

Acute and delayed transcriptional responses following livestock-environment interactions

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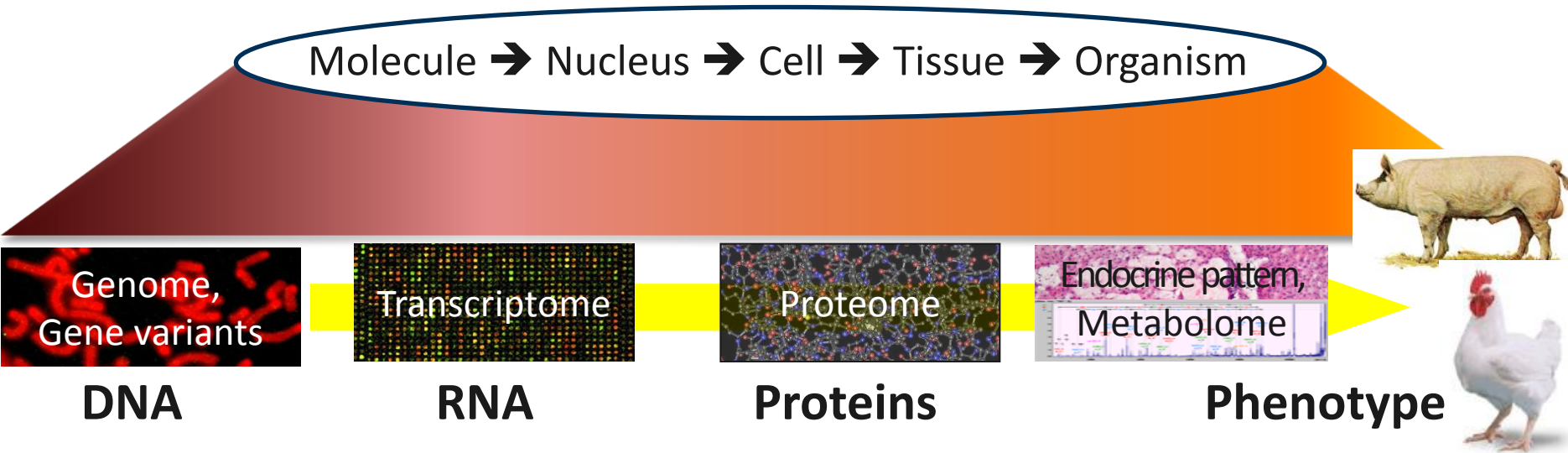
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FOR FARM ANIMAL BIOLOGY

