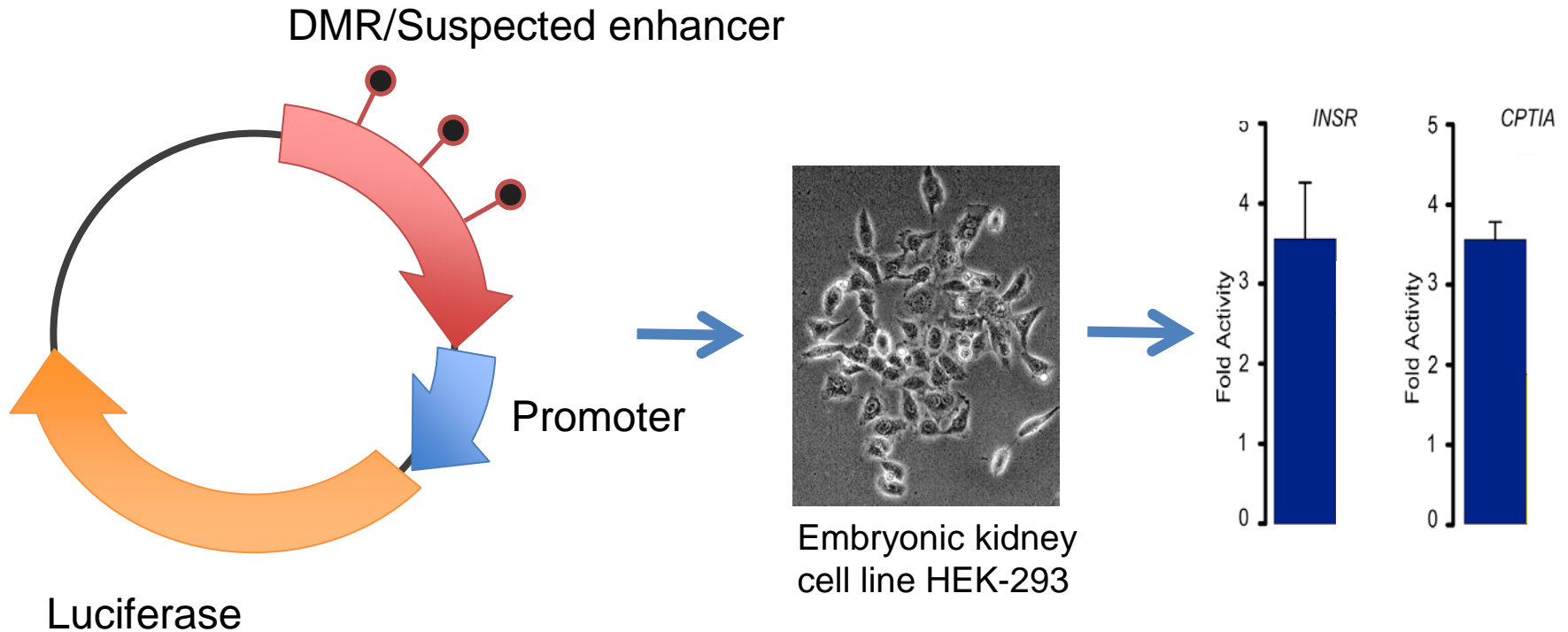
Towards causality

- *In silico* annotation-based predictions of DMR functionality

Towards causality

- *In silico* annotation-based predictions of DMR functionality
- *In vitro* testing DMR functionality and evaluate epigenetic plasticity (specific exposures and beyond DNA methylation)

DMR/Suspected enhancer

Promoter

Luciferase

Embryonic kidney
cell line HEK-293
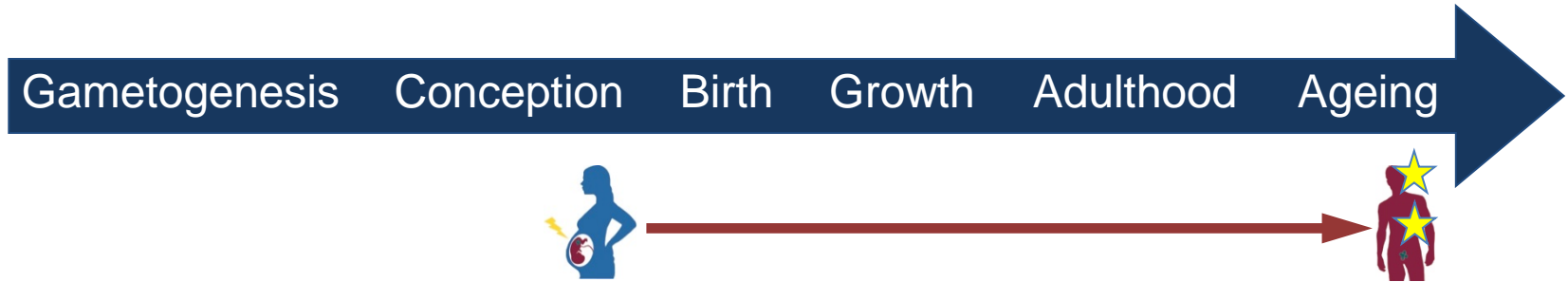
Towards causality

