Vincent COUSTHAM – UMR83 INRA-Univ. Tours, France

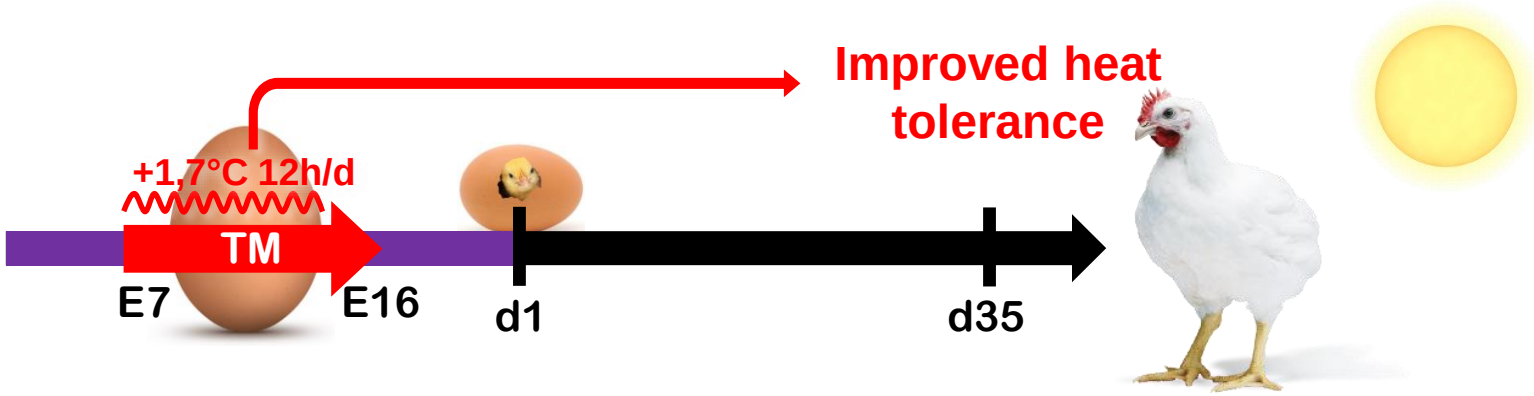
Adebiotech / EPIGEN 2018

Mécanismes épigénétiques de l'acquisition précoce de la tolérance à la chaleur chez les oiseaux

INRA SCIENCE & IMPACT Centre Val de Loire

PROJET FINANCÉ PAR L'ANR - PROJECT FUNDED BY THE ANR

Thermal Manipulation (TM) during embryogenesis impacts broiler later-life heat tolerance



Reduced mortality for male broilers when heat-stressed at slaughter age
(Piestun et al., 2008)

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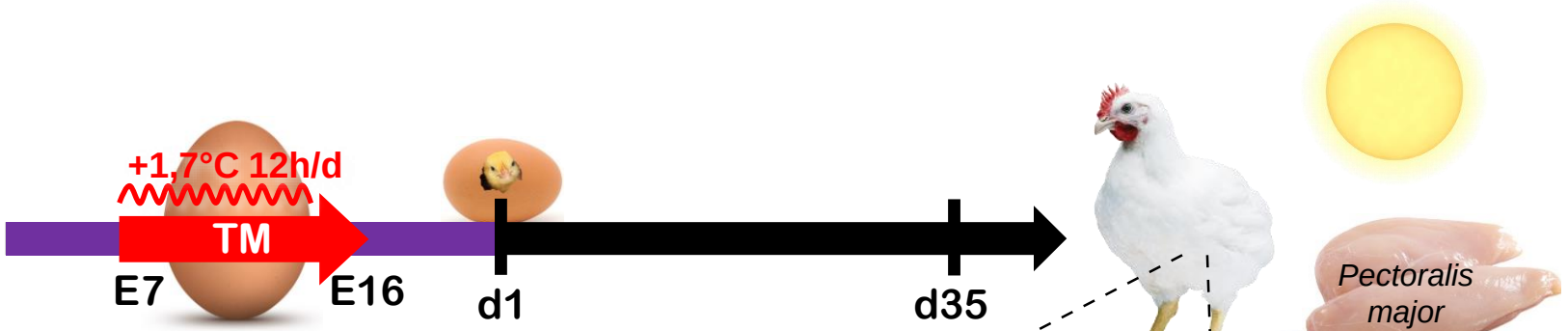
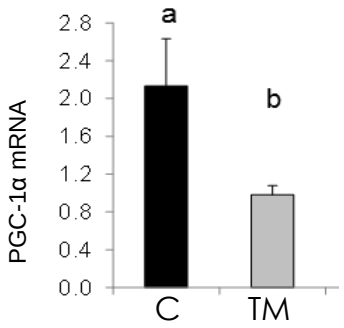


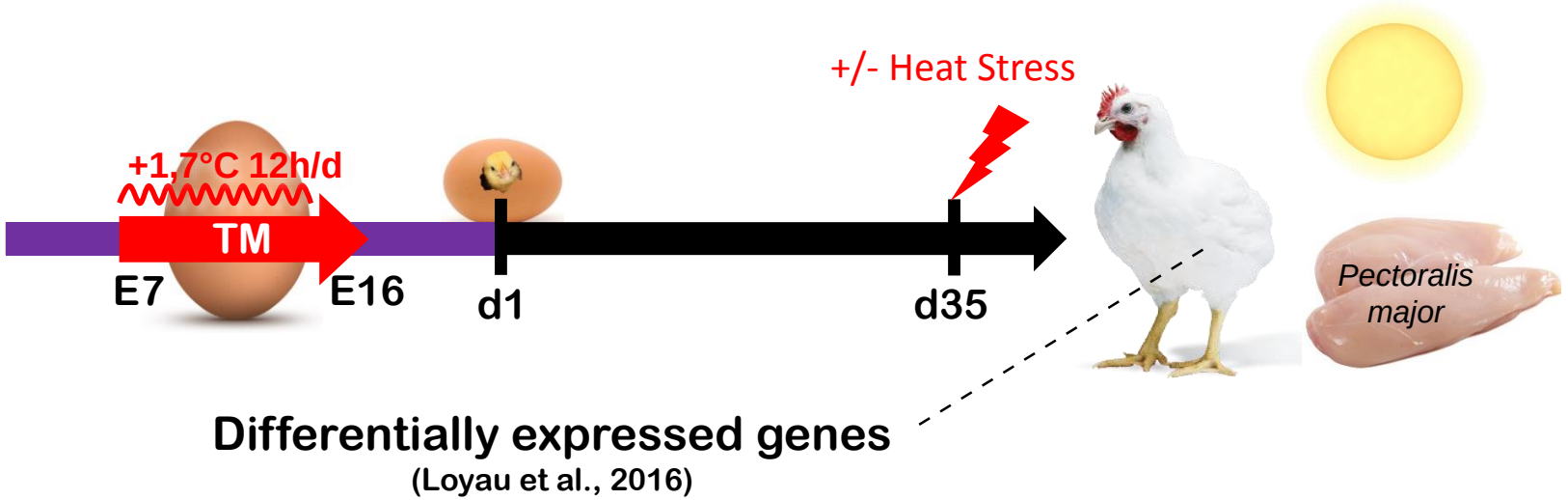
metabolic changes
(Loyau et al., 2014)



physiological changes
(Collin et al., 2007; Loyau et al., 2013)

