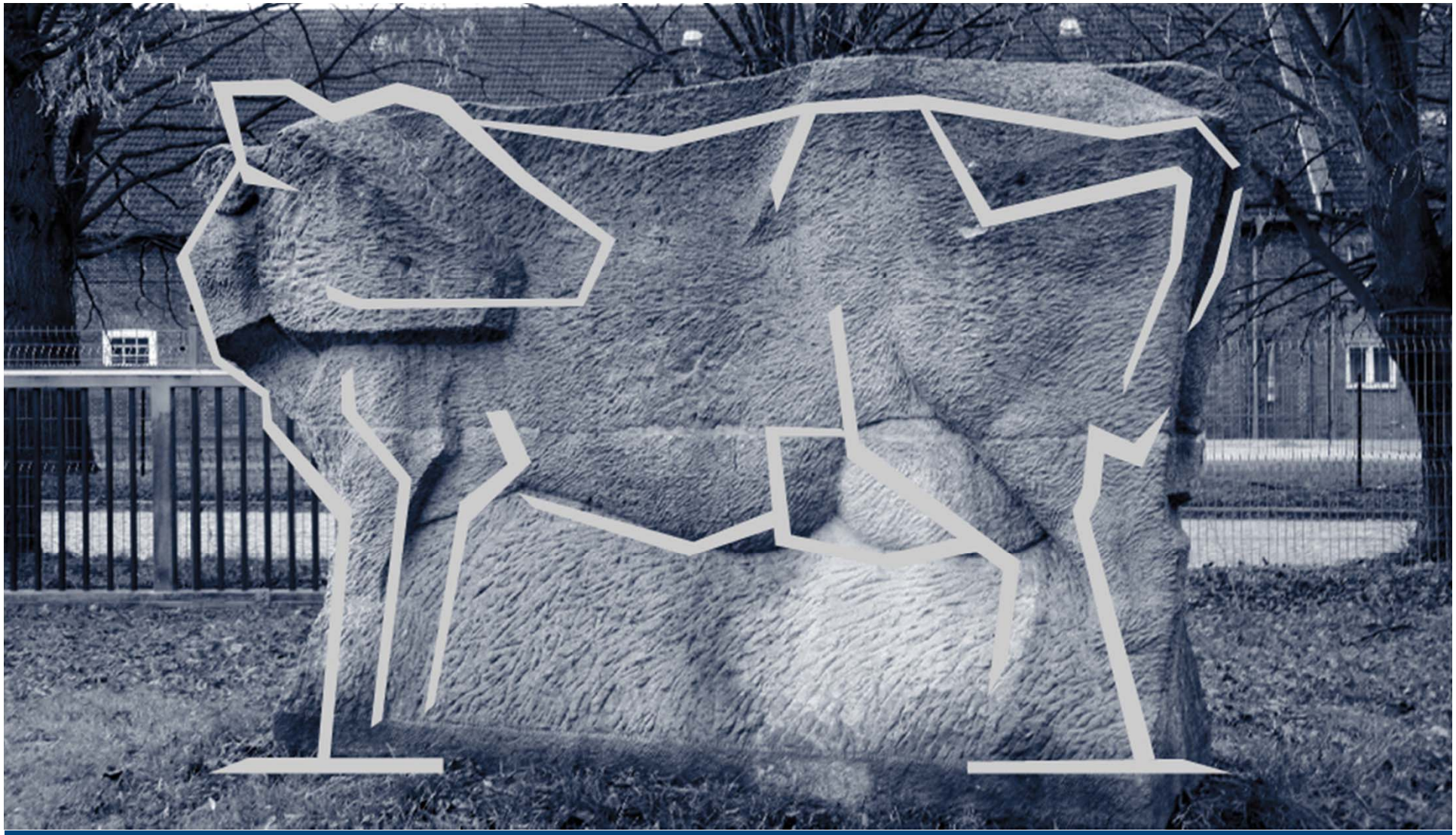


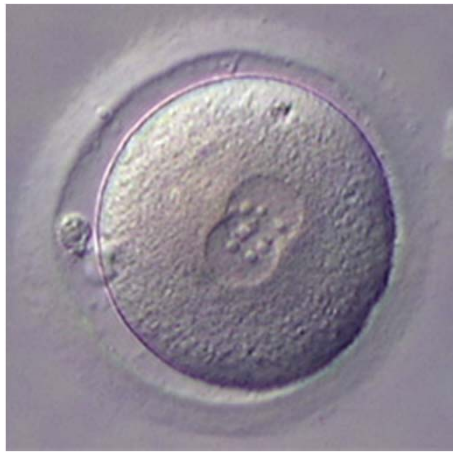
LEIBNIZ INSTITUTE
FOR FARM ANIMAL BIOLOGY

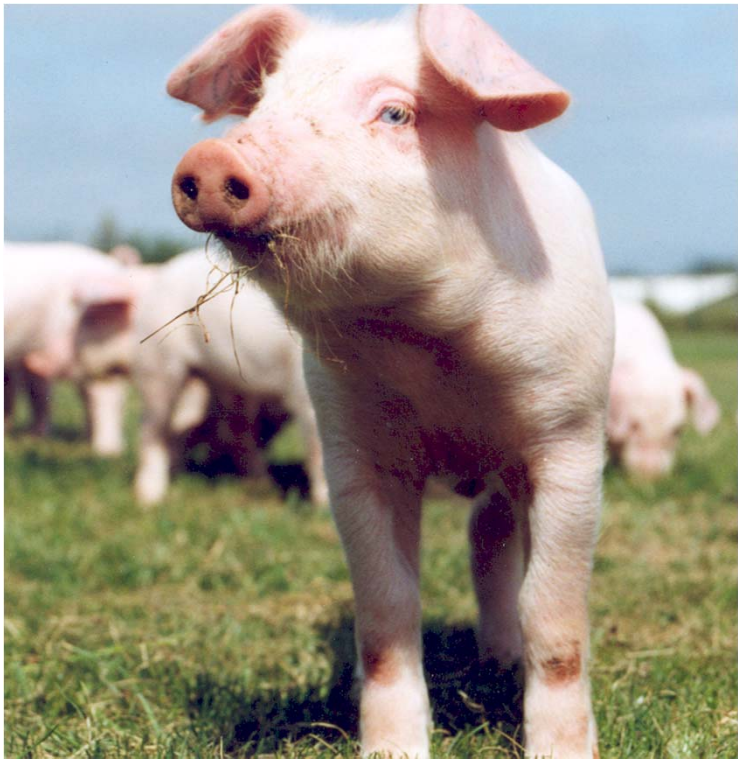
Epigenetic mechanisms and their implications in animal breeding

Klaus Wimmers, Nares Trakooljul, Michael Oster, Eduard Murani, Siriluck Ponsuksili

Leibniz Institute for Farm Animal Biology (FBN), Germany
Institute for Genome Biology

The fate of cells





Zygote:
a single cell with genetic information

becomes a

complex organisms with 10^{14} cells all containing the same genetic information

genetic + epigenetic information

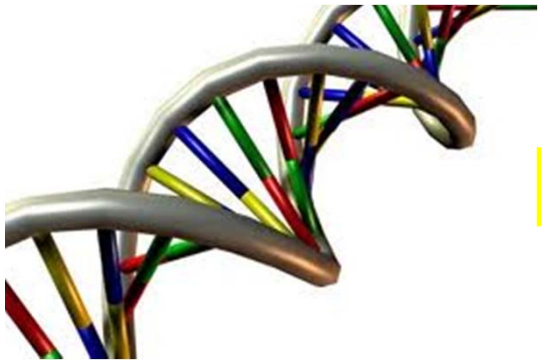
➔at least two forms of information in the cell nuclei:

1. Genetic information: general instructions for the manufacture of all proteins – DNA sequence

2. Epigenetic information: additional instruction on how, when and where these information should be used – epigenetic marks

Genotype-Phenotype-Mapping

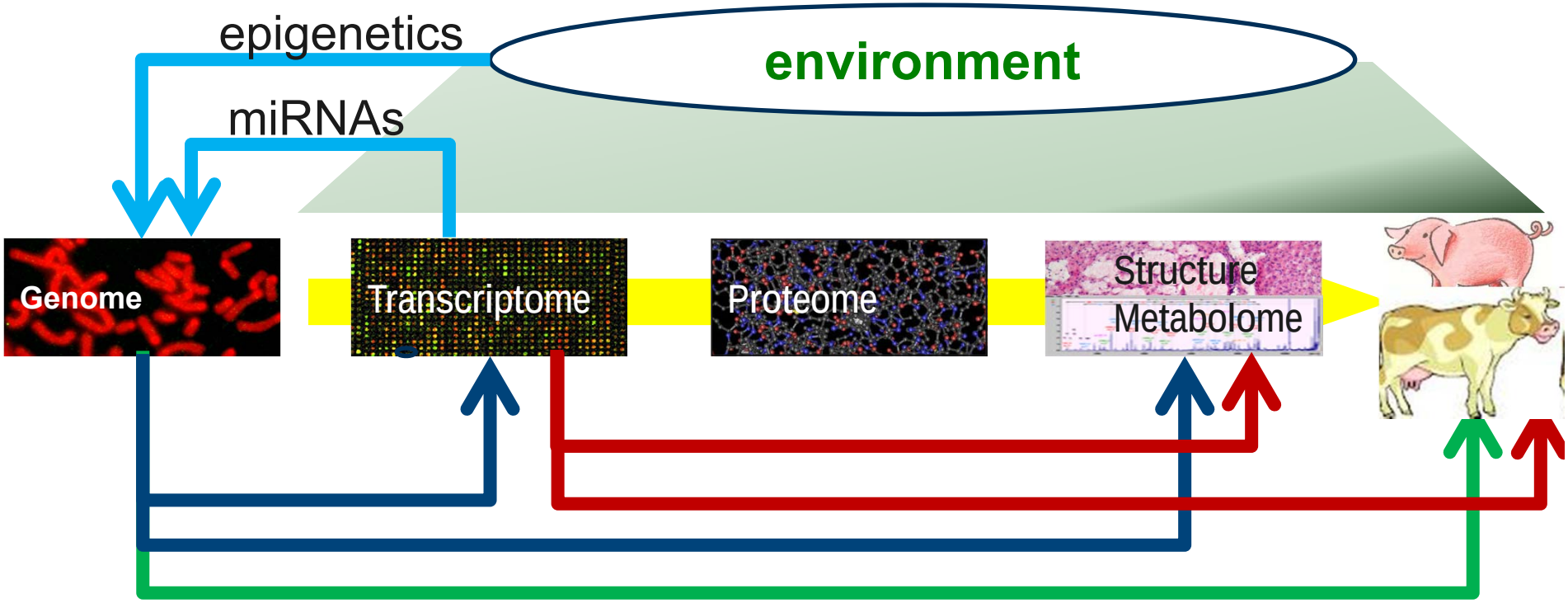
Enviroment + **Genotype** = **Phenotype**



Genome/Genotype/DNA

Phenotype

Genome + Epigenome ➔ Phenotype



- Epigenetic variation contributes to phenotypic variation; knowing it could improve the prediction of the phenotype
- Epigenetic mechanisms link environment and genome: environment x genotype interactions

Epigenetics

Use of the term epigenetics and its definition has changed throughout history.
Conrad Waddington, 1942 – study of epigenesis; how genotypes give rise to phenotypes in development

Robin Holliday, 1990 - temporal and spatial control of gene activity during development of complex organisms.

Our current definition (NIH Roadmap Epigenomics)**:**
Epigenetics is the study of mitotically (meiotically) heritable changes in gene expression that occur without changes in DNA sequence and of stable, long-term alterations of the transcriptional potential of a cell that are not necessarily heritable.

Biochemical reactions which are operating in Epigenetics

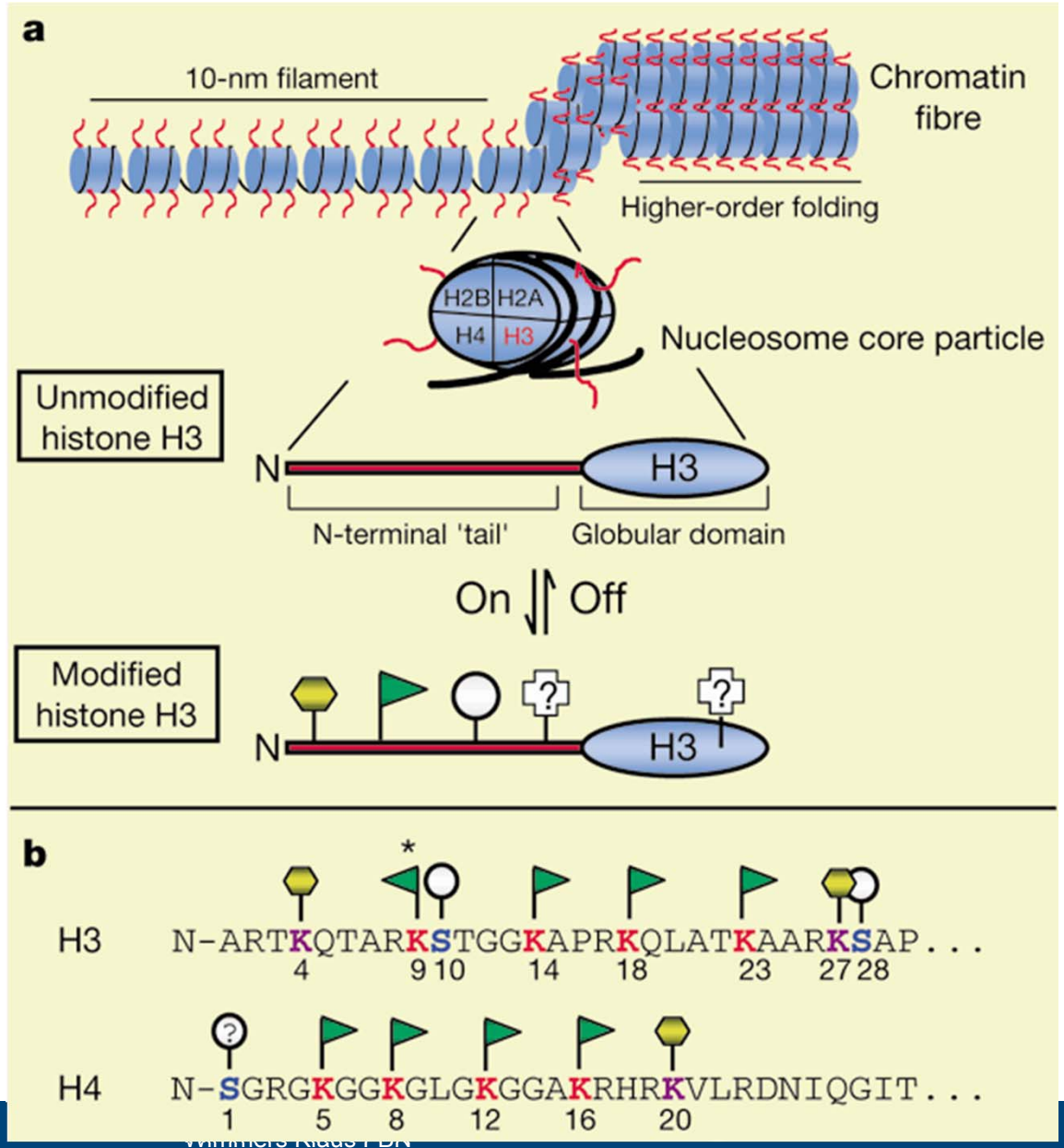
A. Modification at the DNA level

cytosine methylation

B. Histone modification - the histone code

histone acetylation, ~ methylation, ~ phosphorylation, ~ ubiquitination, ~ sumoylation