- *In silico* annotation-based predictions of DMR functionality

- *In vitro* testing DMR functionality and evaluate epigenetic plasticity (specific exposures and beyond DNA methylation)

- *In vivo* experiments in animals (moving from principles to testing specific human outcomes), short-term interventions in humans

- Integrative genomics from genome and epigenome to transcriptome and further

- Causal inference testing statistical approach to evaluate whether DNA methylation mediates associations bewteen prenala adversity and later-life outcomes

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Filling in the gaps

- Combining evidence from complementary studies
  Overcoming limitations of natural experiments
  To early life maternal folate associated with higher *IGF2* methylation (Steegers-Theunissen et al. *PLoS ONE* 2009)
  To clinical outcomes famine DMRs associated with coronary heart disease (Talens et al*. IJE* 2012, Guay et al. *Epigenetics* 2012)

Mill and Heijmans. *Nat Rev Genet* 2013
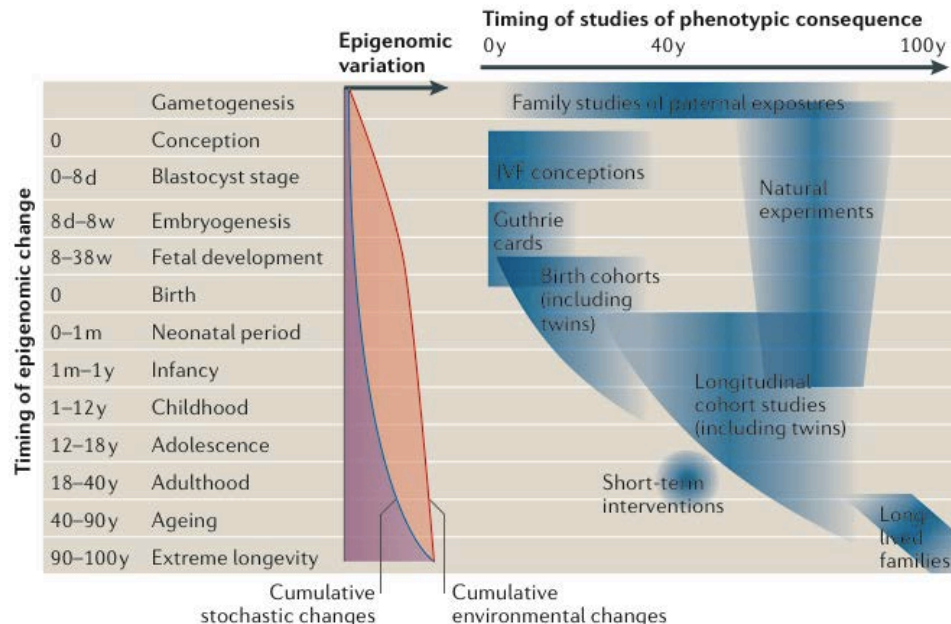
Epigenetic differences after prenatal famine exposure

| Gametogenesis | Conception | Birth | Growth | Adulthood | Ageing |

- Exposure to famine during early development is associated with persistent epigenetic differences in humans.

- DNA methylation differences are modest but extend into biological pathways.

- DNA methylation signatures identified link prenatal famine exposure to growth and metabolism.

Population epigenomics

Epidemiology	Biology
Cases/controls from same study base Longitudinal studies and rich data Bias and (residual) confounding (Reverse) causation Combine designs for causal inference Mendelian randomization	Interrogated portion of the epigenome Cell and tissue specificity Genomic context measured regions Relevance small effect sizes Gene expression effect Functional studies to infer causality