Body T°C d0-d28
Triiodothyronine (T3) d0-d28

Thermal Manipulation (TM) is associated with later-life gene expression changes



TM vs C : **28** differentially expressed (DE) genes at d35

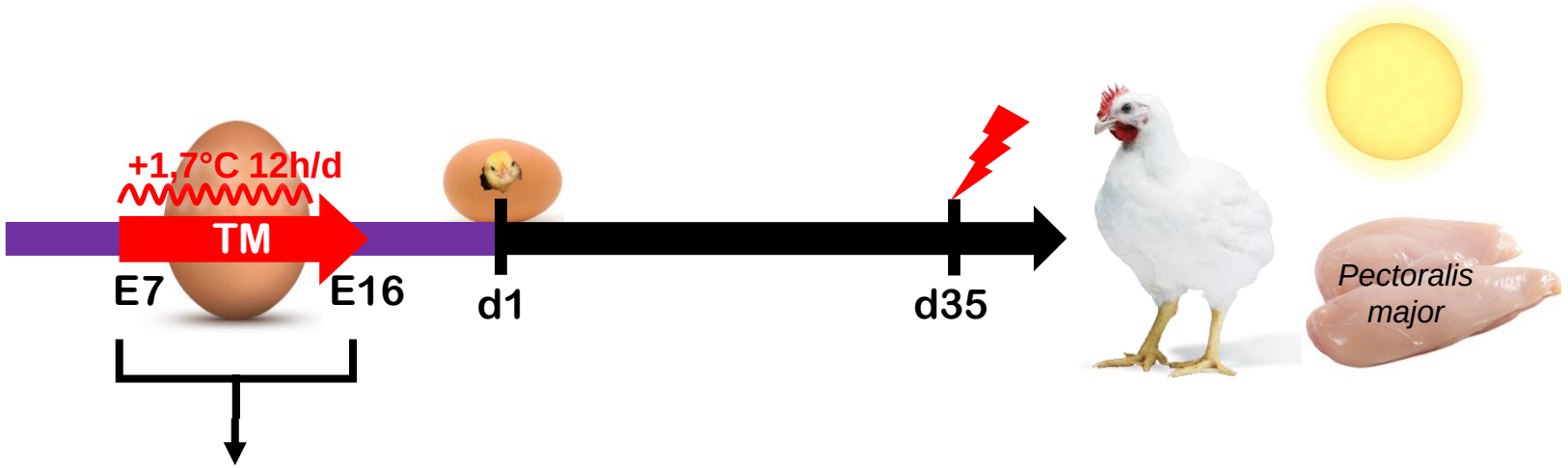
When heat-stressed at d35:

- **128** DE genes for Control incubation (ctrl vs ctrl+HS)
- **759** DE genes for TM incubation (TM vs TM+HS)

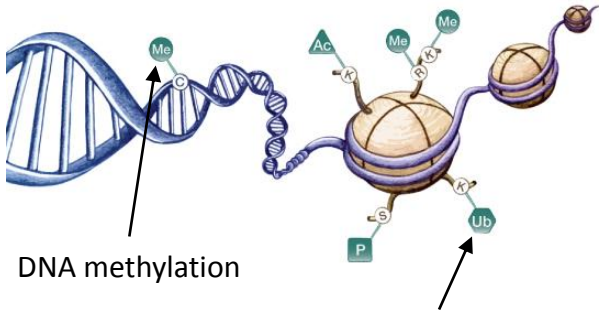
→ 5X more genes responding

Affymetrix chicken microarray

Thermal Manipulation (TM) impact on genes mediated by epigenetic reprogramming?

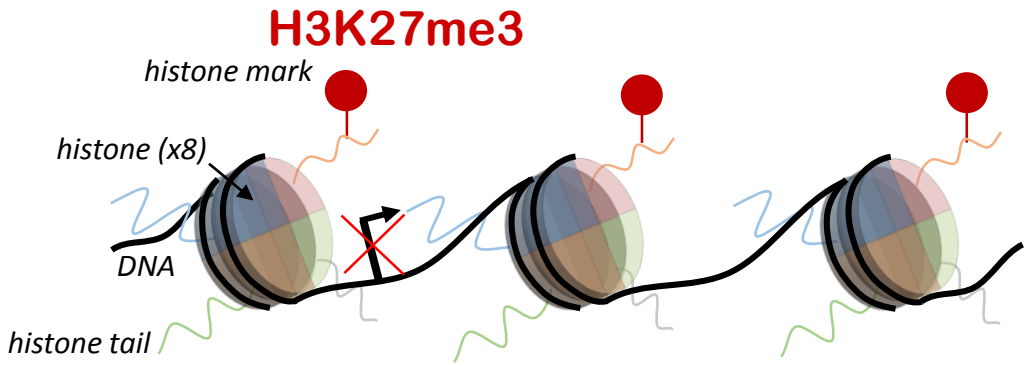


Epigenetic changes? → ***Adapted gene response***

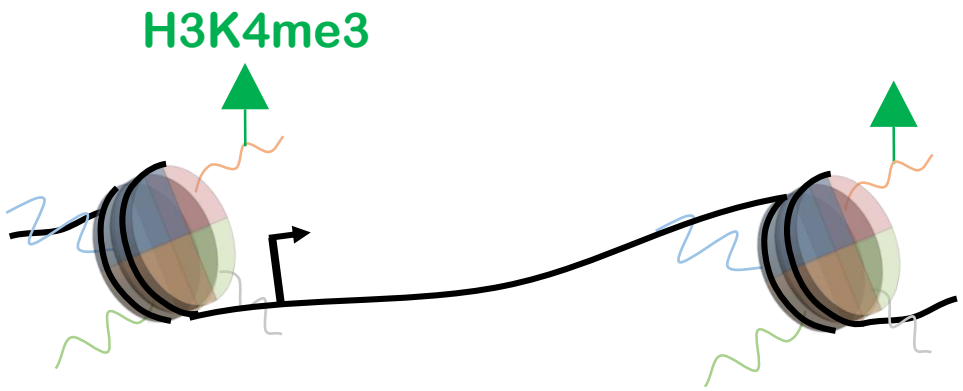


Genome-wide analysis of two histone marks

- **H3K27me3** : repressive histone mark (established by PRC2 complex)