Histone modification



Functional Annotation of Animal Genomes FAANG

- Histone H3 lysine 4 trimethylation (H3K4me3), which correlates with promoters of active genes and transcription start sites;
- Histone H3 lysine 4 monomethylation (H3K4me1), which marks regulatory elements associated with enhancers and other distal elements, but is also enriched downstream of transcription start sites;
- Histone H3 lysine 27 trimethylation (H3K27me3), which marks genes that have been silenced through regional modification;
- Histone H3 lysine 27 acetylation (H3K27ac), which marks active regulatory elements, and may distinguish active enhancers and promoters from their inactive counterparts;

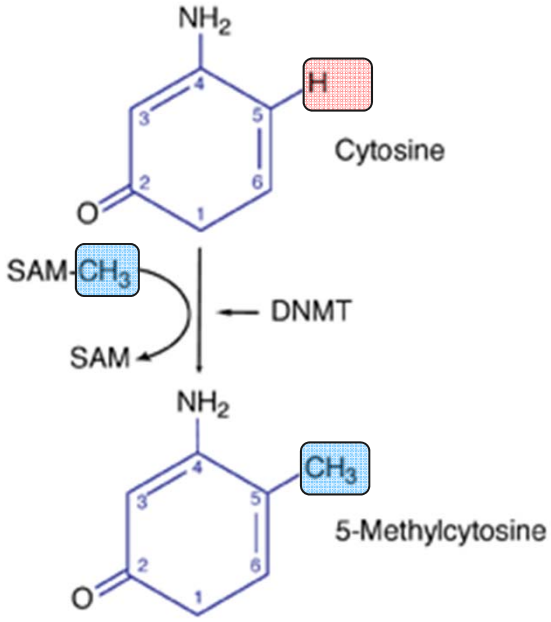
DNA methylation reactions

DNA-Methylation of CpG-di-nucleotides

→causes stable gene inactivation

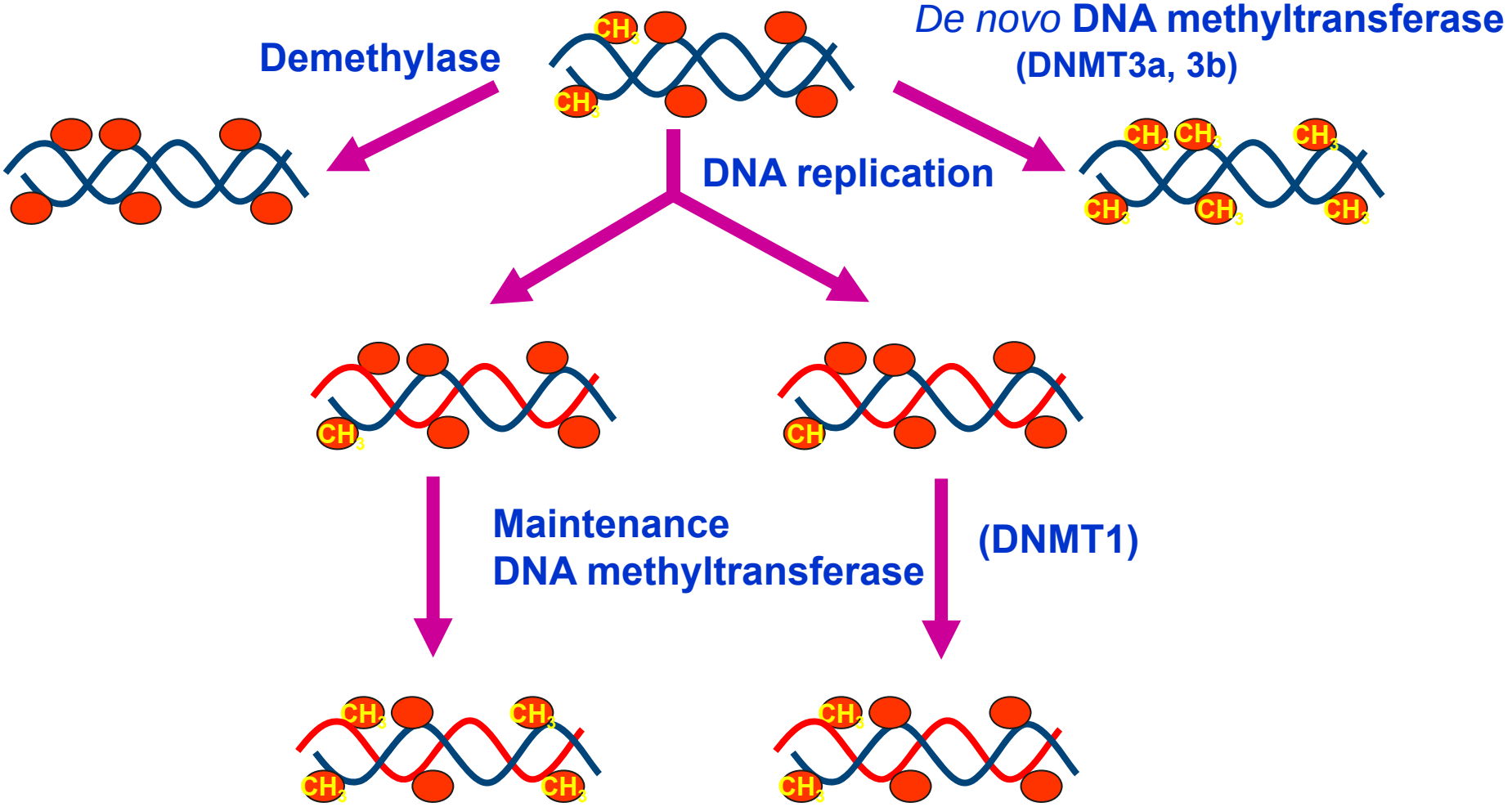
→allows long-lasting gene expression control; imprinting

→ mechanism of acute gene regulation



DNMT – DNA Methyltransferases;
SAM – S-Adenosyl-Methionine

DNA methylation reactions



DNA-Methylation in Vertebrates

CpG-di-nucleotides ~1% of vertebrate genome

60-80% of all CpG are methylated (5% cytosines)

CpG-Islands: ≥200bp, ≥60% CpG

- promoter-associated; 50-60% of genes with CpG-Islands
- usually hypomethylated

CpG outside of CpG-islands strongly methylated

- Maintaining genomic stability
- long-term inactivation of repeats, retrotransposons,
- deamination of methCpG to TpG (C-T-Transition) !!!

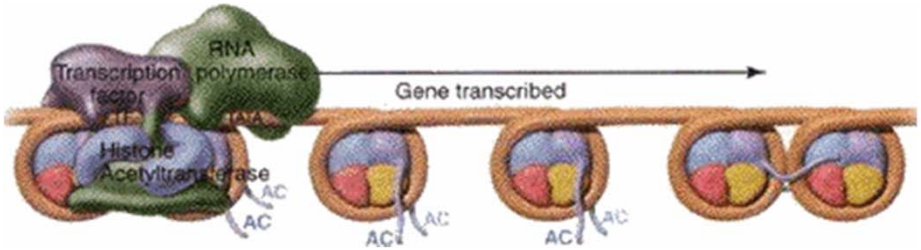
Santos et al. 2005
Gardiner-Garden and Frommer 1987
Doerfler et al. 1992

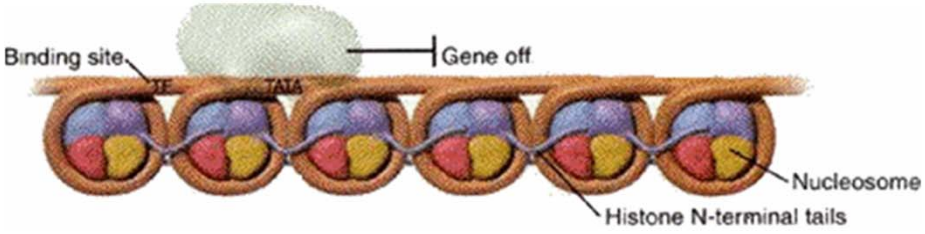
Repression of Gene Expression by DNA-Methylation

1. Direct blocking of TFBS by methyl-group of CpG

2. Blocking of TFBS by methylcytosine binding proteins (MBP)

3. Recruitment of Histone Deacetylases by MBP leads to deacetylation of core histones → change in chromatin structure to heterochromatin



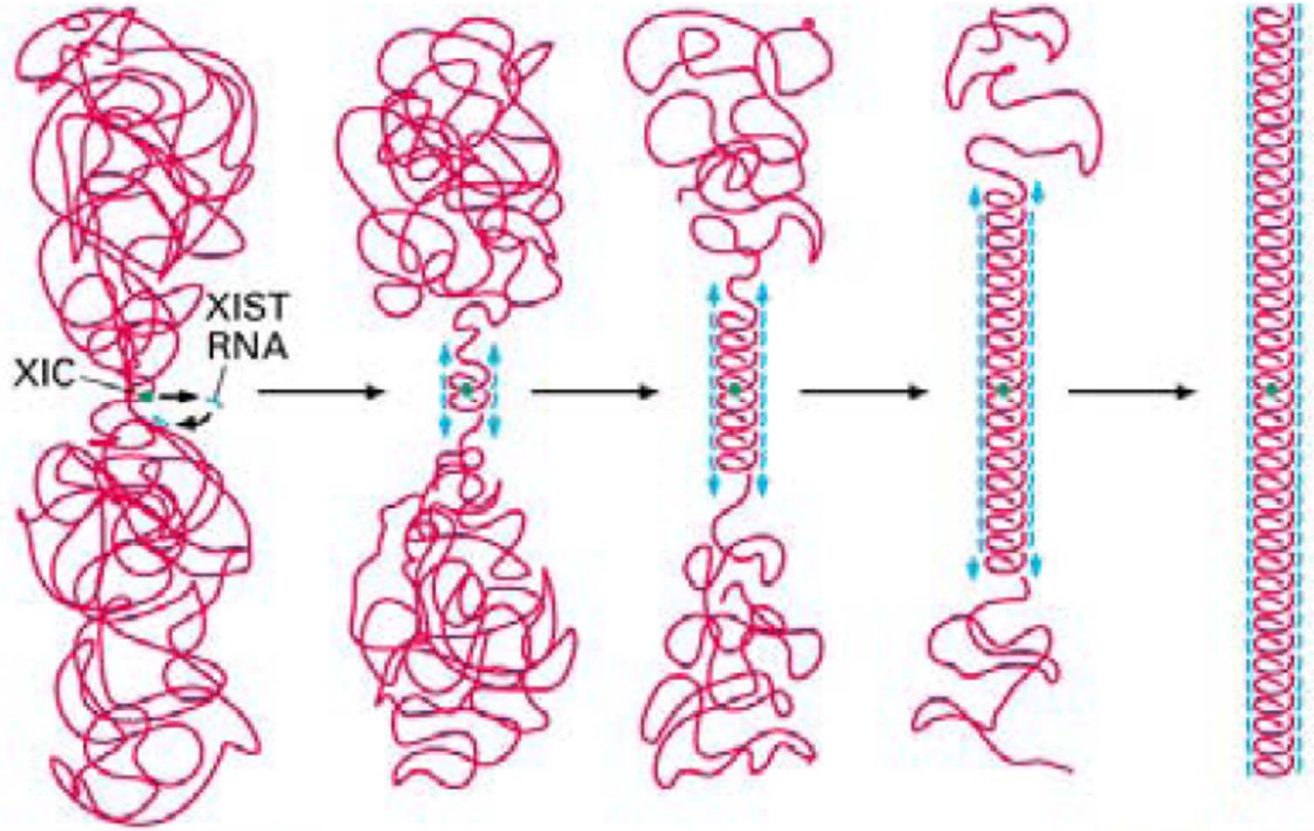


Iguchi-Ariga and Schaffner, 1989; Tierney et al., 2000
Ballestar and Wolffe, 2001; Nan et al., 1998;
Fuks et al., 2003; D'Alessio and Szyf, 2006

Epigenetic phenomena

- X chromosome inactivation
- Genomic imprinting
- Gene inactivation (specific genes, transposable elements, repeats…)
- Tissue specific expression
- Acute regulation of expression
- Centrometric heterochromatin, organisation of chromatin
- Cancer

X chromosome inactivation



XIC= X inactivation center

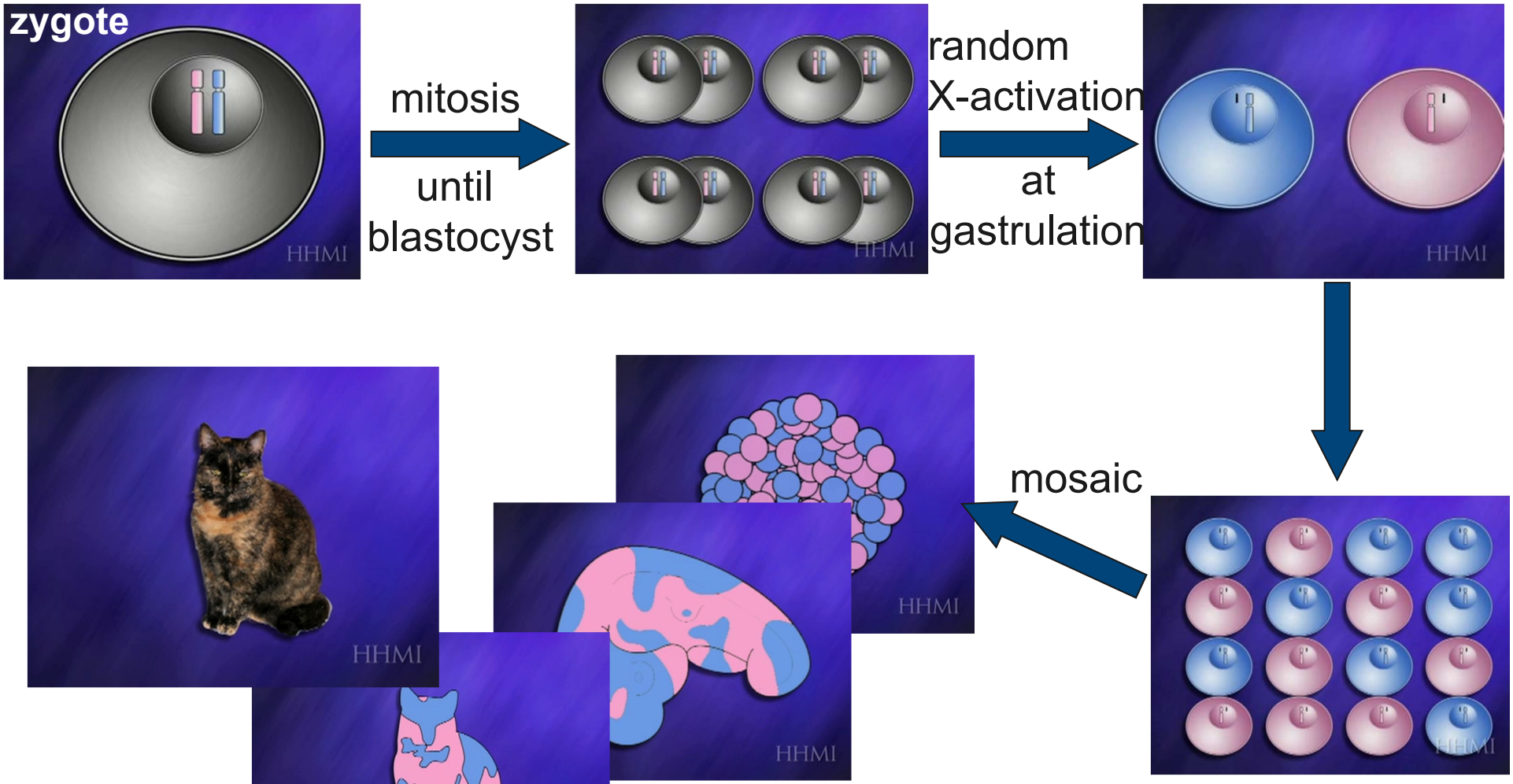
XIST= X inactive specific transcript 17kb noncoding RNA