Need to elucidate molecular phenotypes



Genotype + Environment = Phenotype

Genotype-phenotype map



Recording of molecular traits via 'omics'- techniques

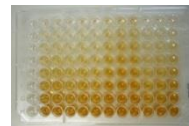
Classical traits



SNP-Chips



Microarray
RNA-Seq



MagPix
Elisa

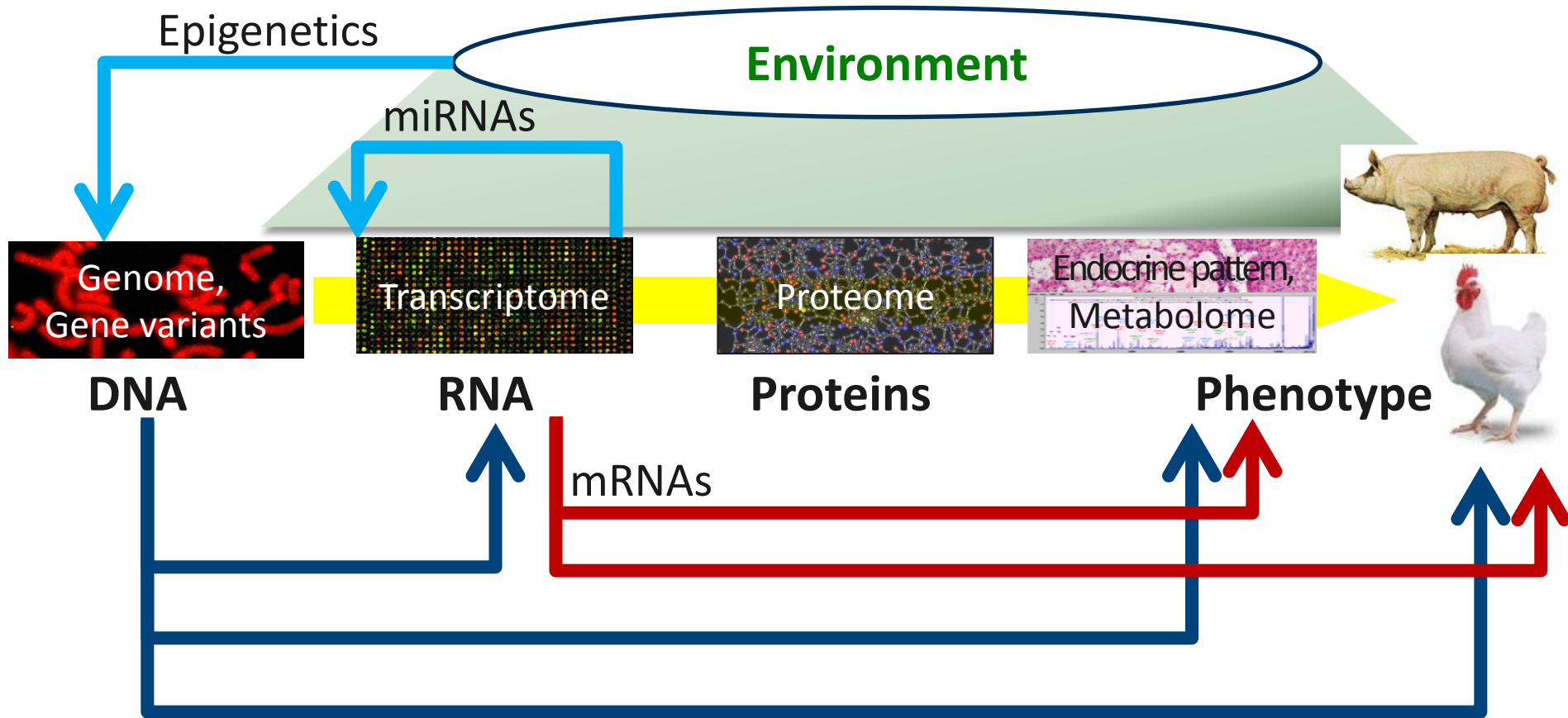


Serum
analytics



Meat quality

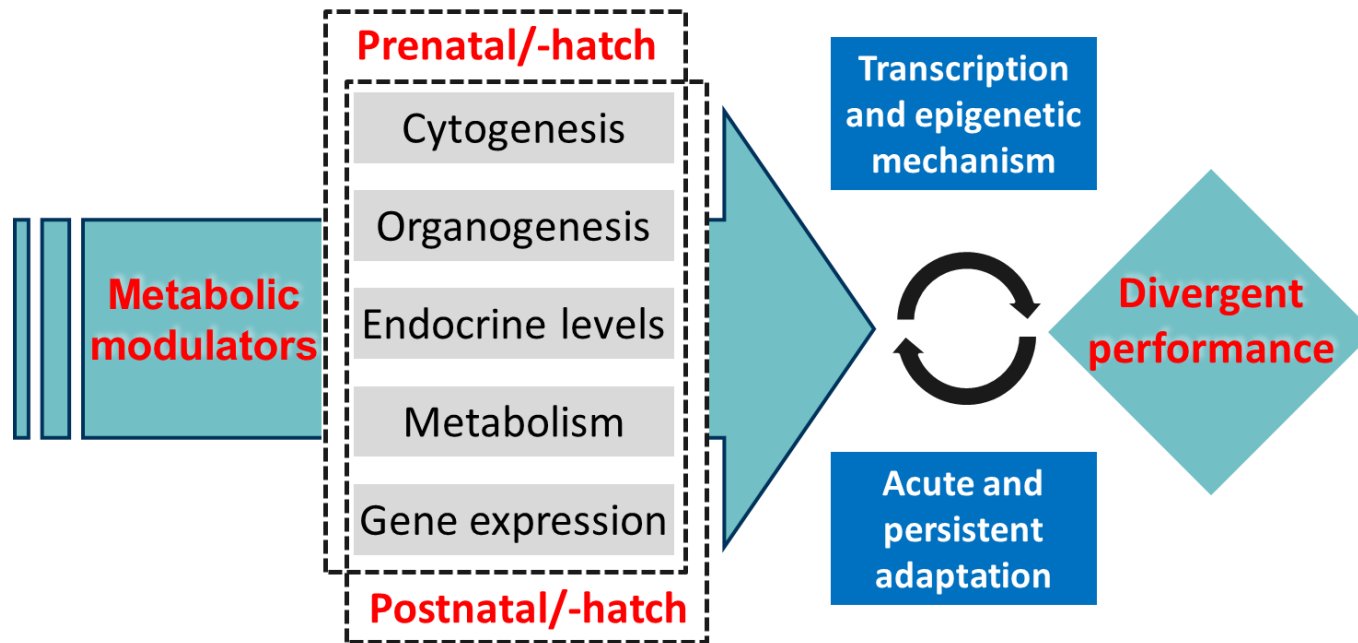
Analyses along the genotype-phenotype map