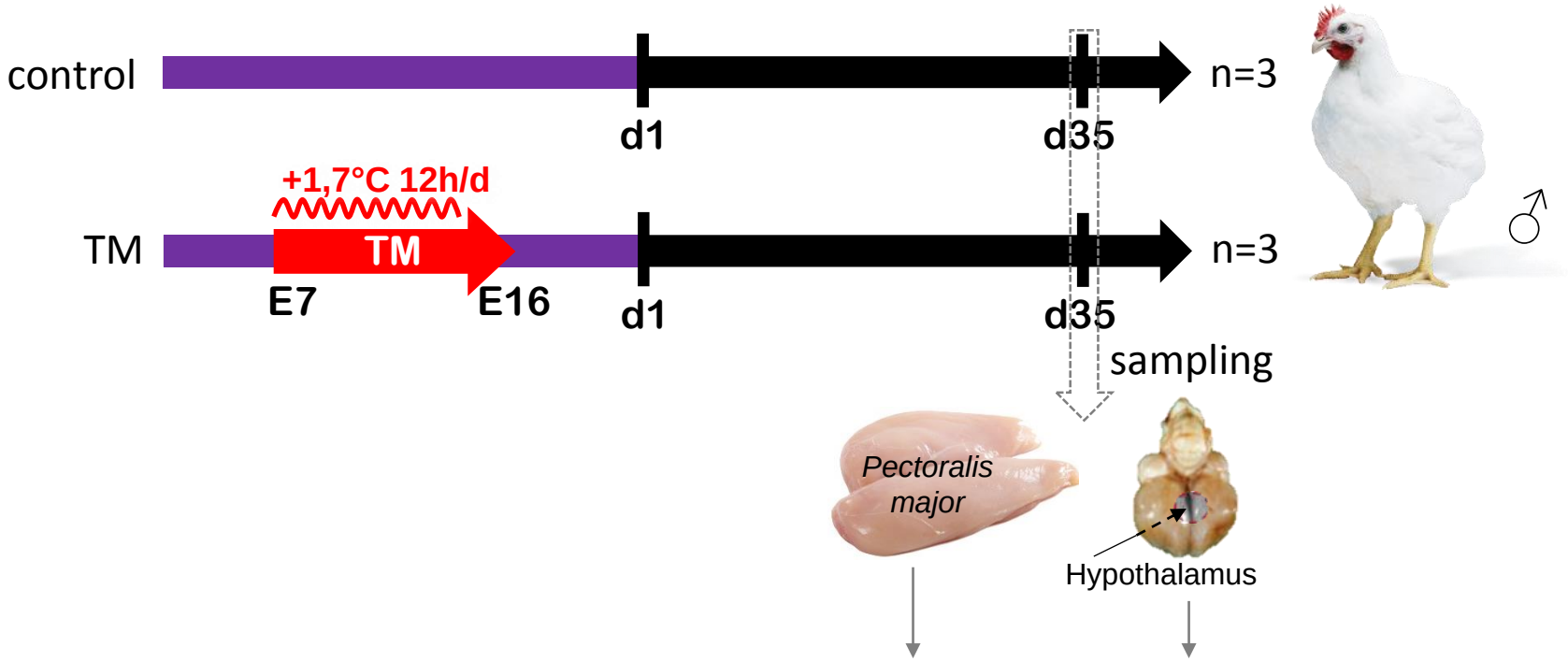


- **H3K4me3** : histone mark permissive to gene expression (established by Trx/MLL complex)

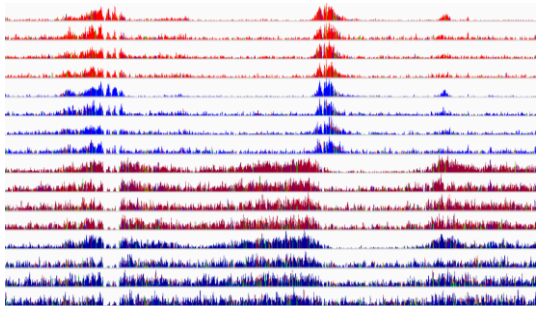


H3K27me3 involved in the memory of cold exposure during winter (Coustham et al 2011) and H3K4me3 in the memory of heat stress in plants (Lämke et al 2016)

Experimental design to analyse the impact of TM on histone marks

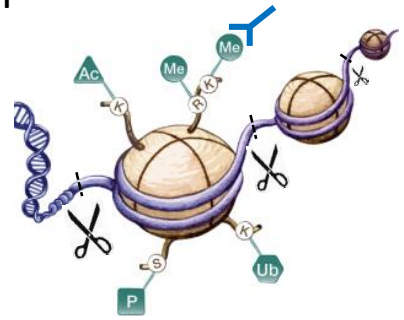


-seq: High-throughput sequencing (Hiseq 4000)

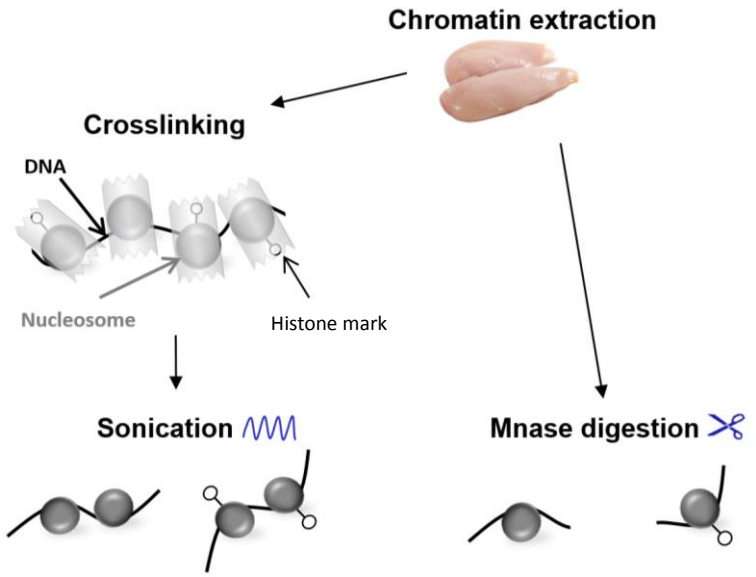


Immunoprecipitated DNA

ChIP: Chromatin extraction followed by immunoprecipitation with anti-H3K4/27me3



Chicken muscle ChIP-seq required native chromatin

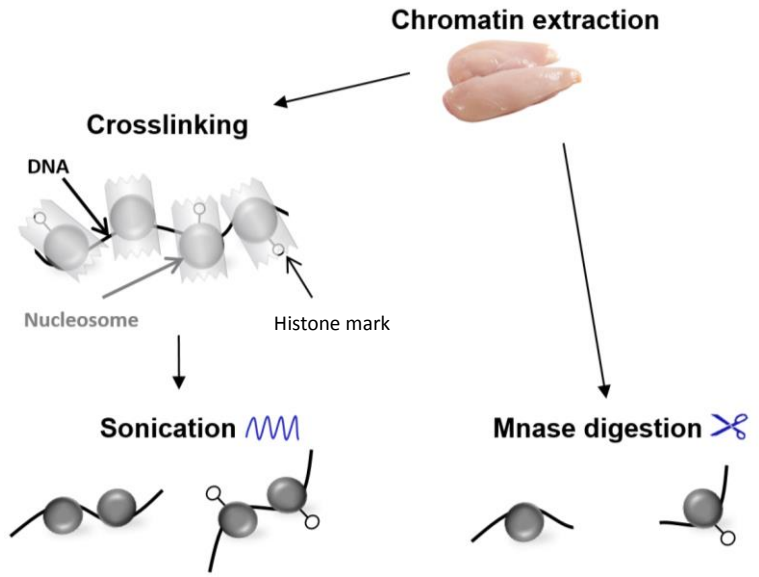


« crosslinked » chromatin « native » chromatin

David, Mersch et al, Epigenomes 2017

Chromatin interactions are usually fixed before analysis, but strong chromatin interactions (such as histone marks) can also be studied without fixation.