stable expressed from inactive X

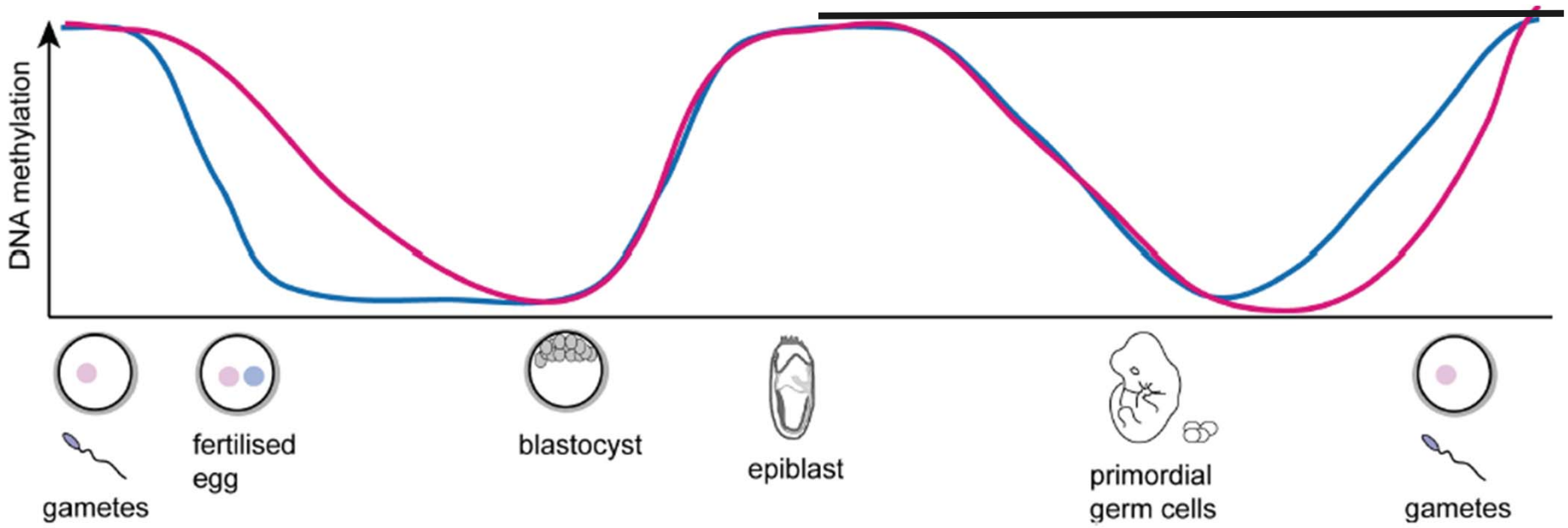
“paints” inactive X chromosome (cis)

its own activity is affected by DNA methylation

X chromosome inactivation

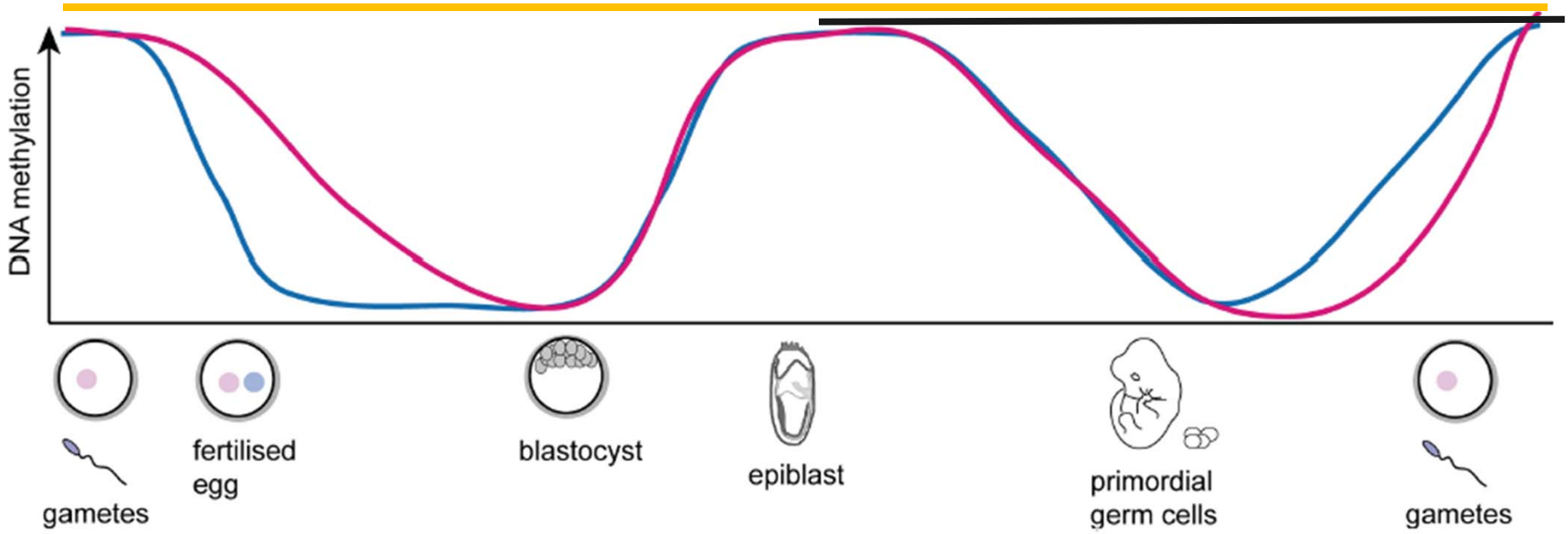


Epigenetic reprogramming



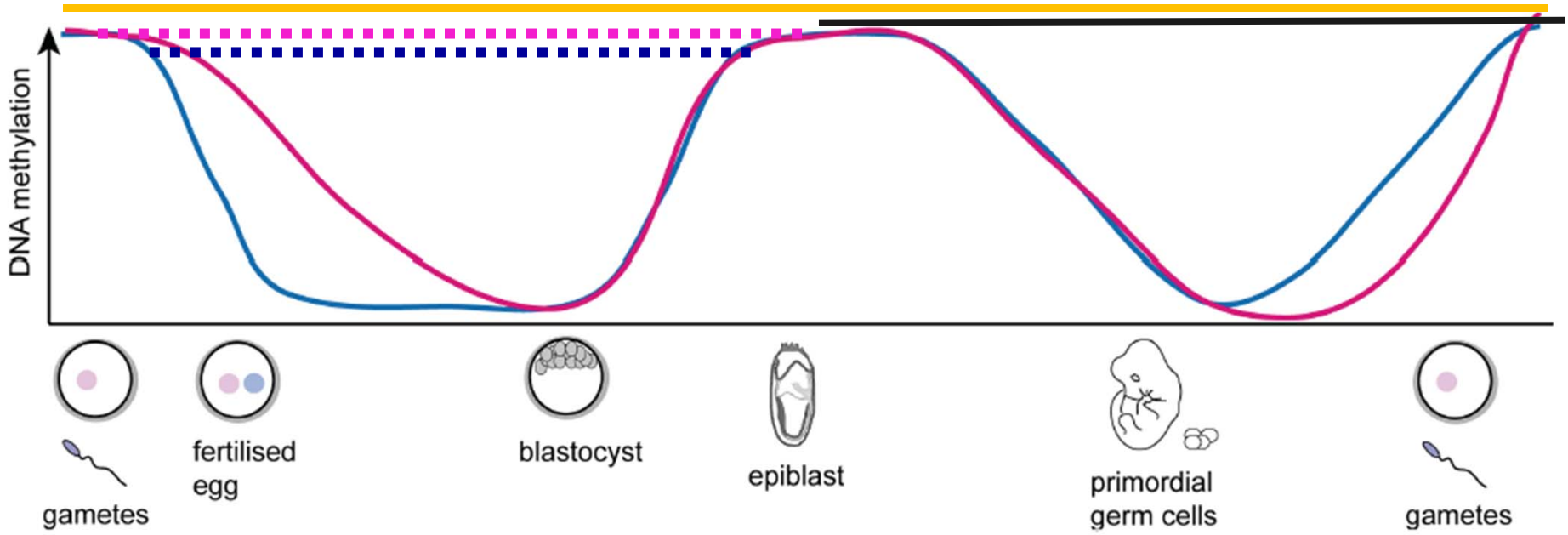
mod.from Blewitt M., course slides

Epigenetic reprogramming



paternal genome
maternal genome
offspring somatic genome
repeats

Epigenetic reprogramming



paternal genome
maternal genome
offspring somatic genome
repeats

paternally imprinted
Maternally imprinted

Imprinting

diploid embryos derived from either only paternal or only maternal pronuclei failed to survive

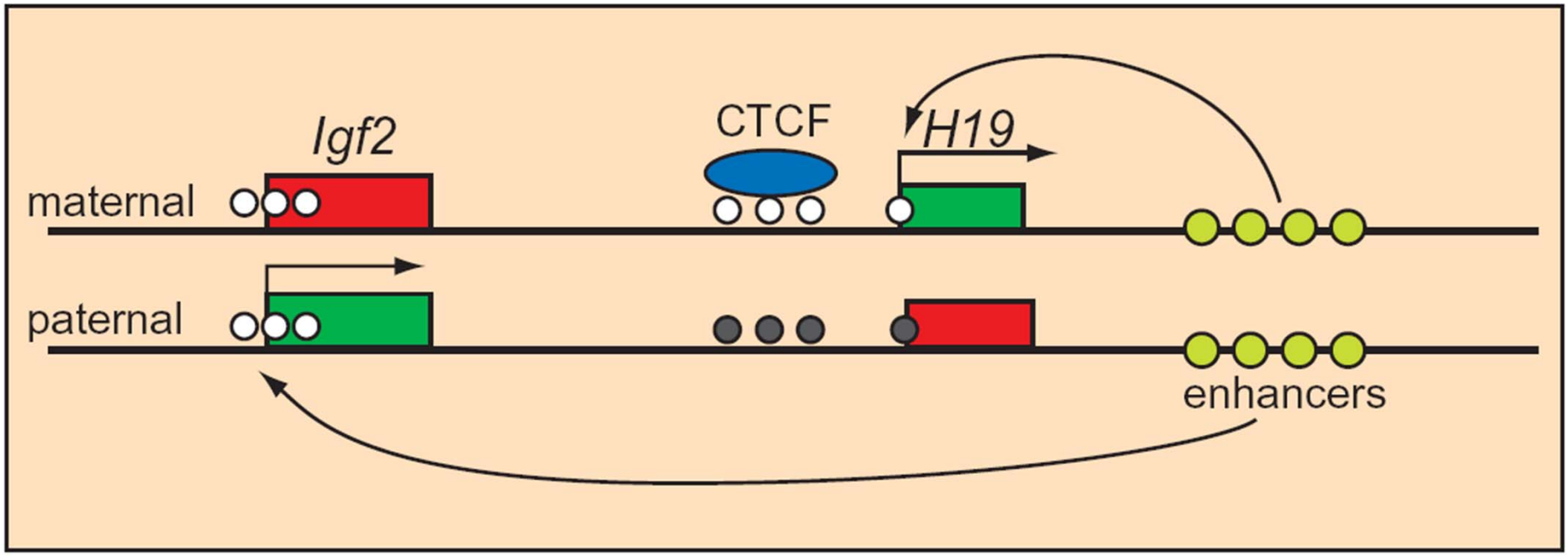
Imprinted genes:

Mouse	132	cattle	25
Human	79	pig	21
		sheep	14

- Mouse and human share only 40 imprinted genes
- Imprinting control region, ICR
- Often located in clusters
- Often coding for embryonic development, metabolism, behavior; relatively few but large effects

QTL with parent-of-origin effects

Imprinting of IGF2

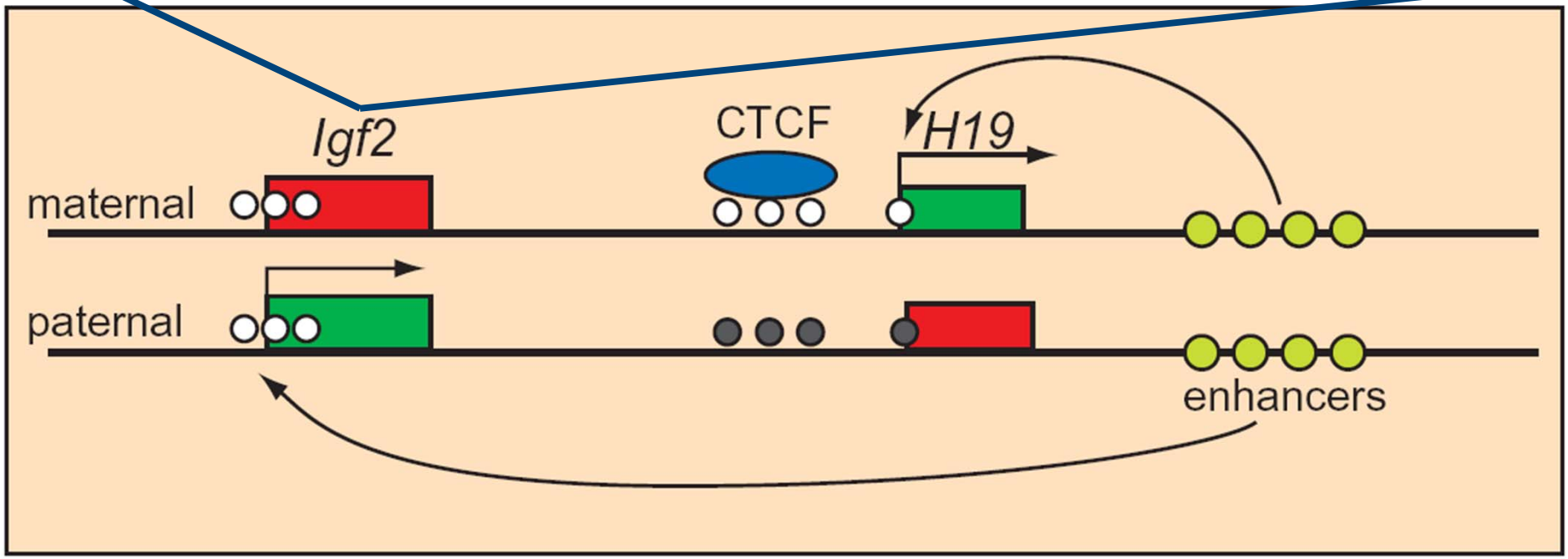


Setting up boundaries. When the CTCF protein binds to DNA, it blocks regulatory DNA downstream from interacting with the *Igf2* gene and only the *H19* gene is expressed. If methyl groups (black) prevent CTCF binding, *Igf2* is active, but *H19* is silenced.