Integration of hypotheses-generating & holistic approaches

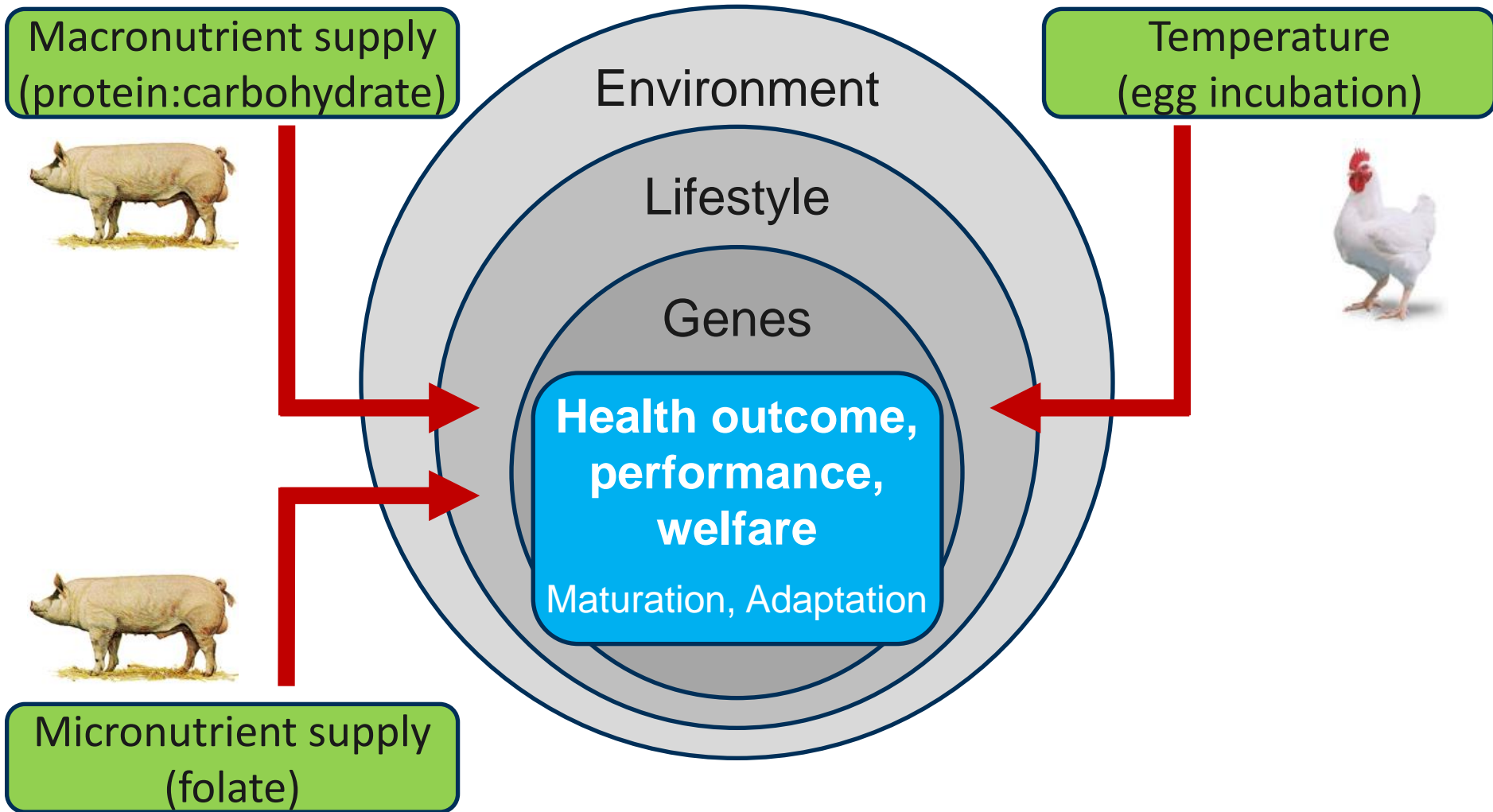
Environmental factors shape the phenotype

- Factors such as nutrition, temperature, light regimen, germ pressure, and stress affect the progeny's development
 - **Adaptive** responses & **long-term** consequences for offspring
 - Primary mechanisms? Health & welfare aspects?