Chicken muscle ChIP-seq required native chromatin

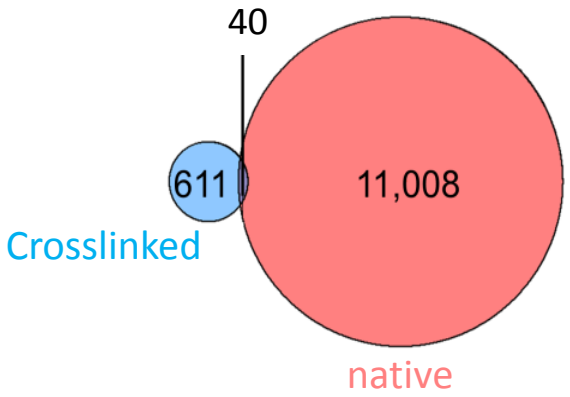


« crosslinked » chromatin

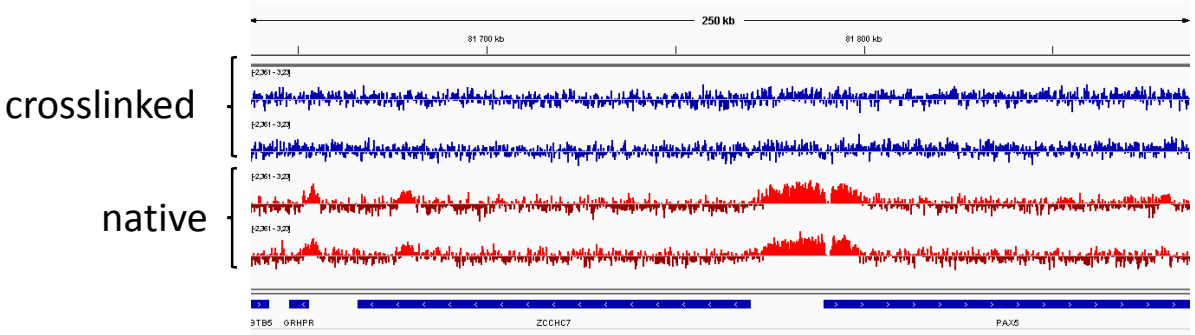
« native » chromatin

David, Mersch et al, Epigenomes 2017

Total ChIP-seq peak counts:



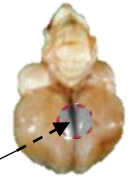
ChIP-seq signal (normalized):



David et al, Biol. Proc. Online 2017

TM impacted H3K4me3 and to a lesser extent H3K27me3 in brain but had a reduced impact in muscle

n=3 TM, 3 controls

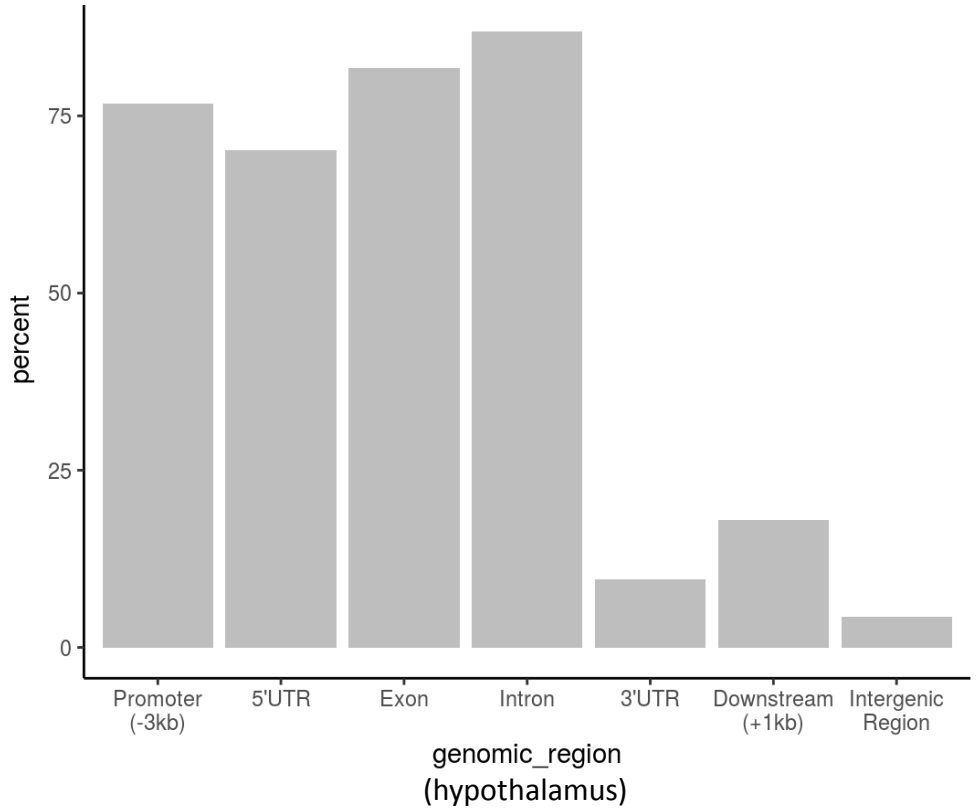


	H3K4me3 PePr(ws240)_union_edgeR	H3K27me3 epic_union_edgeR	H3K4me3 PePr(auto)_union_edgeR	H3K27me3 epic_union_edgeR
Peaks number	75943	26562	30590	23098
Genome coverage	6,44%	6,95%	2,90%	10,89%
Differential peaks TM vs C	**45**	**20**	**785**	**148**
…up	**7**	**4**	**147**	**37**
….down	**38**	**16**	**638**	**111**

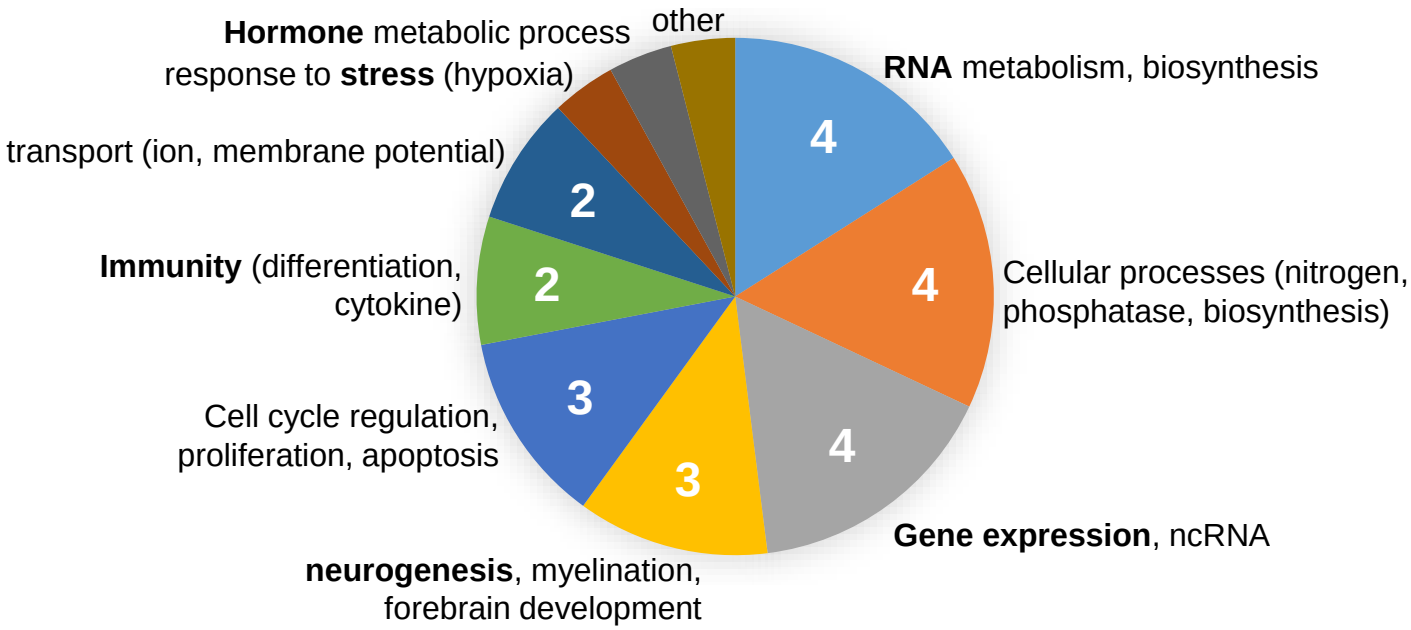
Analysis in progress, final results may vary *David et al, in preparation*