Study design

Hypothesis

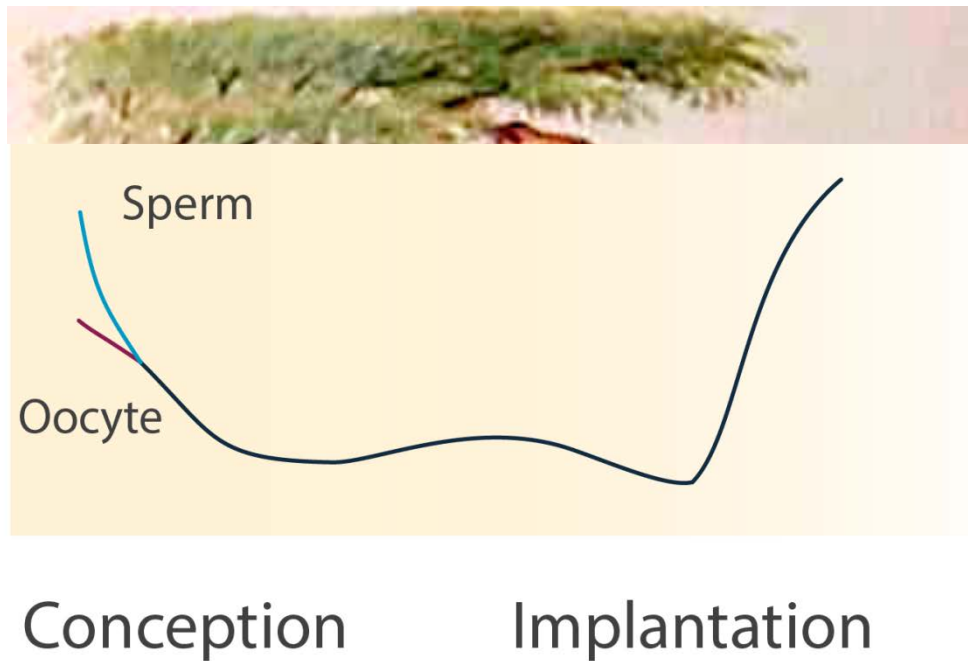(And bigger is better is not good enough)

Mill and Heijmans. *Nat Rev Genet* 2013

Transgenerational epigenetics?

Lamarckism revisited – the inheritance of acquired traits
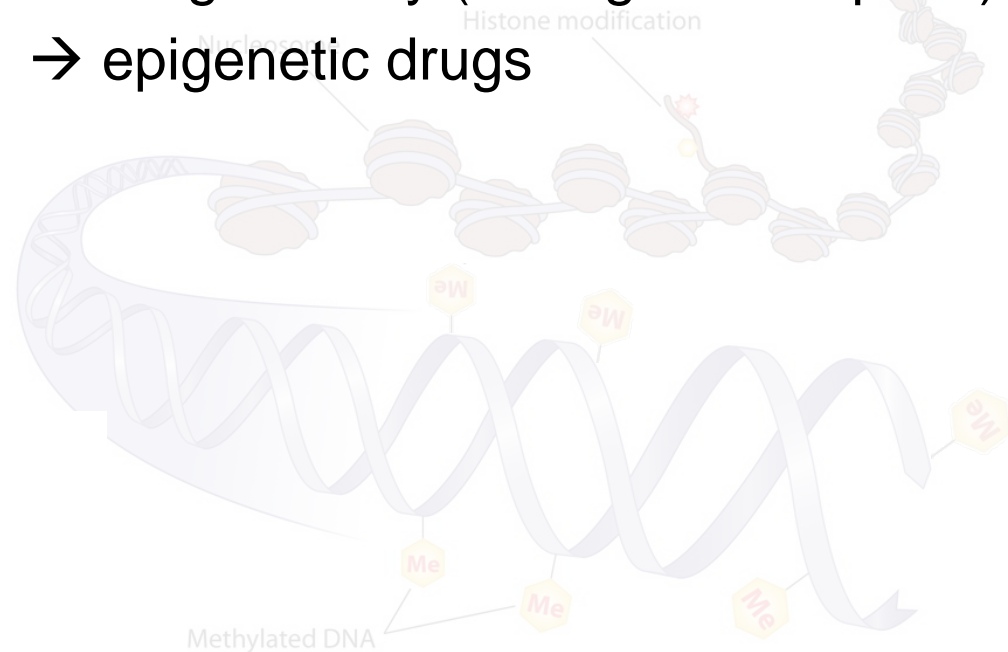

Jean-Baptiste Lamarck
(1744-1829)

Insights from epigenomics

1. Fundamental biology → development & cell differentiation
2. Disease mechanisms → dysregulation of the genome
3. Disease risk → interface genome & environment
4. Biomarkers/ Surrogate markers → (past) exposure, diagnosis, prognosis or drug efficacy (surrogate end-point)
5. Treatment → epigenetic drugs

Learning objectives

- The molecular basis of epigenetics.

- The role of epigenetics in fundamental biology.

- Epigenetics as integrator of environmental signals.

- Challenges in designing human epigenetics studies.

Acknowledgements

Roderick Slieker

Elmar Tobi