Rand & Cedar, 2003

Imprinting of IGF2

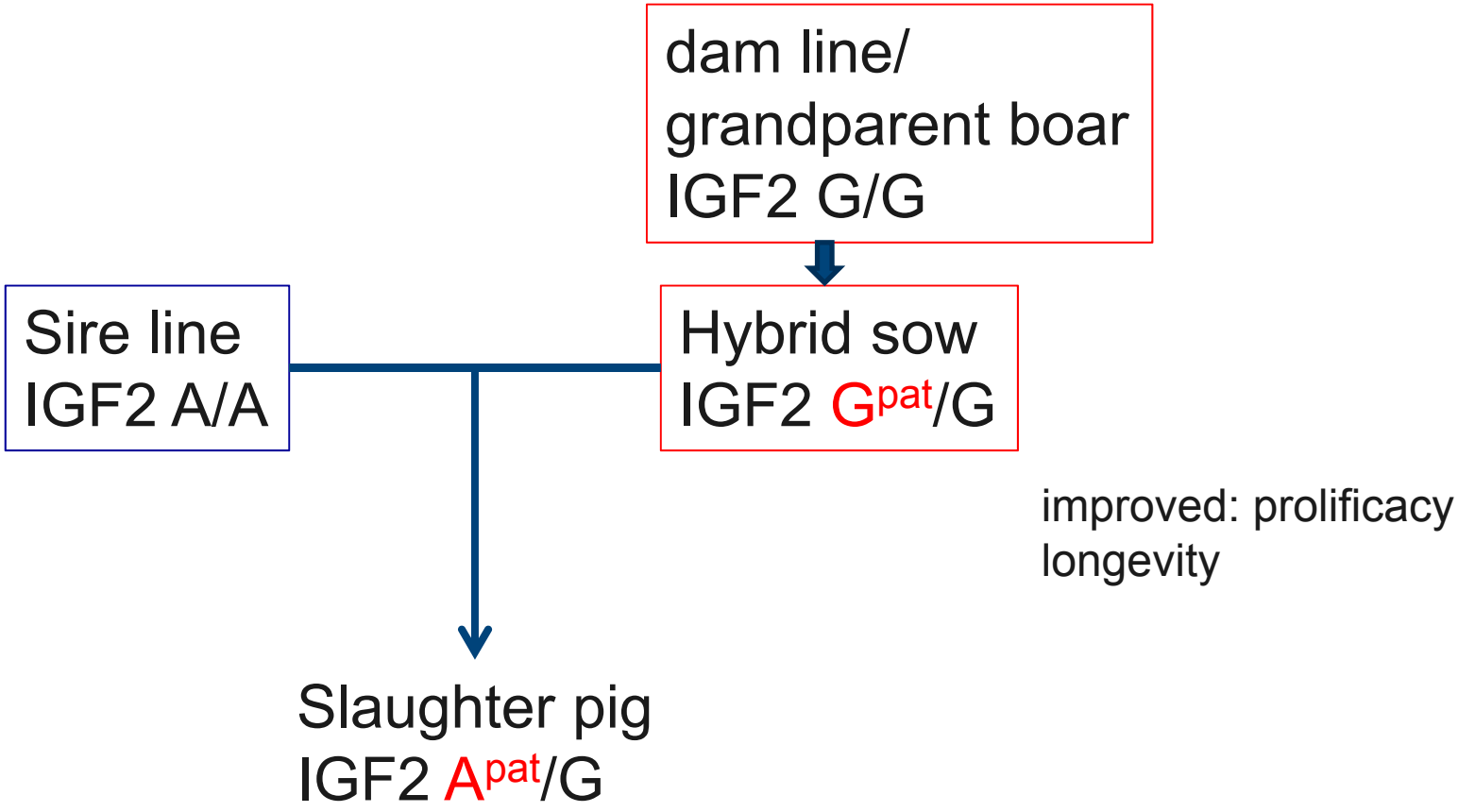
A>G transition in intron 3, CpG-island
A = Q, does not bind a repressor (ZBED6)
G = q, binds repressor; G^{met} does not binds repressor
Explains 30% variance in lean meat
➔inheritance of the A-allele from the sire 3-fold increase in IFG2,



mod. from Rand & Cedar, 2003

Jeon et al., 1999; de Koning et al. ,2000
Nezer et al., 1999; Van Laere et al., 2003

Imprinting of IGF2



Other implications of epigenetics

Genome + Epigenome ➔ Phenotype

Additional source variation:
Knowledge may contribute to predict the phenotype

- **biomarker ➔management tool**
- **selective breeding ➔ refinement of estimates**
  imprinted locus = functionally hemizygous

Other implications of epigenetics

- **missing heritability & missing causality**

- **if the epimutation is stable:**
  likely to be in LD with SNP (no implication for genomic selection)
- **if epimutation is unstable:**
  no good selection criterion,
  does not contribute much to missing heritability
  identity by descent does not imply identity in state

but if epimutation is causal, it will not be detected by DNA sequencing

Goddard & Whitelaw, 2014
Slatkin, 2009
Gonzales-Recio, 2012

Other implications of epigenetics

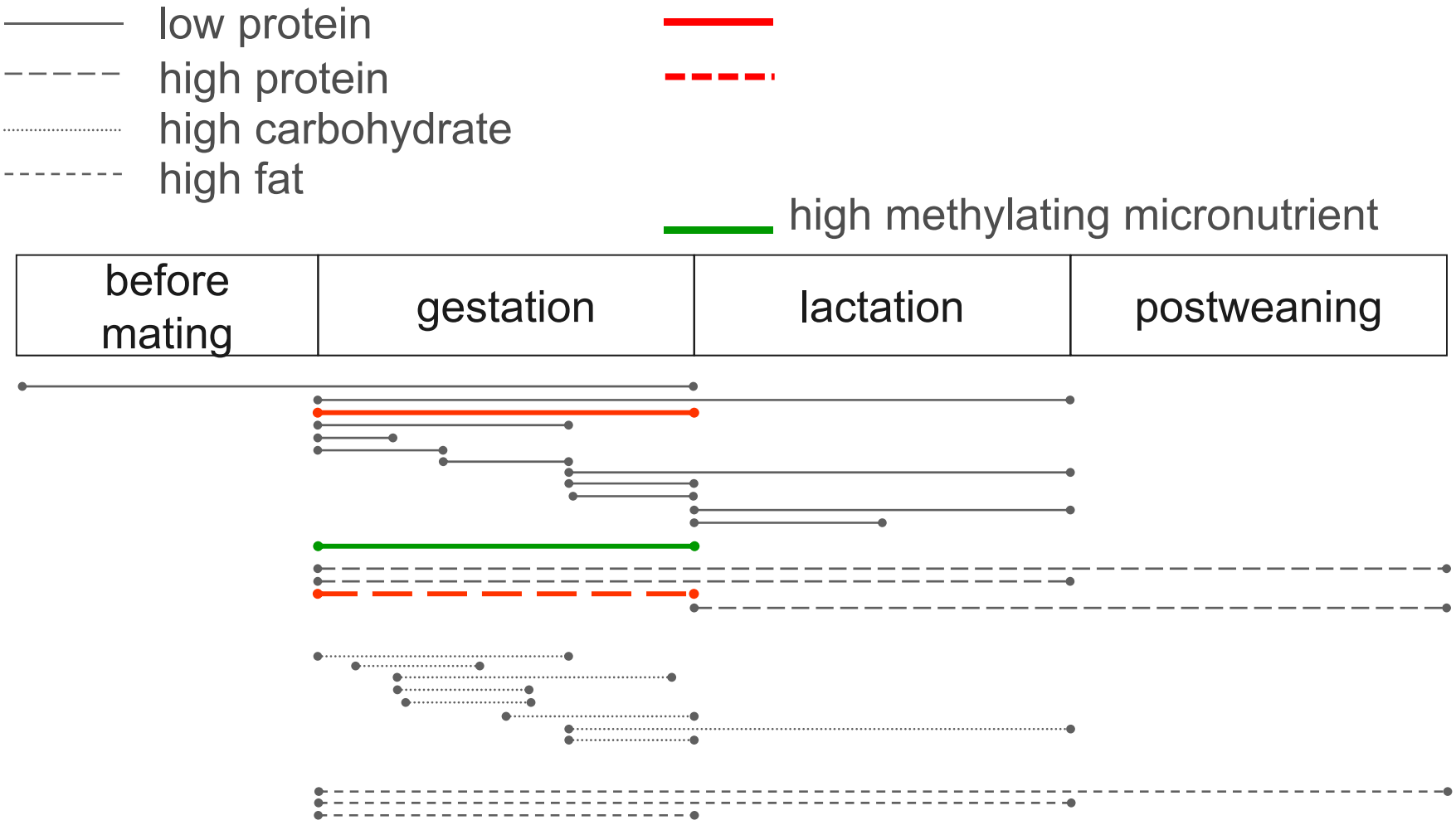
- **Assisted reproductive technologies: for example LOS**
- **Fetal programming (nutritional programming (conditioning)):**

Epidemiological data and experiments in model and farm animals revealed that environmental effects during gestation impact the phenotype of offspring

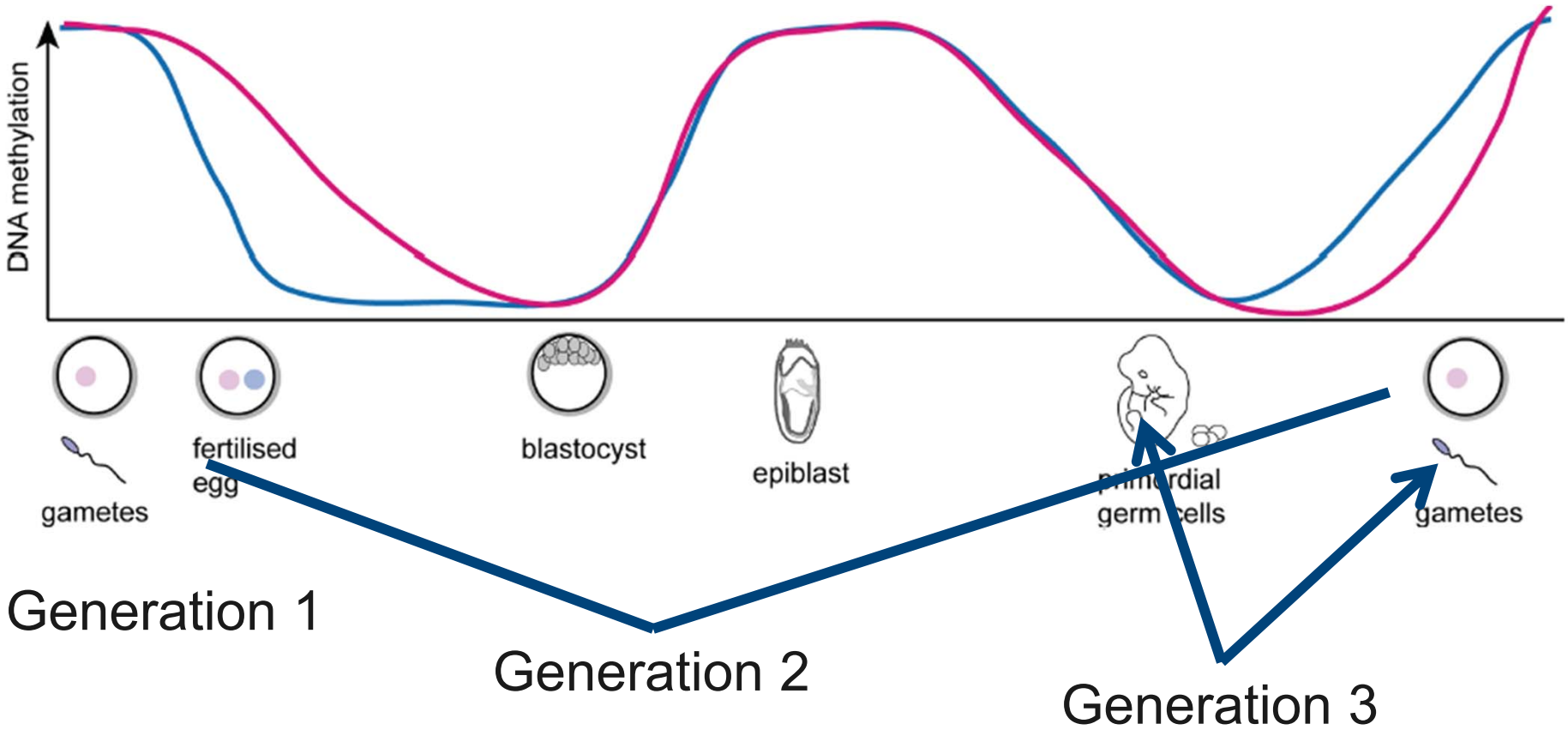
Thrifty Phenotype Hypothesis

Epigenetic mechanisms as a molecular memory are involved

Impact of gestation diets



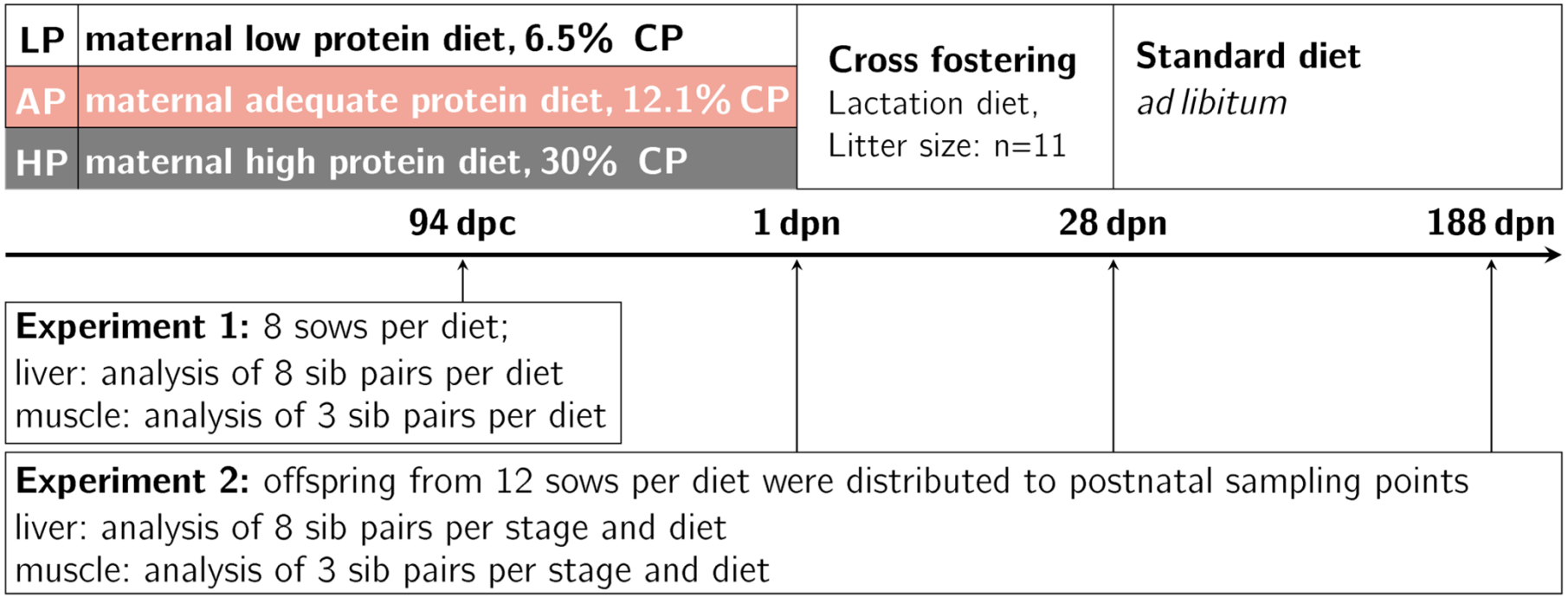
Epigenetic transgenerational inheritance