Caracterisation of H3K4me3 differential peaks

Distribution of differential peaks (ChIPpeakAnno)



Functional analysis : 25 GO clusters
(Biological Processes)



The enrichment tests were performed with the topGO R package, and enriched GO terms were considered with p<0.01

Analysis in progress, final results may vary

Conclusions (chicken, histone marks)

TM is associated with histone post-translational modifications in the hypothalamus of male broilers that may alter the response of genes involved in several biological pathways that may contribute to heat acclimation;

The lack of differential peaks in muscle is consitent with a **genome-wide analysis of muscle DNA methylation on 5 TM and 5 control**: difficulties to reliably identify regions of differential methylation (Whole-Genome Bisulfite Sequencing, Illumina Hiseq 2000, 2x100b).

► **Muscle epigenome may not be reprogrammed by TM (unlike hypothalamus)**

The inbred INRA quail line: an ideal avian model to study avian environmental epigenetics?

ConsD line © INRA

Same bird family as chicken: Phasianidae
2n=78, high degree of synteny (Kayang 2006, Recoquillay 2016)

Small size, fast generation time, prolific

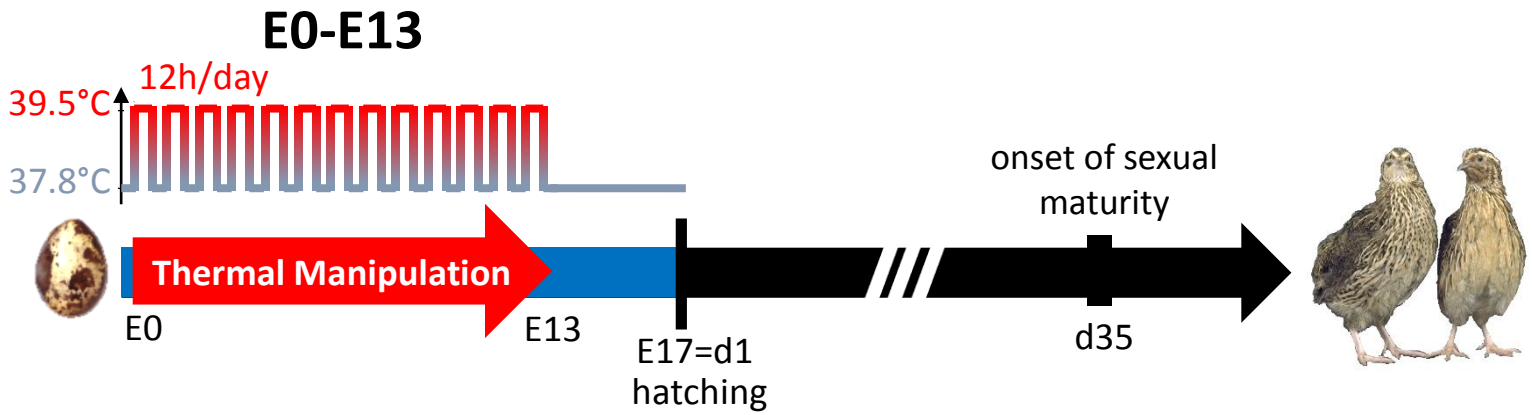
INRA **inbred line ConsD**
Limited genetic variability, full pedigree history, ancestors rearing conditions controlled

High quality genome available (ConsD)
Illumina GA IIx (73X) + PacBio long reads (20X)
Genome size : ~ 0,92Gb
15281 annotated transcripts so far (NCBI)

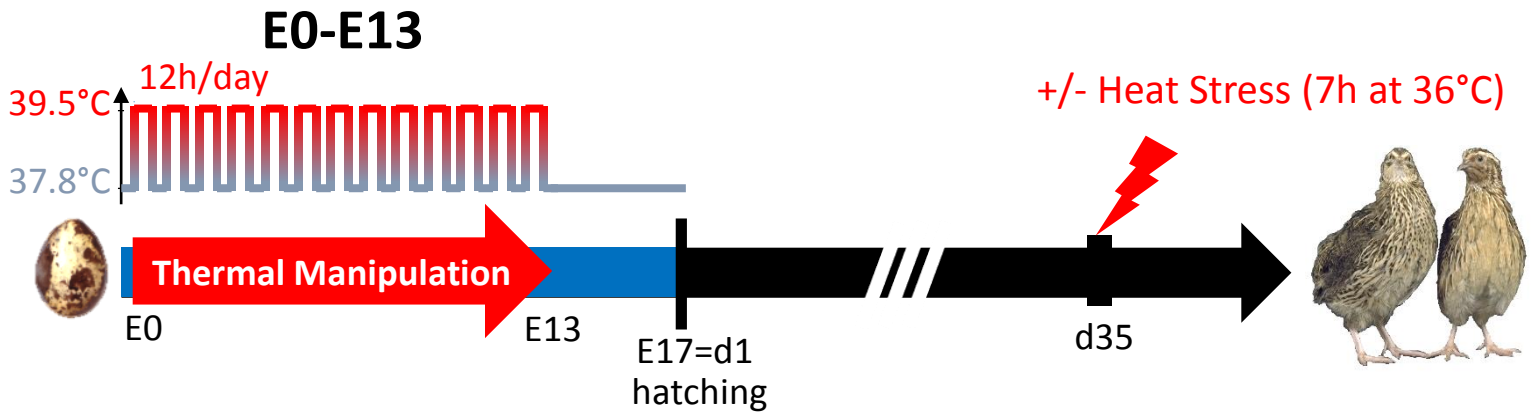
TM-like treatments impact quail physiology
Hemid et al., 2010
Alkan et al., 2013
El-Daly et al., 2013

ANR Project « QUAILHEATE » 2015-2019
EPIGENETIC MECHANISMS OF EMBRYONIC **HEAT** CONDITIONING IN **QUAIL**

The TM treatment in quail



The TM treatment in quail: some results

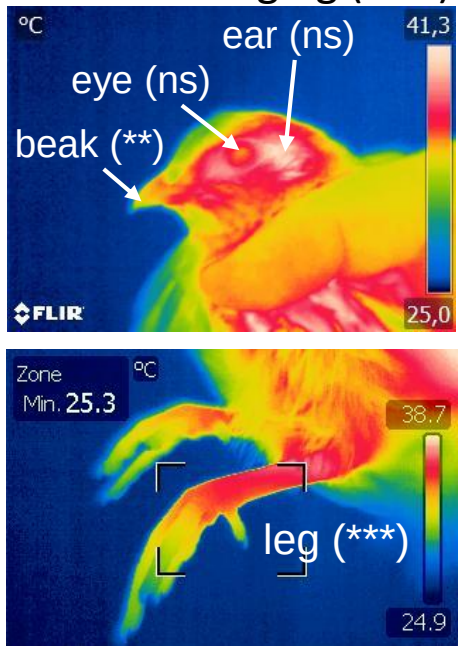


Body weight

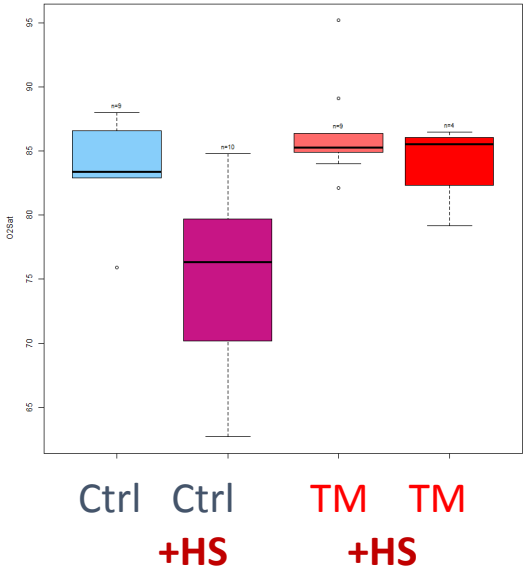
↗ at hatching (**)