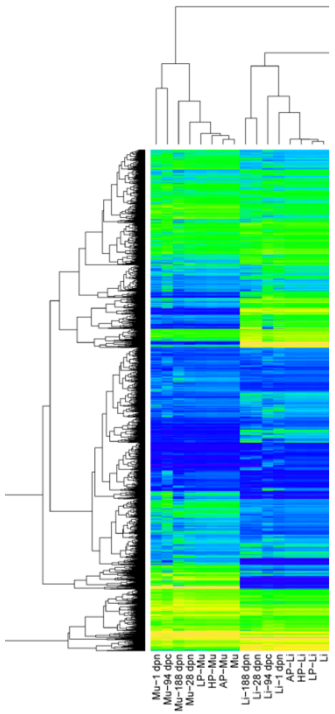
Experimental feeding to mid gestation

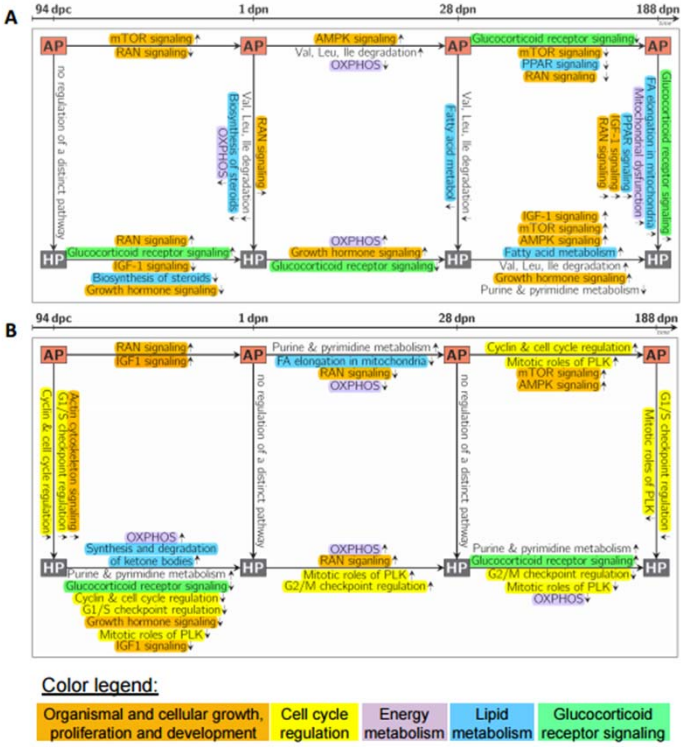
Experimental feeding to end gestation

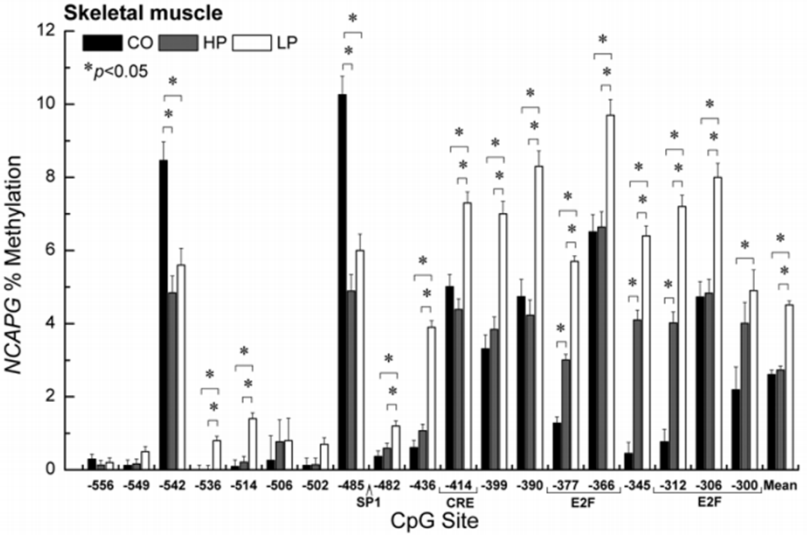
Foetal Programming



- Impact of gestation diets with high and low protein content on gene expression
- Involvement of epigenetic mechanisms/DNA methylation



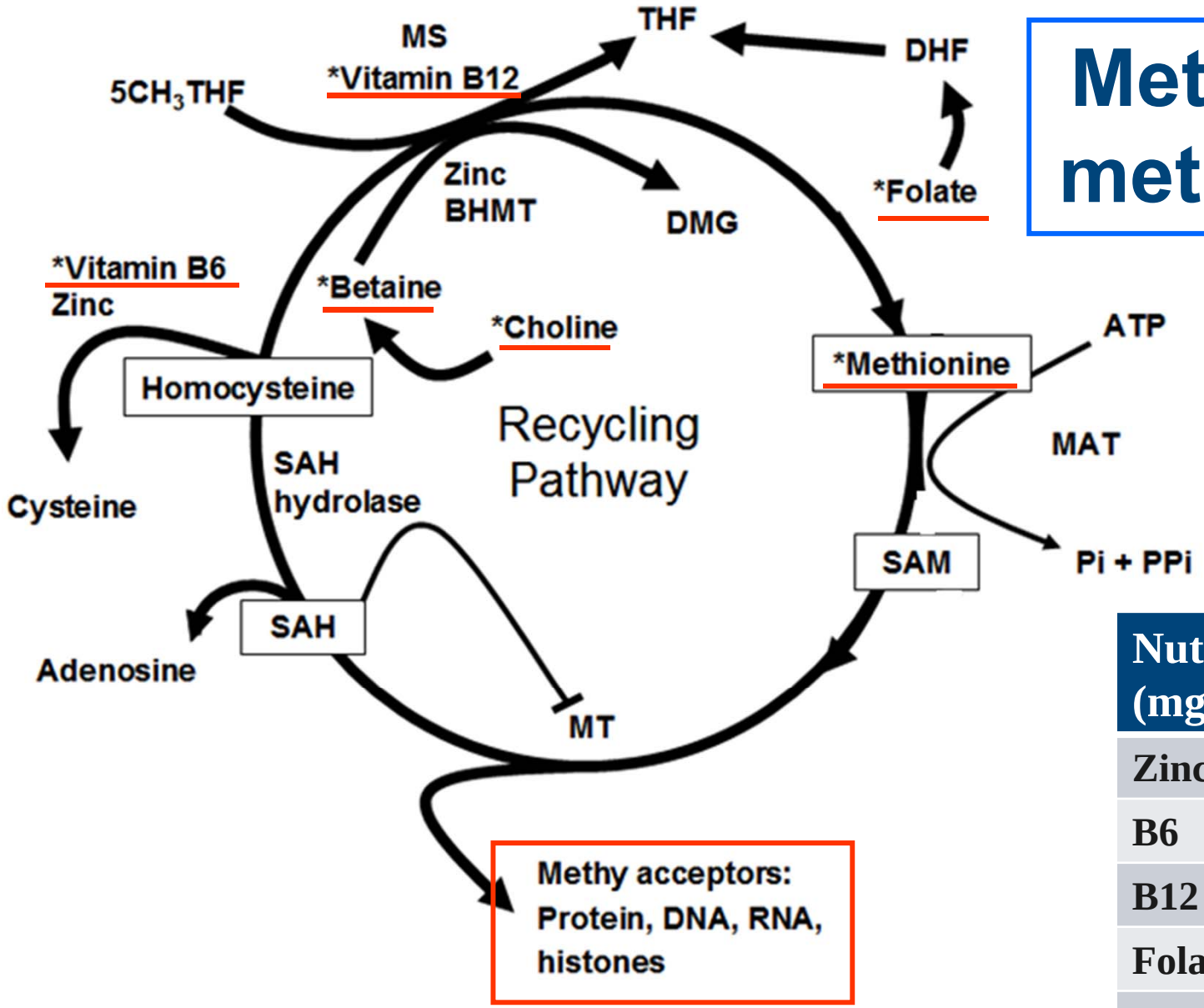




- hierarchical influence of tissue, ontogenetic stage, and diet on transcript levels
- Muscle appeared to be a less resistant to nutritional modulation than liver
- no gatekeeper pathways/genes were obvious
- Differential expression of DNMT1, DNMT3a and DNMT3b
- Differential DNA-methylation of PPARα, NR3C1, CYP2C34, NCAPG…

Oster et al. 2011, 2012a,b,c,2014
Altmann et al. 2012a,b,c,2013

Methionine metabolism

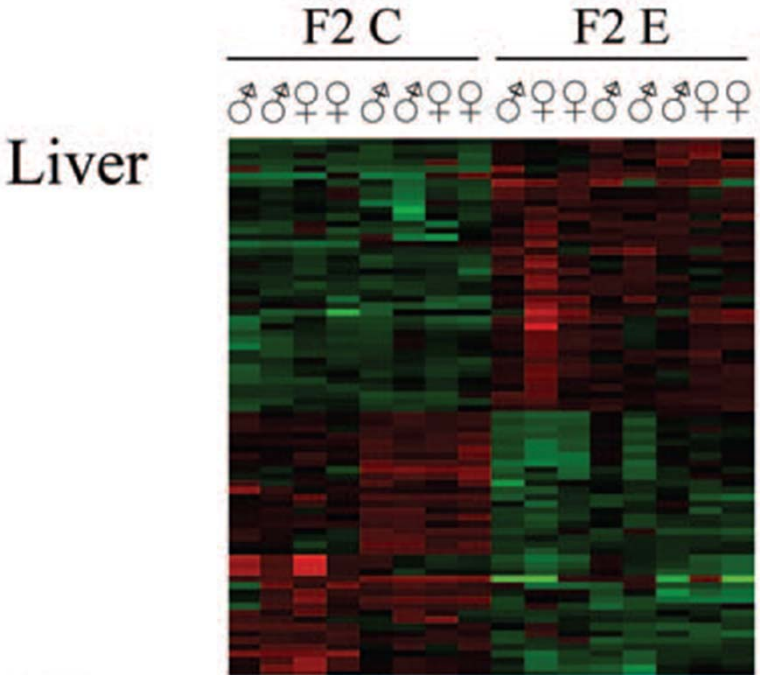


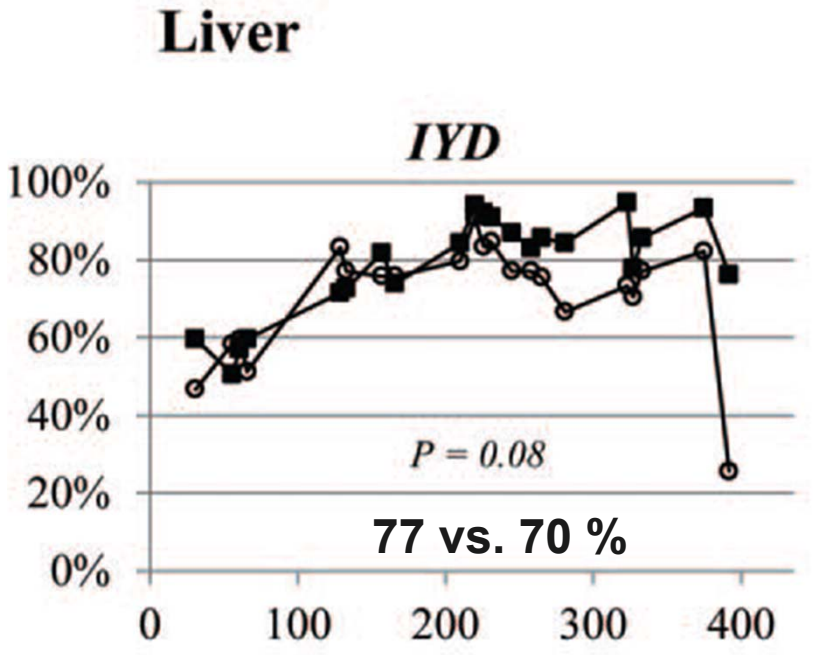
DNA Methylation

Nutrient (mg/kg)	CON	MET
Zinc	21.8	149.0
B6	3	1180
B12	31	5930
Folate	3	92.2
Choline	500	2230
Methionine	2050	4700

Epigenetic transgenerational inheritance

F2 offspring groups differed with respect to backfat percentage (P = 0.03)
in liver and muscle differential expression of lipid metabolism and metabolic pathway
a significant difference in DNA methylation at the IYD gene





Methylating micronutrients supplementation

To study the effects of methylating nutrient enriched maternal diet on DNA methylation and transcriptome changes

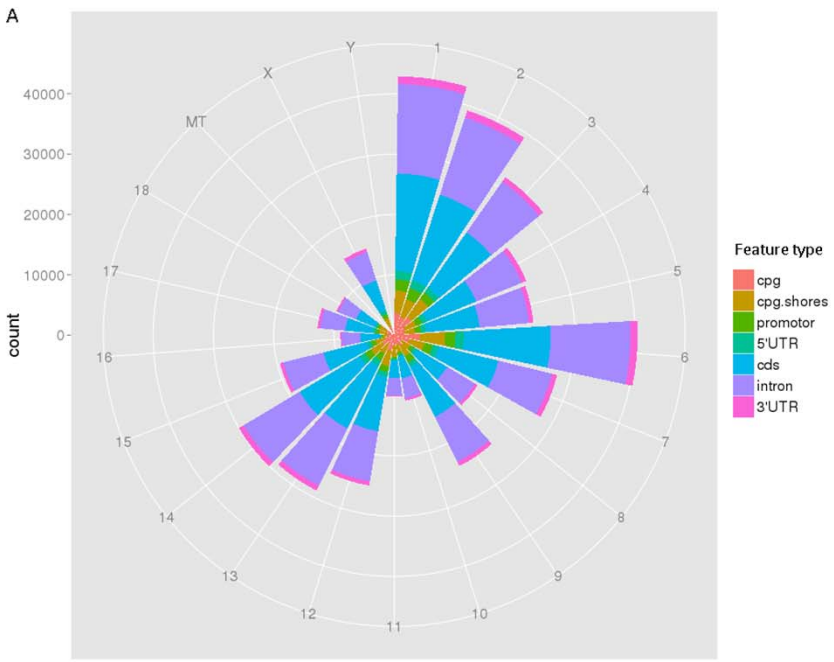
To map differentially methylated regions associated with treatment factors developmental stage, breed and maternal diet

To explore biological significance of DNA methylation changes by integration of DNA methylation and transcription profiles

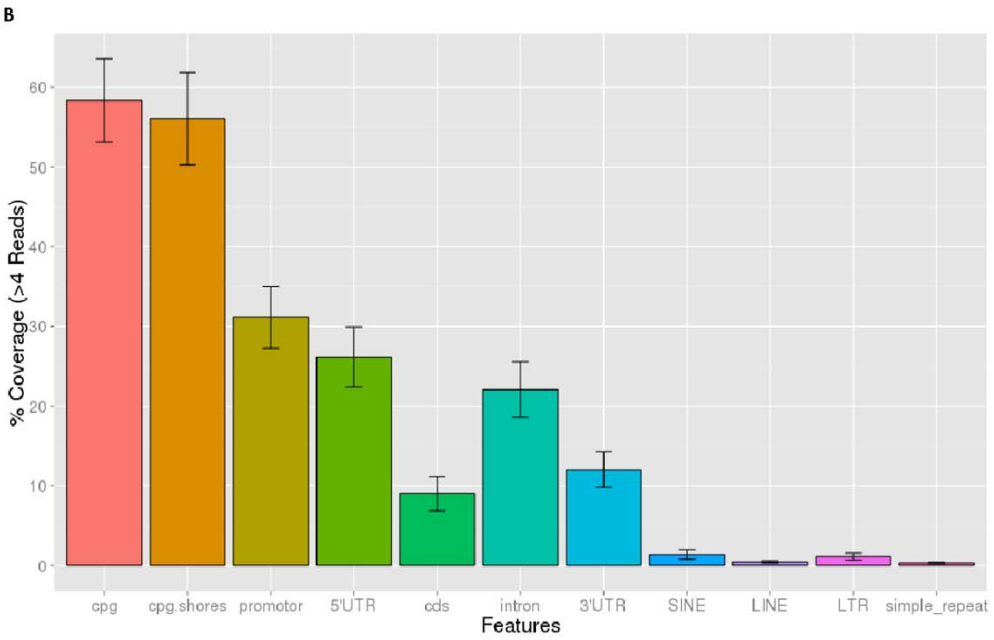
2 breeds (Pi, GL) × 2 diets × 2 stages (91 ppc, 150 dpn)
4 offspring/breed/diet/stage, n=32, liver

Next Generation Sequencing:
RNA⇨Transcriptome (RNA-Seq)
DNA⇨Methylome (RRBS)

Genome coverage of the RRBS library



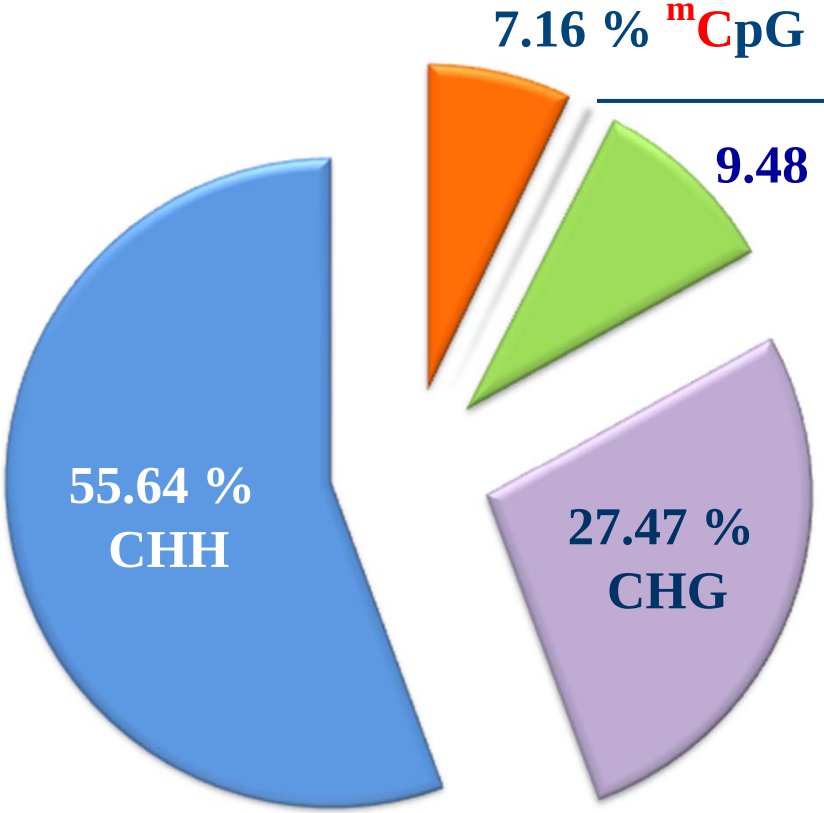
Frequency of annotated features in the pig genome.



Percentage of genomic features covered by greater than 4 reads.

RRBS effectively target specific genomic regions including CpG islands and CpG-island shores (±1kb from CpG island) and CpG rich promoters.

Percentage methylation of cytosines in CpG, CHG or CHH context



43.02 % of CpGs are methylated

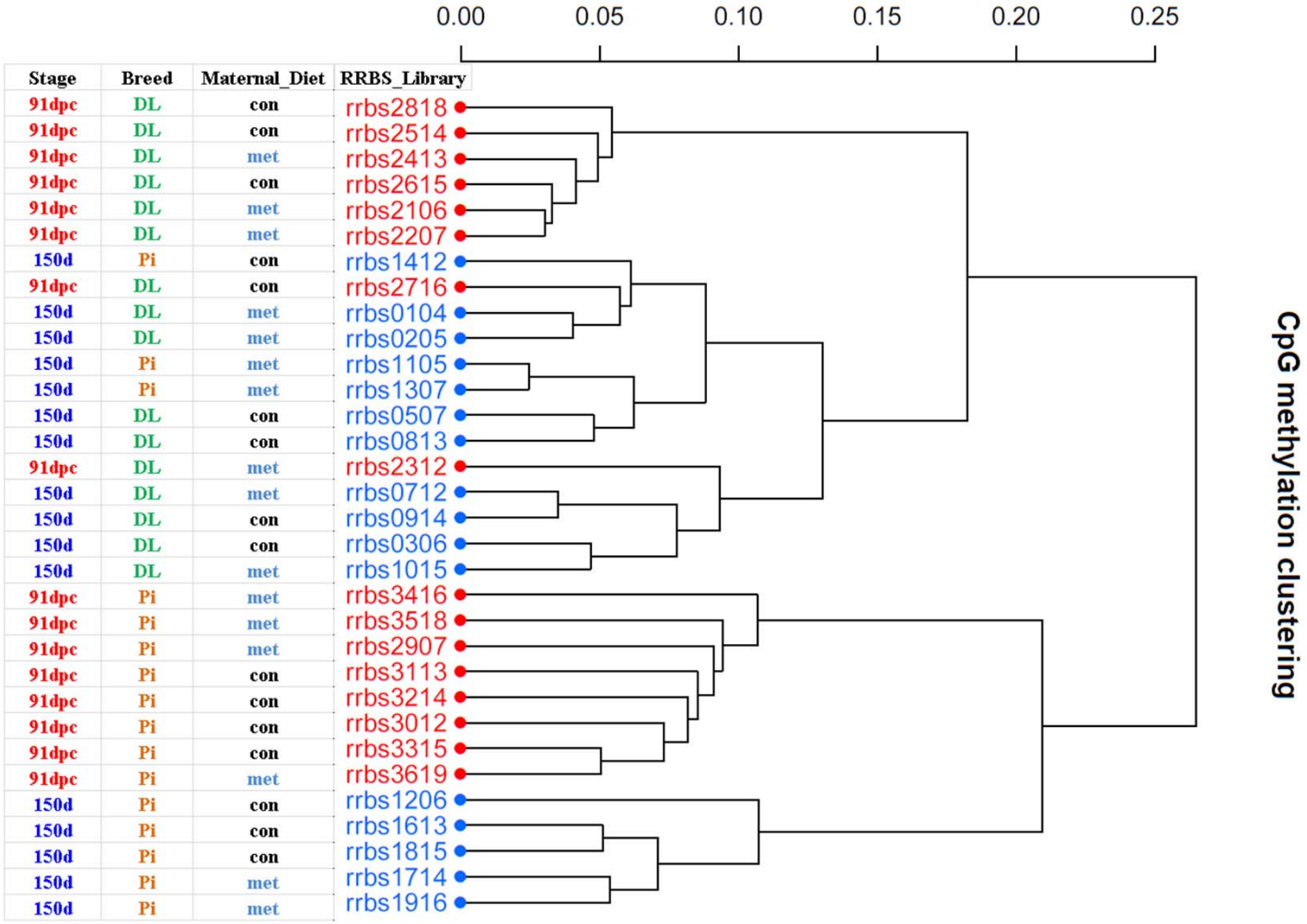
0.05 % ^{m}CHG;

0.24 % ^{m}CHH

H = A, T or C

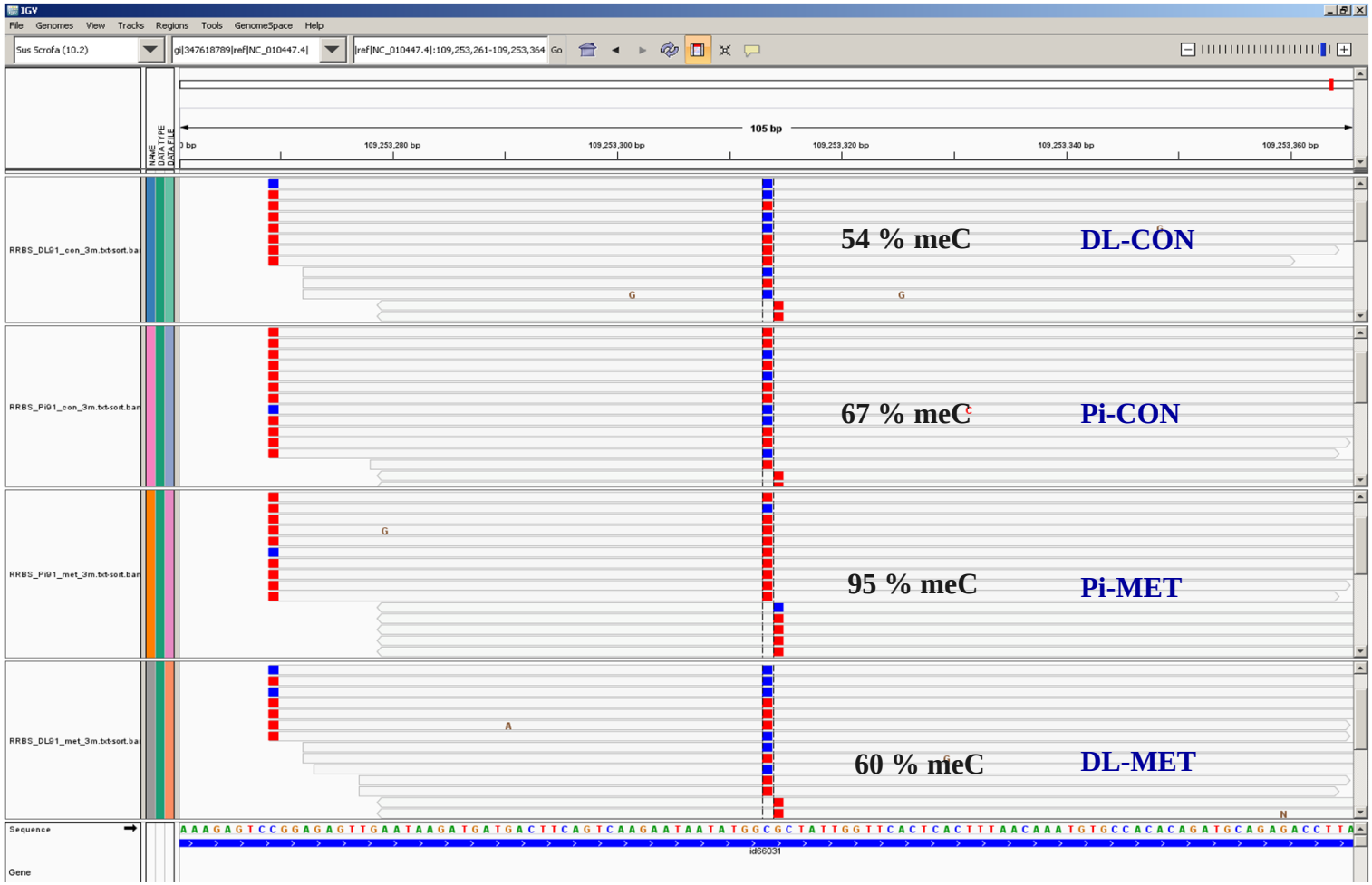
% = median of 32 libraries

Clustering based on the DNA methylation profile



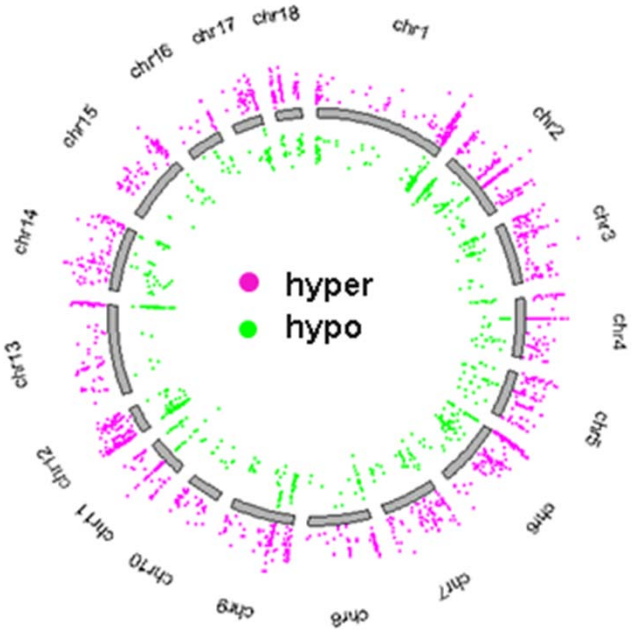
Stage	Breed	Maternal_Diet	RRBS_Library
91dpc	DL	con	rrbs2818
91dpc	DL	con	rrbs2514
91dpc	DL	met	rrbs2413
91dpc	DL	con	rrbs2615
91dpc	DL	met	rrbs2106
91dpc	DL	met	rrbs2207
150d	Pi	con	rrbs1412
91dpc	DL	con	rrbs2716
150d	DL	met	rrbs0104
150d	DL	met	rrbs0205
150d	Pi	met	rrbs1105
150d	Pi	met	rrbs1307
150d	DL	con	rrbs0507
150d	DL	con	rrbs0813
91dpc	DL	met	rrbs2312
150d	DL	met	rrbs0712
150d	DL	con	rrbs0914
150d	DL	con	rrbs0306
150d	DL	met	rrbs1015
91dpc	Pi	met	rrbs3416
91dpc	Pi	met	rrbs3518
91dpc	Pi	met	rrbs2907
91dpc	Pi	con	rrbs3113
91dpc	Pi	con	rrbs3214
91dpc	Pi	con	rrbs3012
91dpc	Pi	con	rrbs3315
91dpc	Pi	met	rrbs3619
150d	Pi	con	rrbs1206
150d	Pi	con	rrbs1613
150d	Pi	con	rrbs1815
150d	Pi	met	rrbs1714
150d	Pi	met	rrbs1916

Base-pair resolution DNA methylation profile

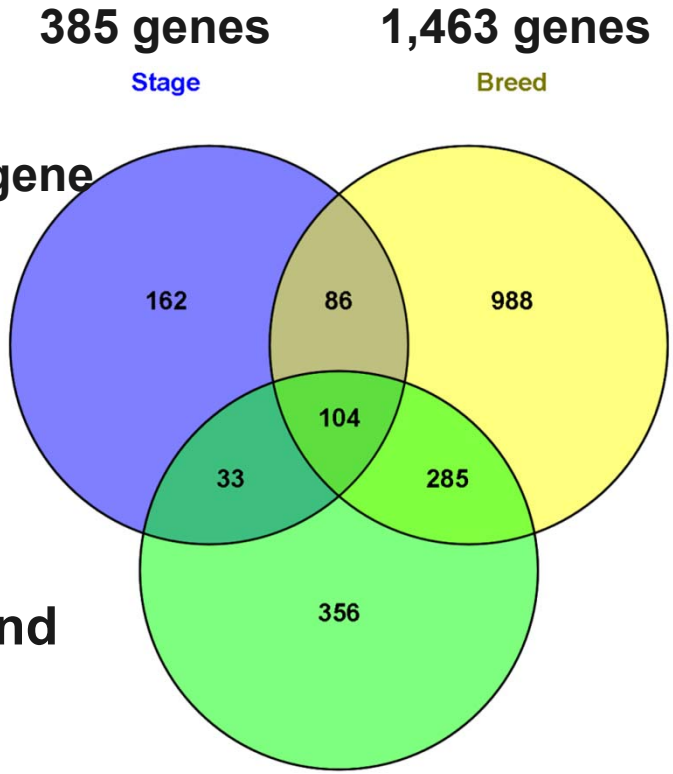


differentially-methylated CpG site 1.9-kb upstream of the E2F7 transcriptional start site compared between control and methyl-donor rich maternal diet groups in DL and Pi pigs at 91-dpc stage

Genome-wide distribution of DMRs associated with stage, breed and diet



link to the nearest gene

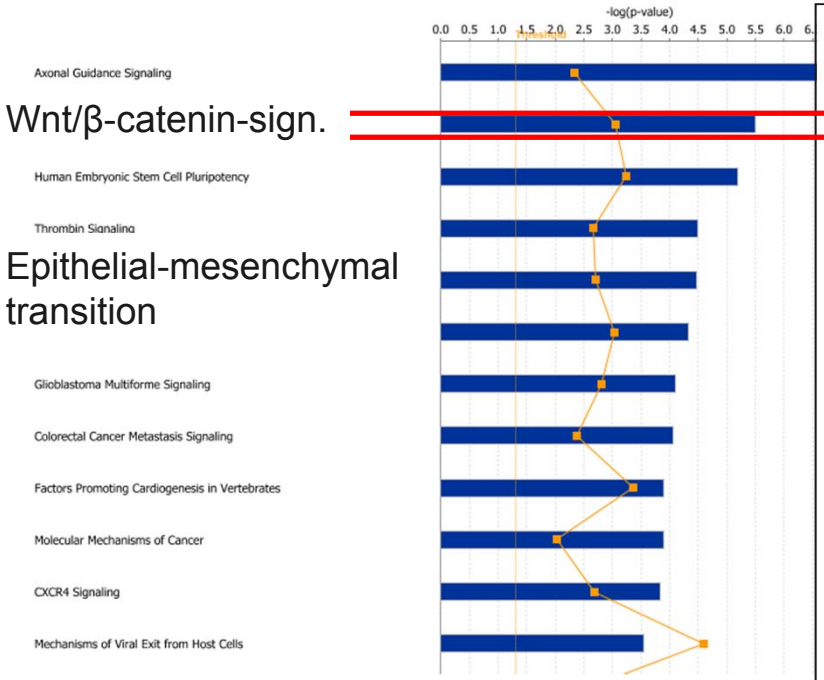


DMRs with 15% differential methylation and *q-value* < 0.05.