↘ at d28-d35 (***)

Thermal Imaging (d35)

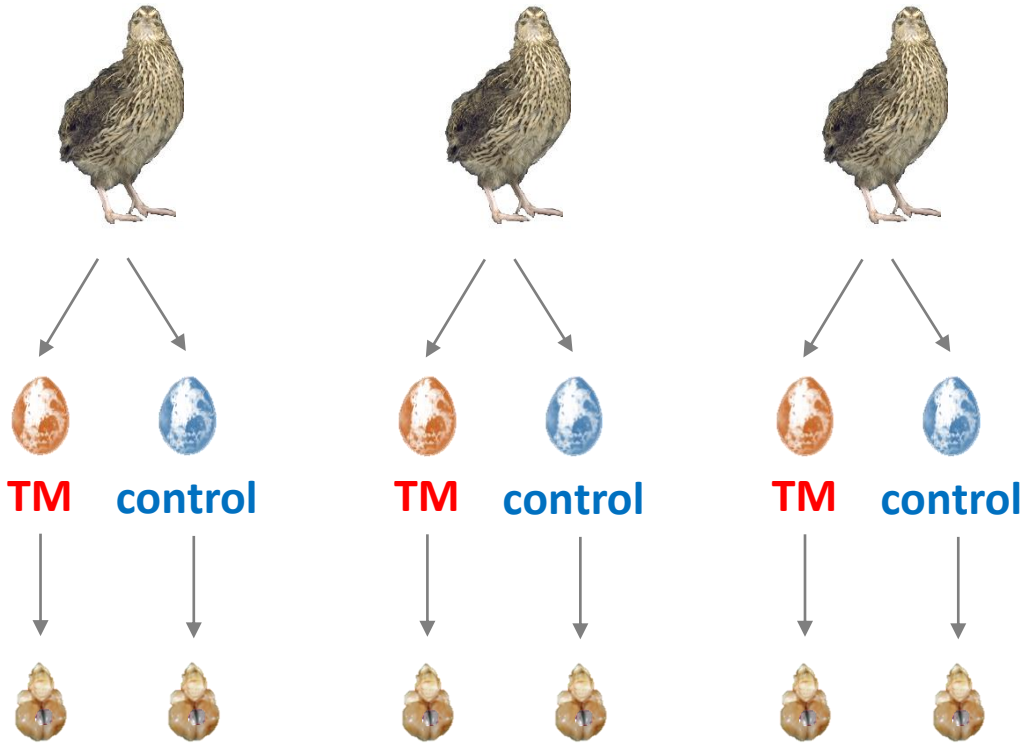


O2sat (females, d35)



Vitorino Carvalho et al, in preparation

Pilot -omics experiments in male quails



3 pairs of brothers

Hypothalamus sampling at **hatching** (d0)

Transcriptome (RNA-seq)
1x50bp, Hiseq4000
45 million reads/sample

Methylome (WGBS)
2x150bp, Hiseq2500
60X genome coverage

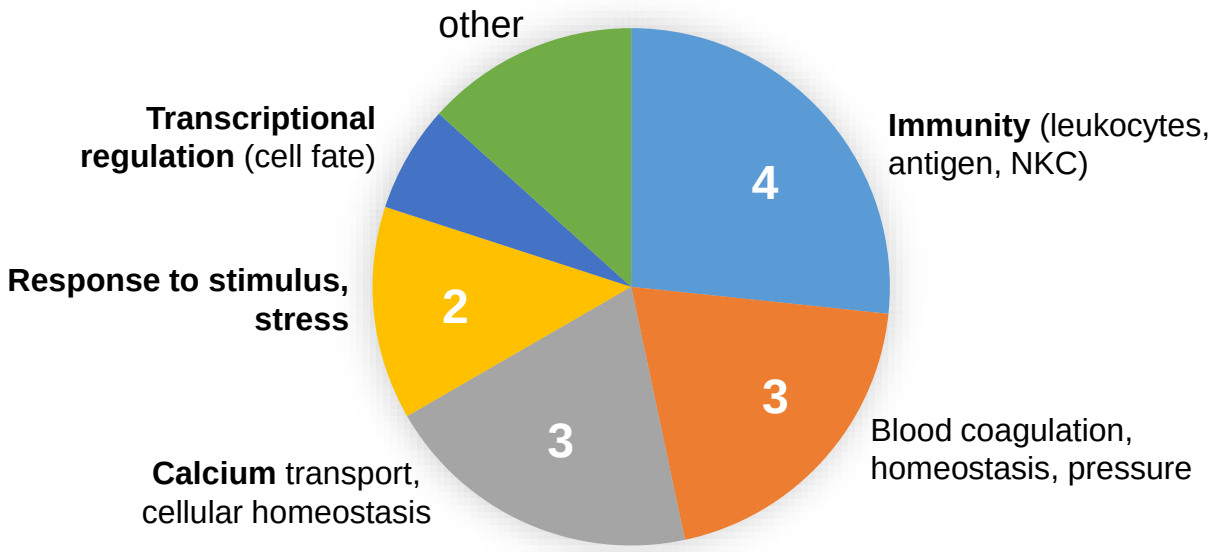
Transcriptome analysis (hypothalamus, ♂ quail, d0)

Number of DE genes
(SARTools edgeR, siblings effect in the model)

	n=	Aligner	Down	Up	Total
Males	**6**	STAR	**176**	**149**	**325**

A BH p-value adjustment was performed (Benjamini, 1995 and 2001) and the level of controlled false positive rate was set to 0.05.