433, 2038, and 932 DMRs were identified to be associated with stage, breed, and diet, respectively.

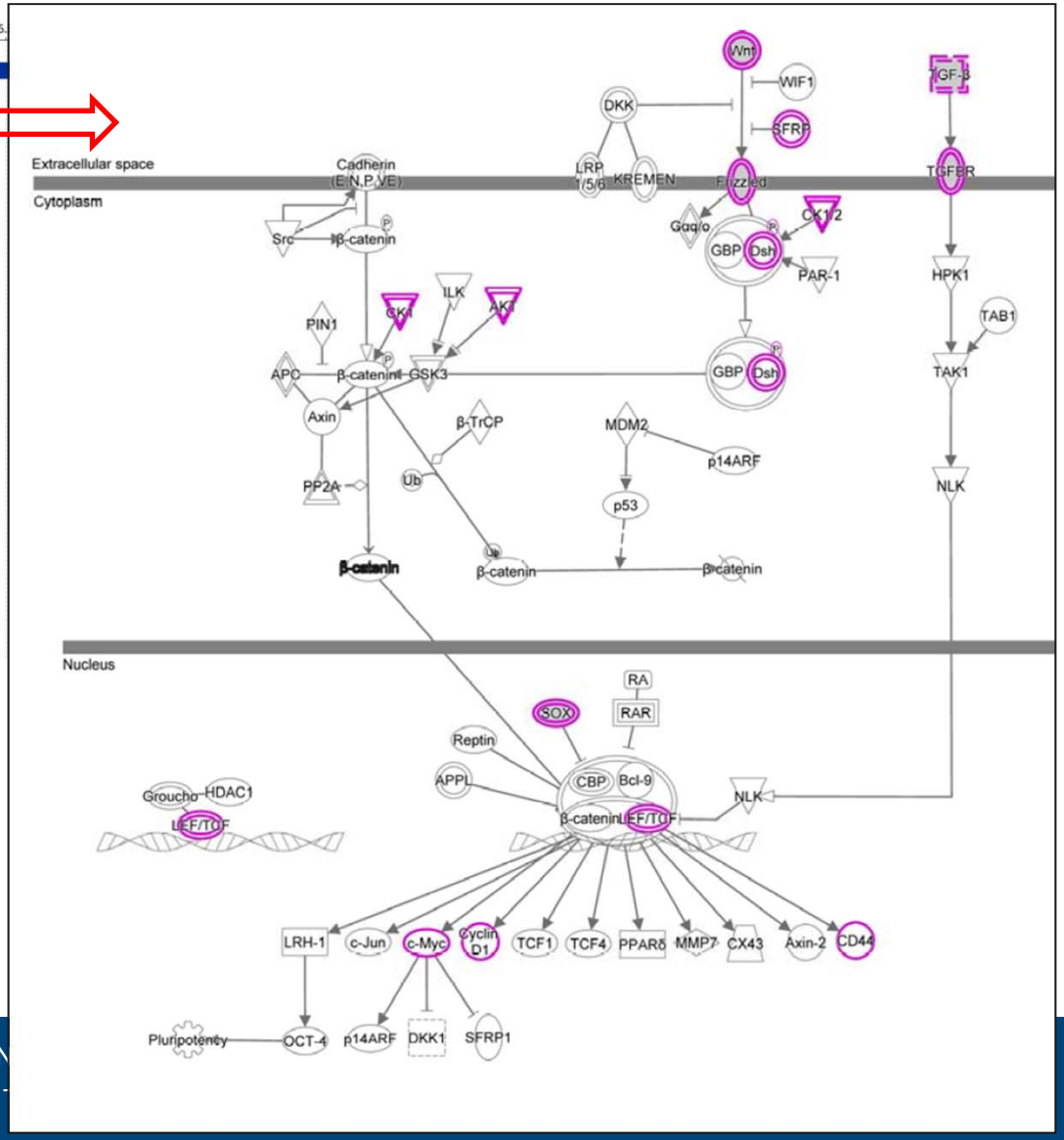
Functional annotation of genes in DMRs

A) Breed-associated DMRs/Genes



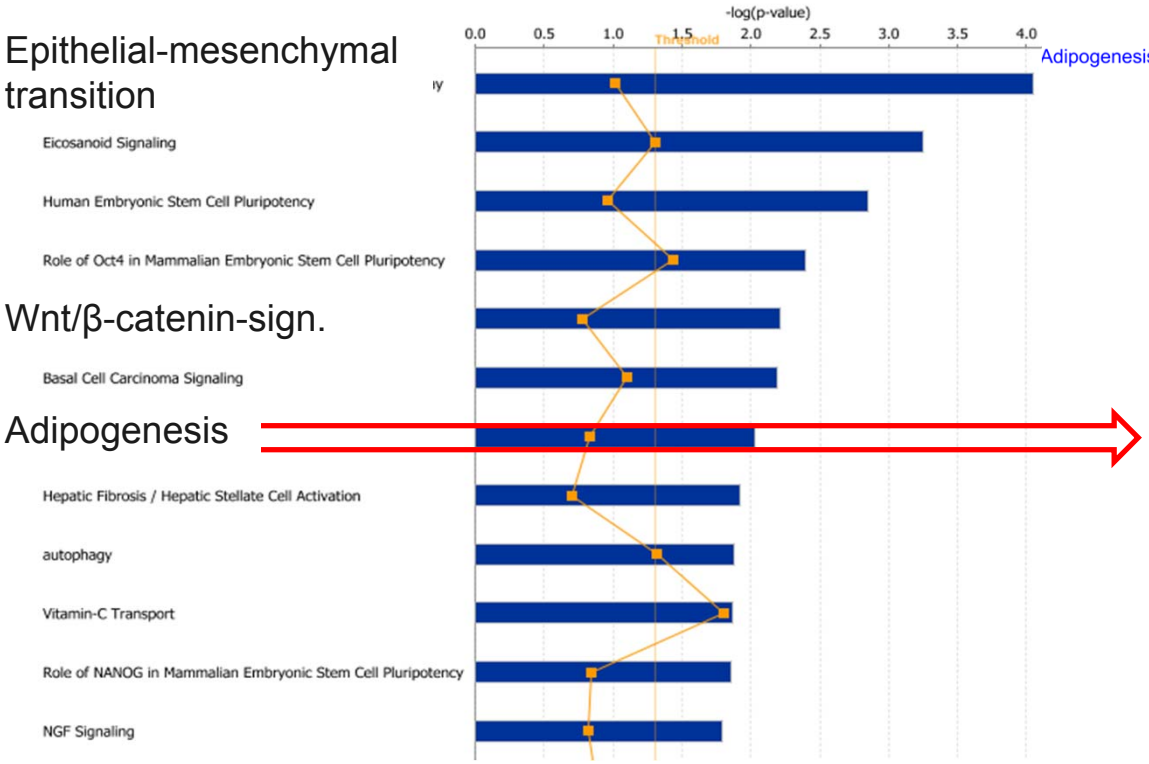
Wnt/β-catenin-sign.

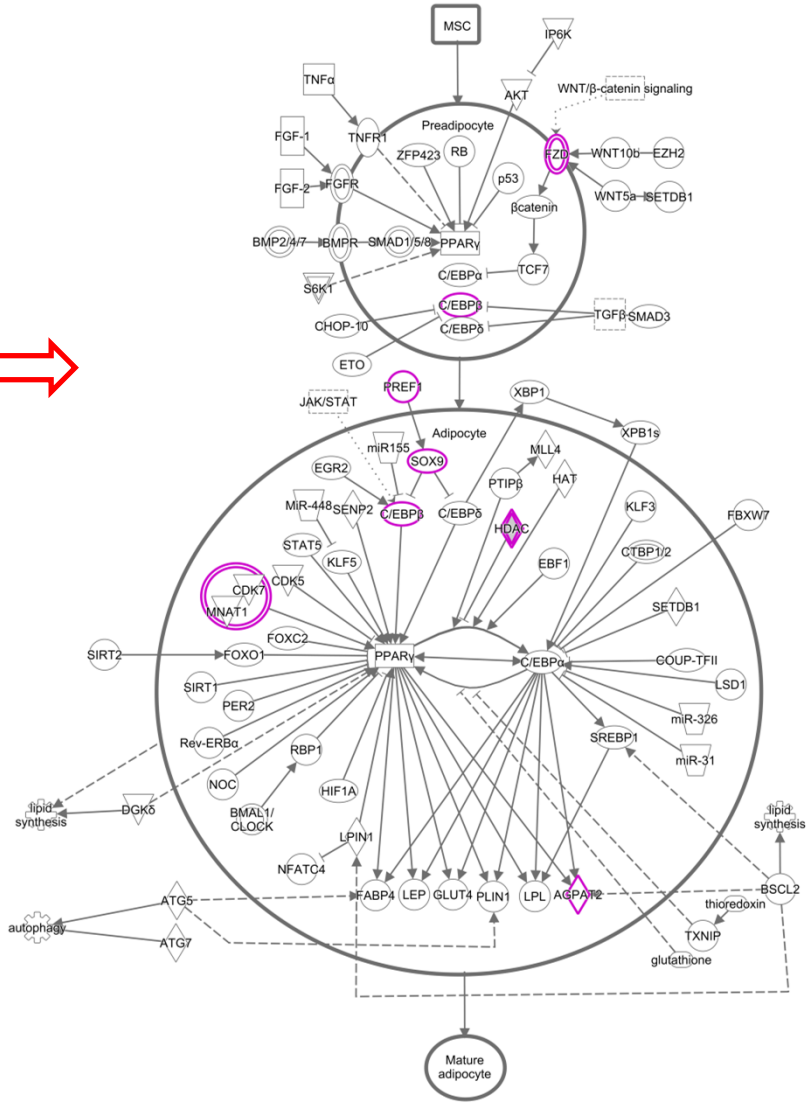
Epithelial-mesenchymal transition



Functional annotation of genes in DMRs

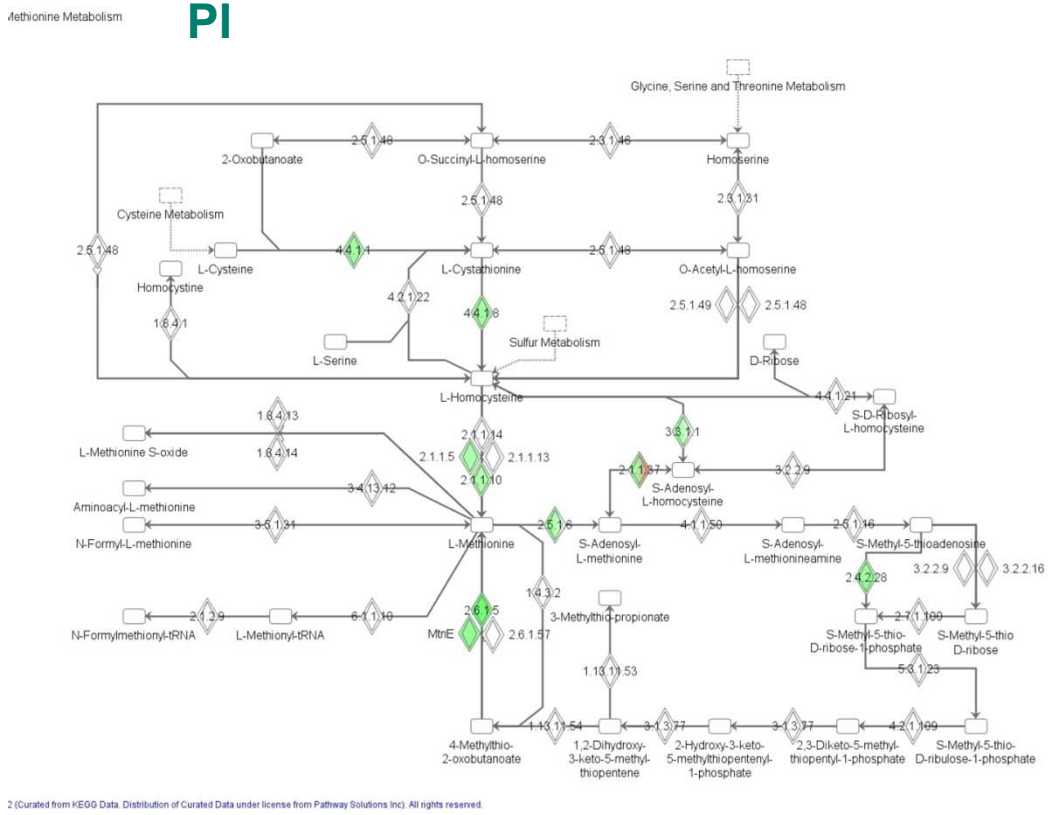
B) Maternal-diet-associated DMRs/Genes





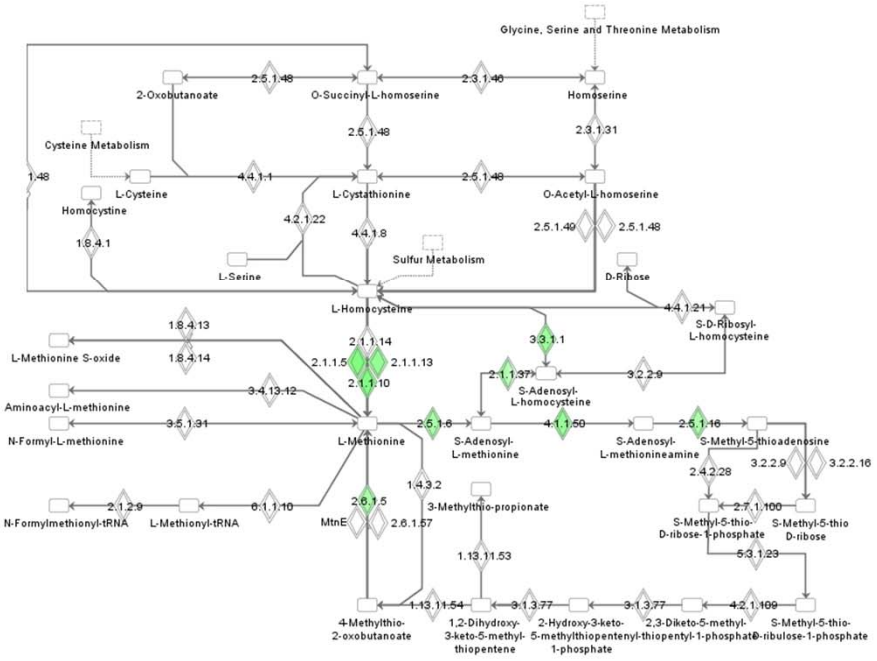
Differential expression due to maternal dietary treatment