Functional analysis : 15 GO clusters
(Biological Processes)



Reference was built using GO terms from known ortholog genes.
The enrichment tests were performed with the topGO R package, and enriched GO terms were considered with p<0.01

Analysis in progress, final results may vary

Methylome analysis (hypothalamus, ♂ quail, d0)

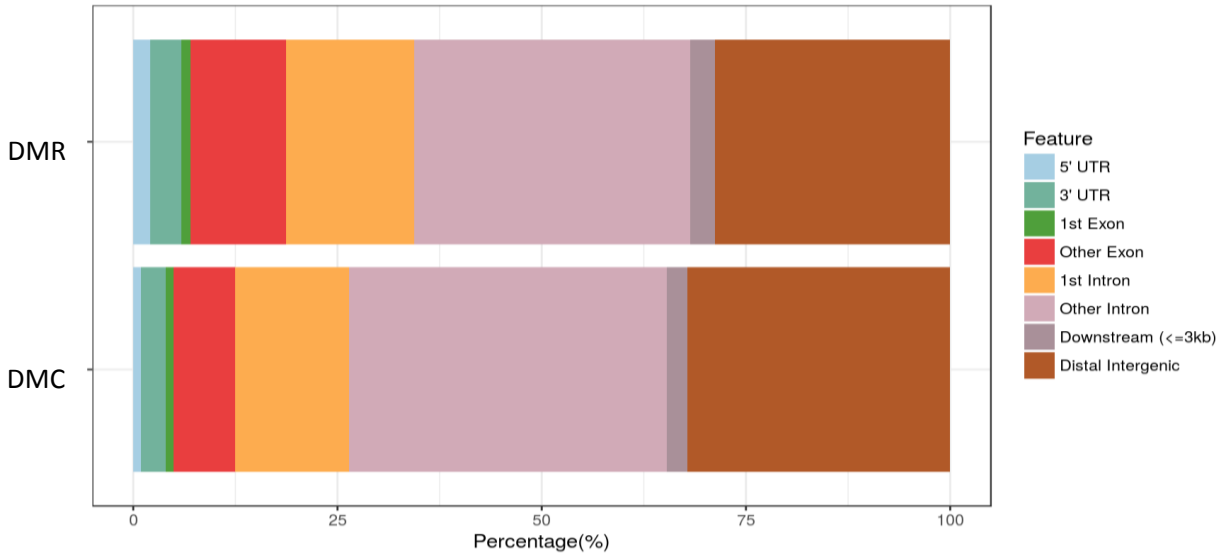
Number of Differentially Methylated Cytosines (DMC) or Regions (DMR):
Bismark, Methylkit, DSS

	n=	DSS
DMC	6	2474
DMR	6	1500

DMR: 3 or more consecutive CpG with at least 50% differential

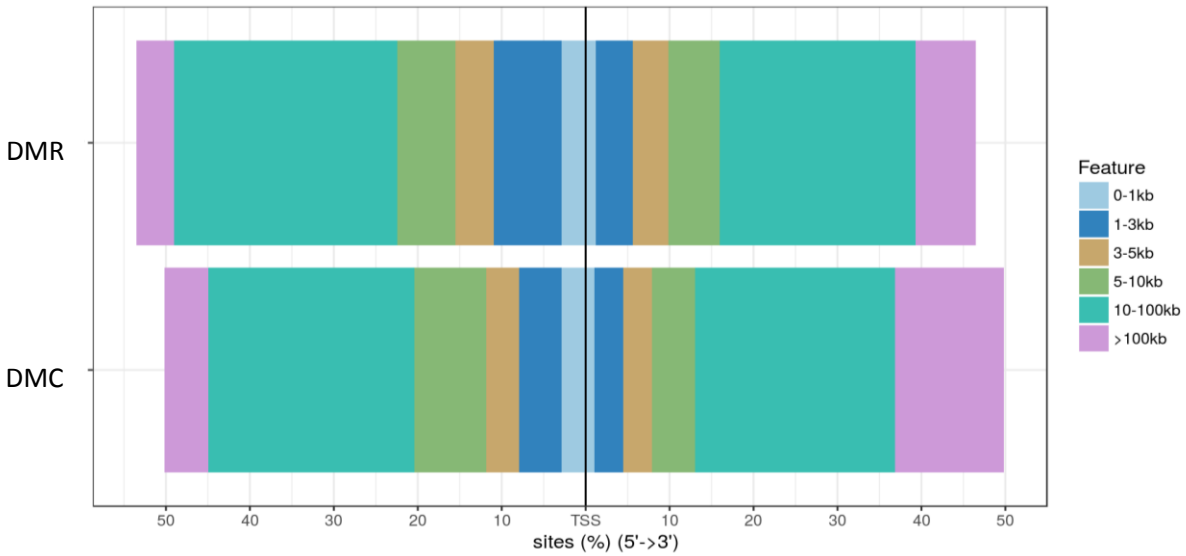
Most of DMC/DMR are located within genes:

Feature distribution:



Differential methylation is not preferentially located at promoters:

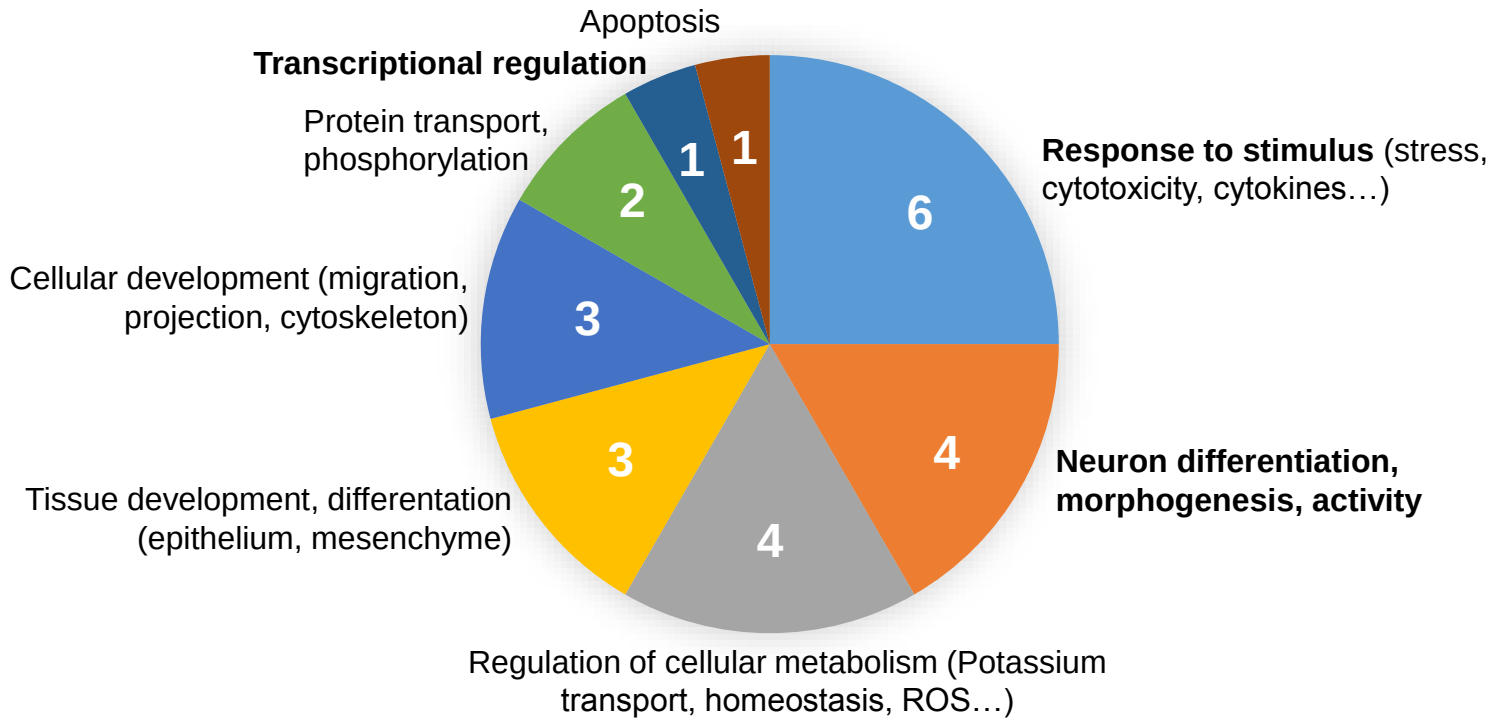
Distribution of DMR and DMC relative to TSS:



Analysis in progress, final results may vary

Methylome analysis (hypothalamus, ♂ quail, d0)

DMC/DMR functional analysis
24 GO clusters identified:



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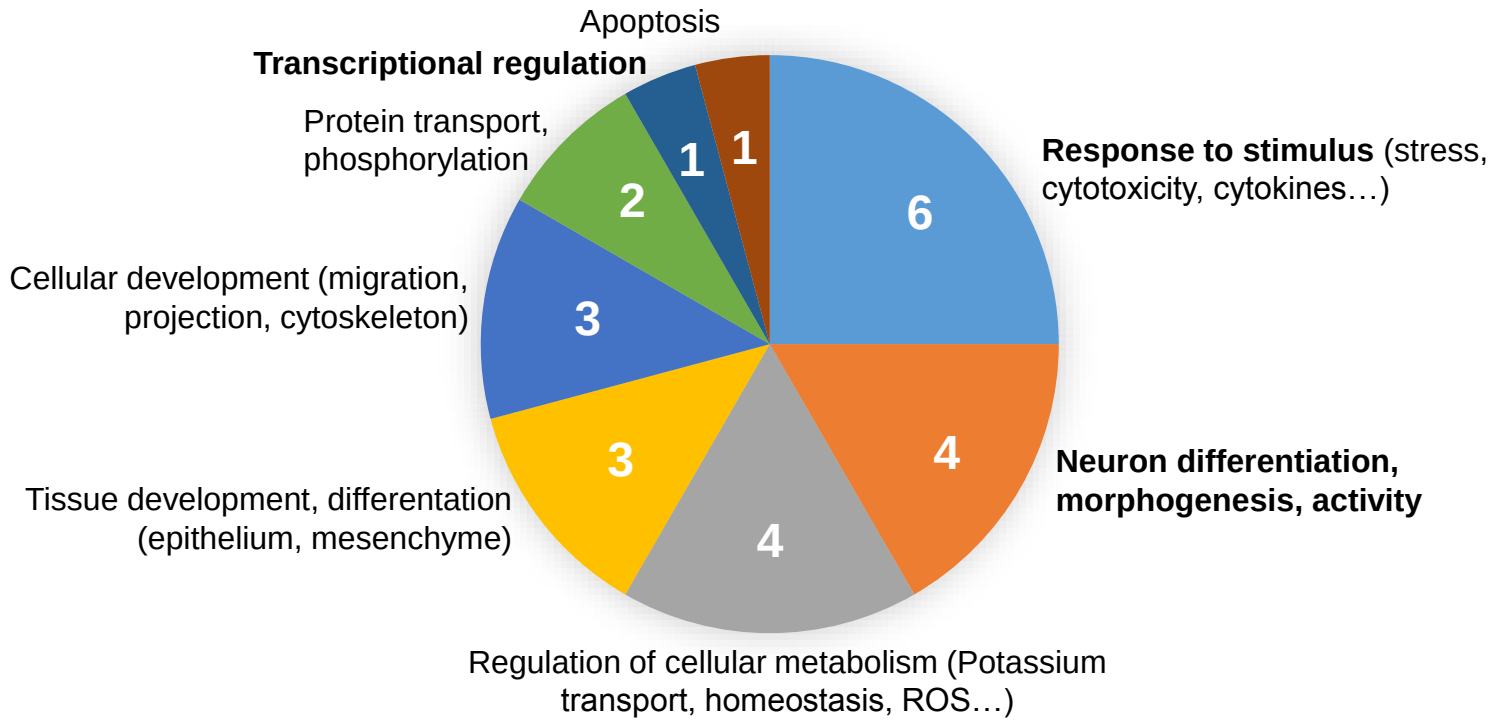
Methylome analysis (hypothalamus, ♂ quail, d0)

DMC/DMR functional analysis
24 GO clusters identified:



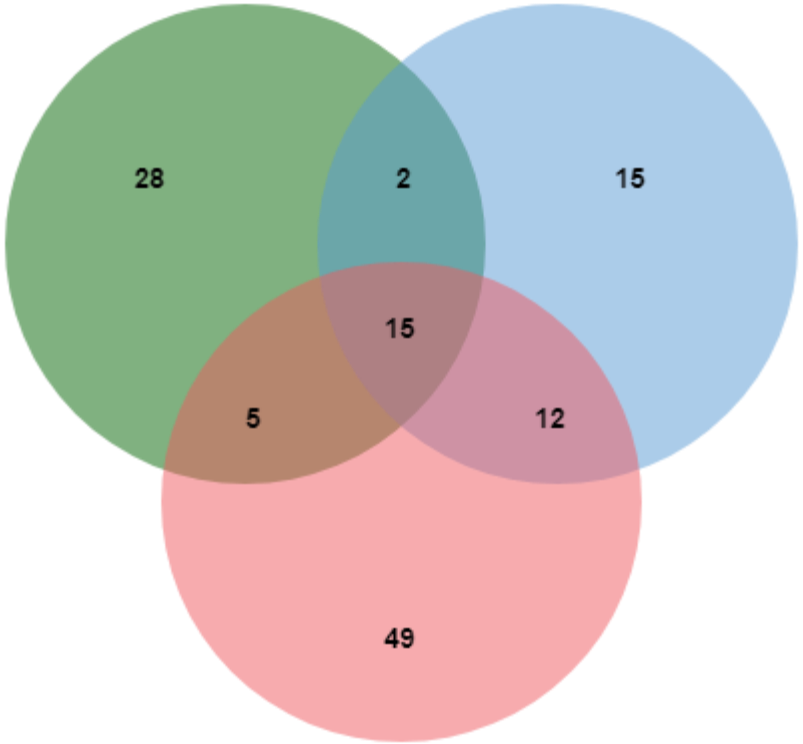
► TM may alter genes related to **transcriptional regulation**, **stress response** and **cellular metabolism** (transcription, homeostasis…) in the 3 analyses despite different marks, species and developmental stages

Analysis in progress, final results may vary

IPA canonical pathways comparison between studies (p<0,1)

RNA-seq (d0)

DMR (d0)

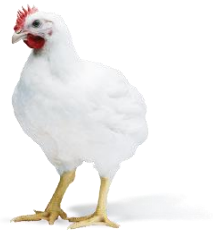


H3K4me3 (d35)

Shared canonical IPA pathways between the 3 studies:

Calcium Signaling (0,05)
Netrin Signaling
Synaptic Long Term Potentiation (0,05)
Opioid Signaling Pathway (0,05)
Corticotropin Releasing Hormone Signaling
Dopamine-DARPP32 Feedback in cAMP Signaling
Synaptic Long Term Depression (0,05)
Gustation Pathway
GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells
Transcriptional Regulatory Network in Embryonic Stem Cells
Cellular Effects of Sildenafil
GNRH Signaling
Neuropathic Pain Signaling In Dorsal Horn Neurons
CREB Signaling in Neurons
GABA Receptor Signaling

Analysis in progress, final results may vary



Conclusions

Both chicken and quail respond to the TM embryonic heat treatment despite variations;

These changes were accompanied by:
- **histone mark alterations** in the hypothalami of male TM chickens
- **transcriptome and methylome alterations** in the hypothalami of male TM quails

that may affect long-term phenotypes through partly similar biological pathways;

The muscle may not be the right place to look for epigenetic reprogramming as a consequence of an embryonic TM treatment;

The inbred Japanese quail is a promising model to study the long-lasting impact of perinatal environment on bird epigenome.

Acknowledgements

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