PI

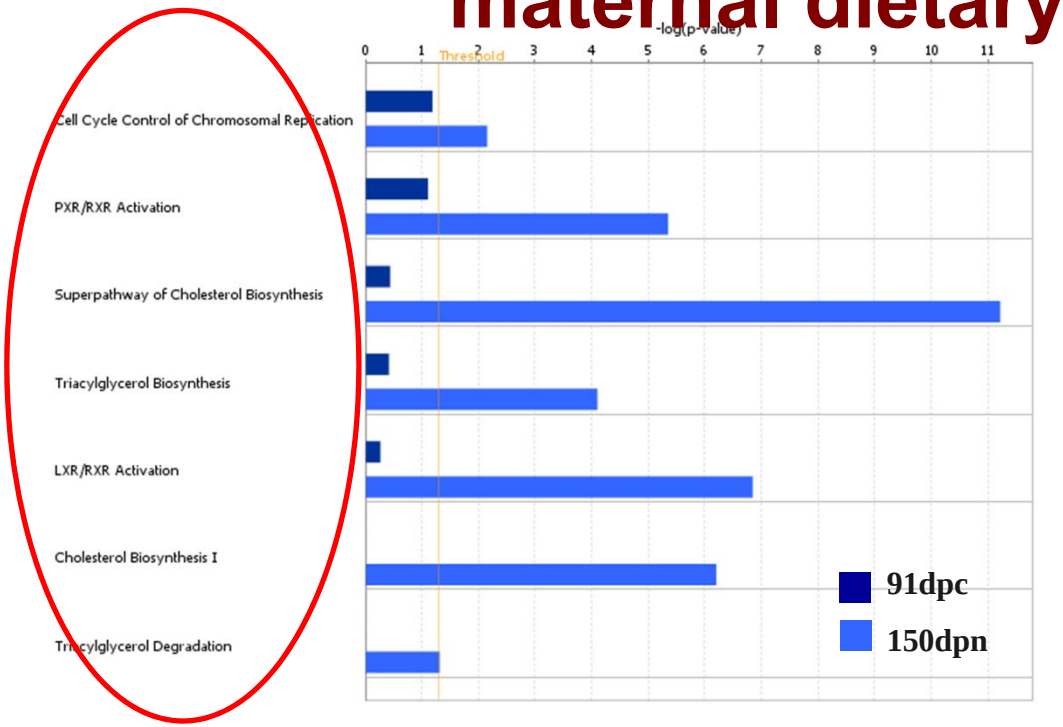


Methionine metabolism: regulated in both breeds including DNMT1, DNMT3a, DNMT3b

DL

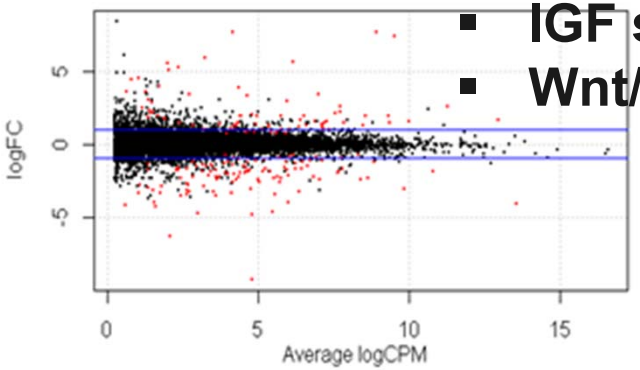


Differential expression due to maternal dietary treatment

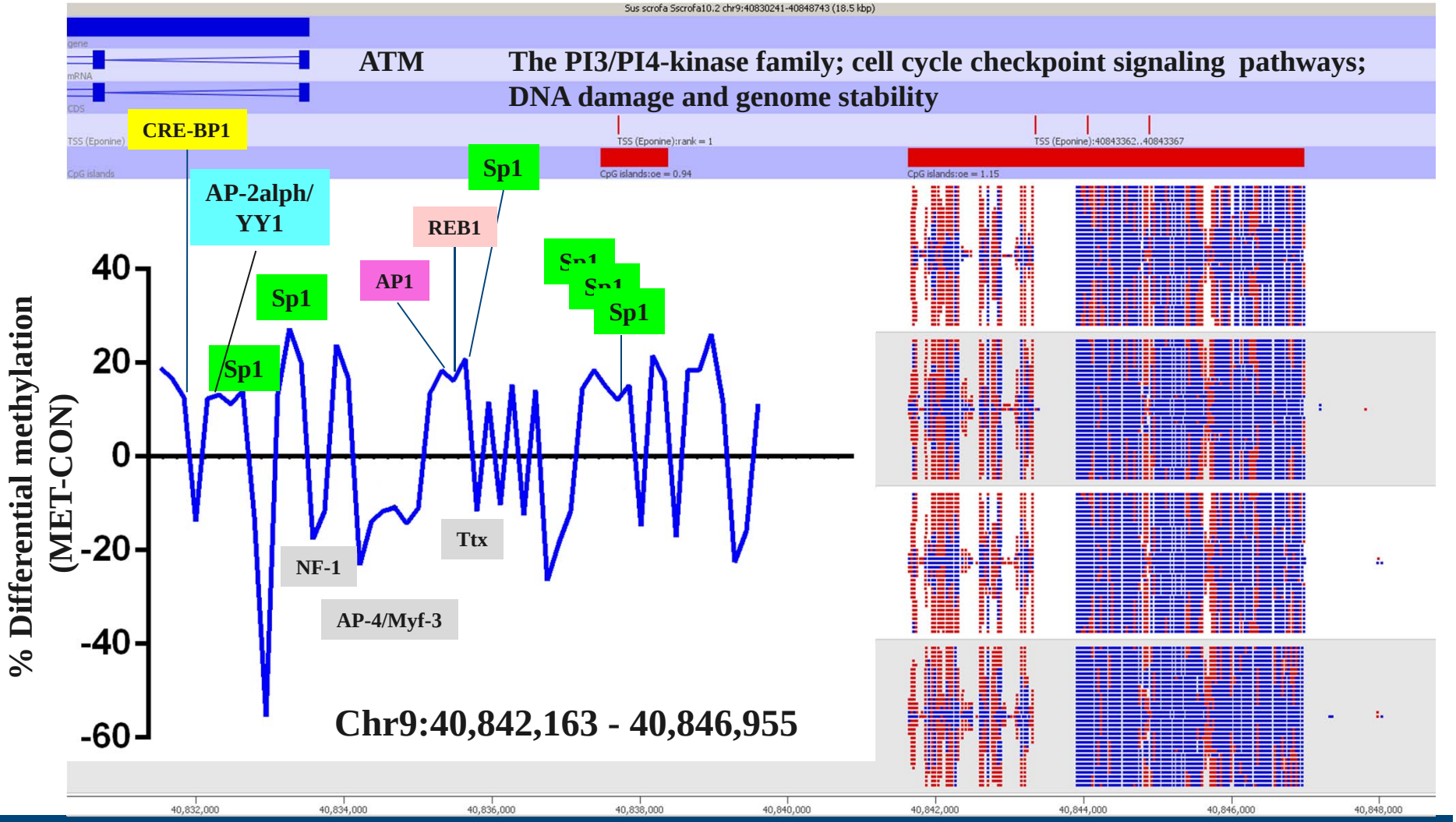


- 150dpn:
  - several lipid metabolism pathways
- 91dpn:
  - GADD45 signaling
  - Pyridoxal 5-phosphate salvage pathway
  - Folate poly-glutamylation
  - IGF signaling
  - Wnt/β-catenin signaling

426 and 363 genes differentially expressed at 91dpc and 150dpn, respectively.

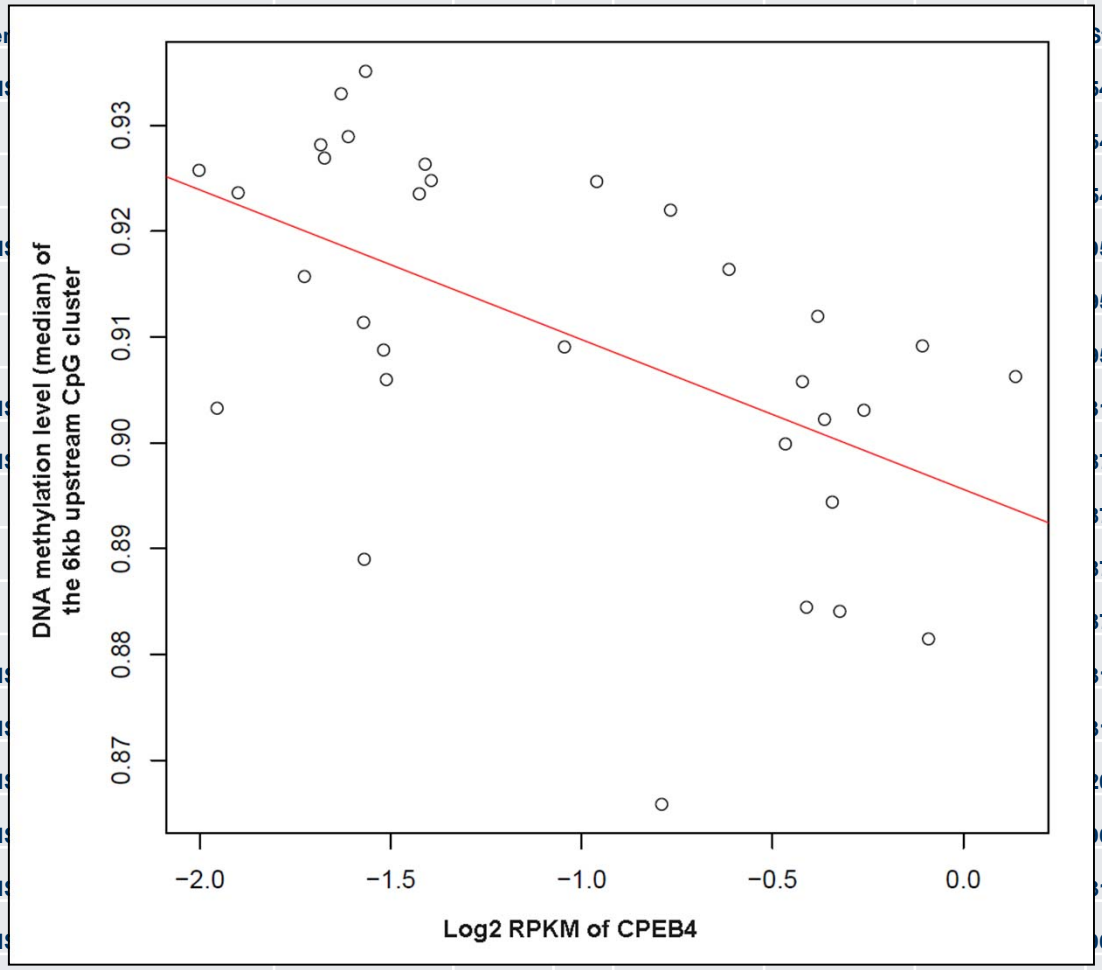


Functional annotation of DMRs: Serine-protein kinase ATM locus



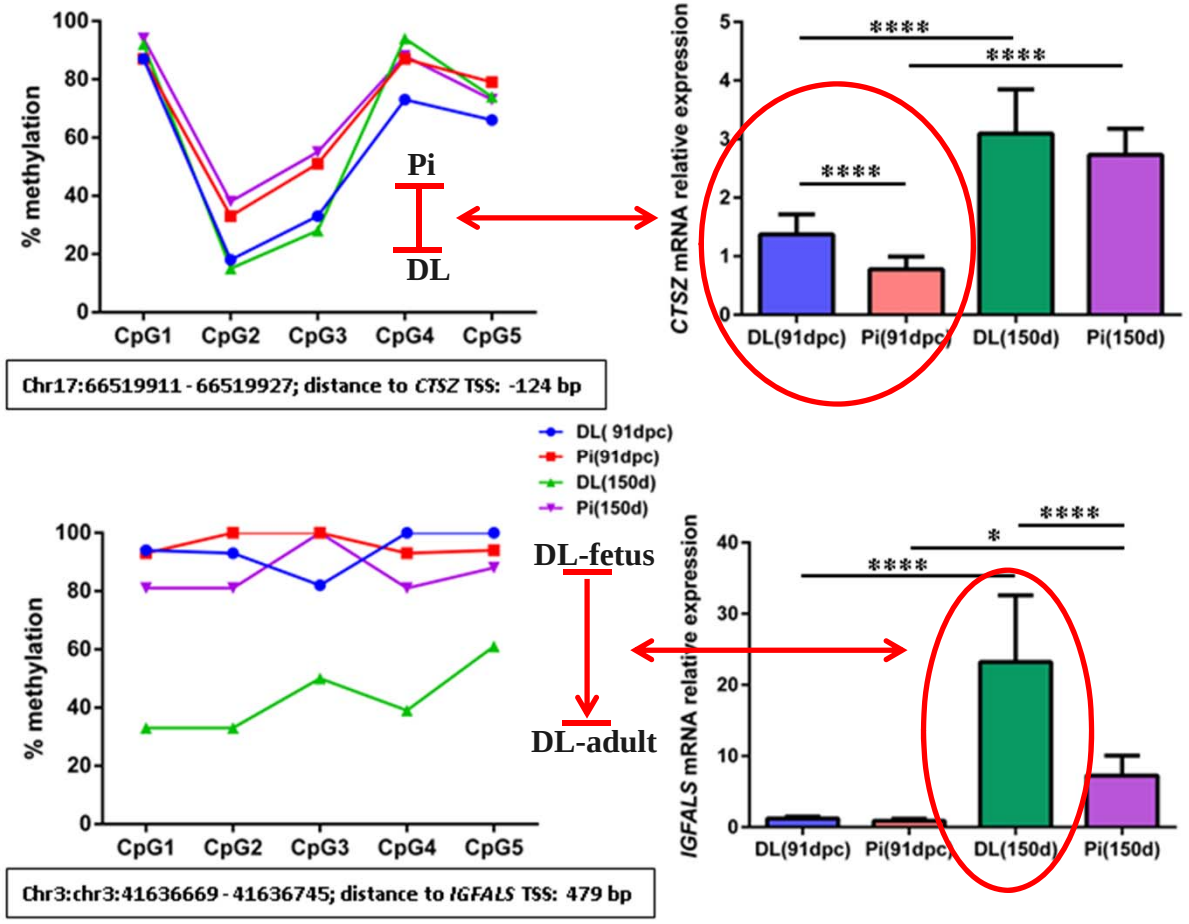
Integration of RRBS and RNA-Seq

Significant correlation between RNA-Seq and RRBS



Ge					Start	End	Upstream (kb)	Mean correlation
EN					54881577	54881654	-4.88	-0.3486
					54883290	54883353	-6.59	-0.6066
					54883879	54883948	-7.18	-0.5185
EN					956292	956548	-66.32	-0.4726
					955689	956001	-66.92	-0.4569
					955133	955334	-67.47	-0.4833
EN					314215889	314215939	-78.00	-0.4348
EN					872977	873957	-0.28	-0.3231
					874404	874578	-1.71	-0.3150
					874784	875098	-2.09	-0.3378
					875540	875758	-2.84	-0.3133
EN					314215861	314215889	-64.34	-0.3924
EN					314215910	314215939	-70.05	-0.3889
EN					20180501	20180635	-88.71	-0.3786
EN					96942120	96942153	-86.52	-0.3754
EN					314215861	314215919	-89.82	-0.3682
EN					96940169	96940258	-31.97	-0.3574
ENSSSCG00000017388	COA3	+	12	20277755	20278918	12_1	20180501	20180598	-97.25	-0.3320

Association between DNA methylation state and gene expression suggesting biological significance of the identified DMRs



Summary

- Epigenetic marks determine the transcriptional potential of the cell
- They represent an additional level of information to explain the phenotype
- Epigenetic marks link environment and genome and represent the molecular equivalent of genotype × environmental interactions
- Knowing and understanding epigenetic marks will contribute to the refinement of estimates in selective breeding
- Transgenerational inheritance and the contribution to “missing heritability” are a matter of debate
- Epigenetic studies will provide biomarker for management and/or breeding
- It will be possible to promote targeted epigenetic marks to develop certain phenotype

Thanks to
my co-authors and lab-team

SABRE
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H. Sauerwein, Uni Bonn

Thank you for your attention!!!