Hales & Barker 1992
Snoeck *et al.* 1991
Lucas *et al.* 1991

Early Environment & Nutrition: FBN trials



Transcriptomic responses of porcine progeny to maternal diets varying in protein:carbohydrate ratios

Species: Pig


Environmental factor: Macronutrients

Hypothesis: Maternal diets which differ in protein:carbohydrate ratios affect phenotype of the progeny and induce adaptive transcriptional responses.



Experimental design

LP	maternal low protein diet, 6.5% CP	Cross fostering Lactation diet, Litter size: n=11	Standardised shoat and fattener feeding <i>ad libitum</i>	
AP	maternal adequate protein diet, 12.1% CP			
HP	maternal high protein diet, 30% CP			
94 dpc		1 dpn	28 dpn	188 dpn



- German Landrace; sib pairs balanced for sex
- Isocaloric diets containing 6.5 %, 12.1 %, and 30 % crude protein
- Sampling of liver & skeletal muscle tissue (*M. longissimus dorsi*)
- n=8 fetuses/offspring per diet, sex, stage, and tissue

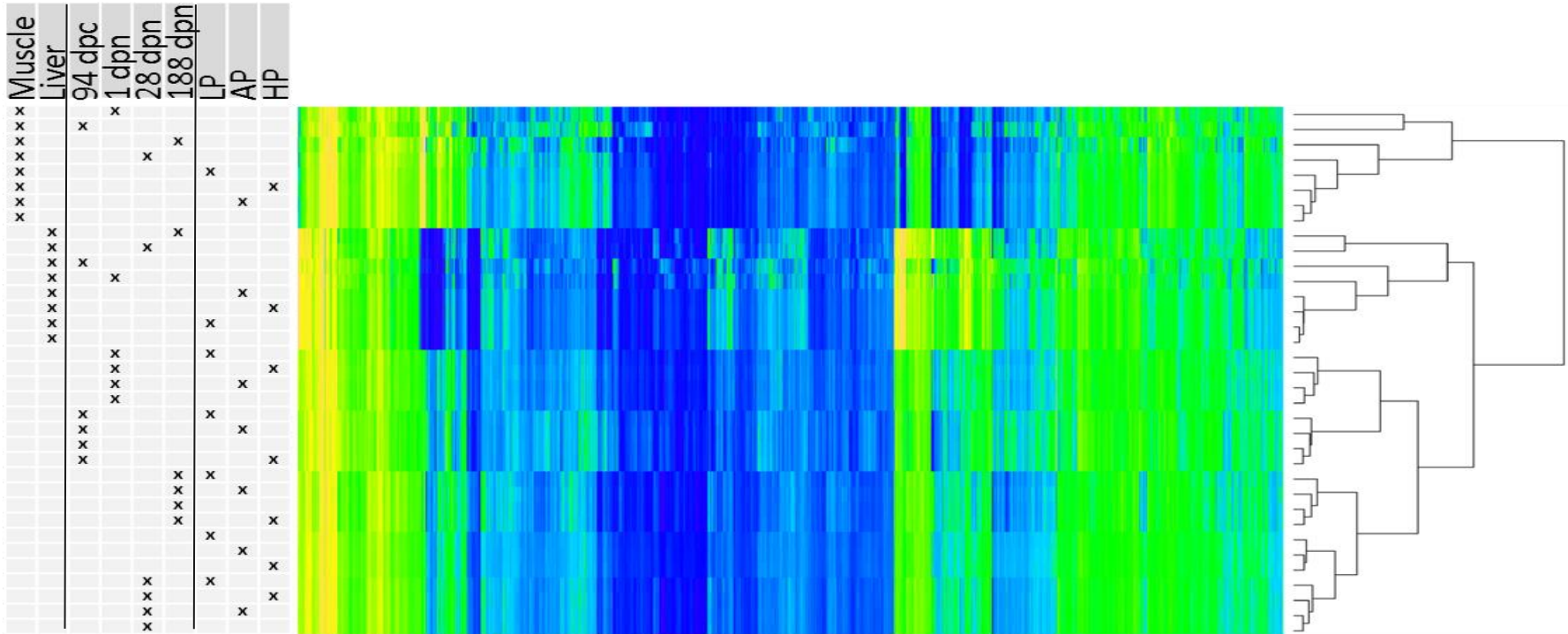
Lowered birth weight due to maternal diets

Table 1 Body weight and weights of tissues and organs of selected neonatal piglets (least squares means \pm SE) born to gilts fed adequate (AP), high (HP), or low (LP) levels of protein throughout gestation

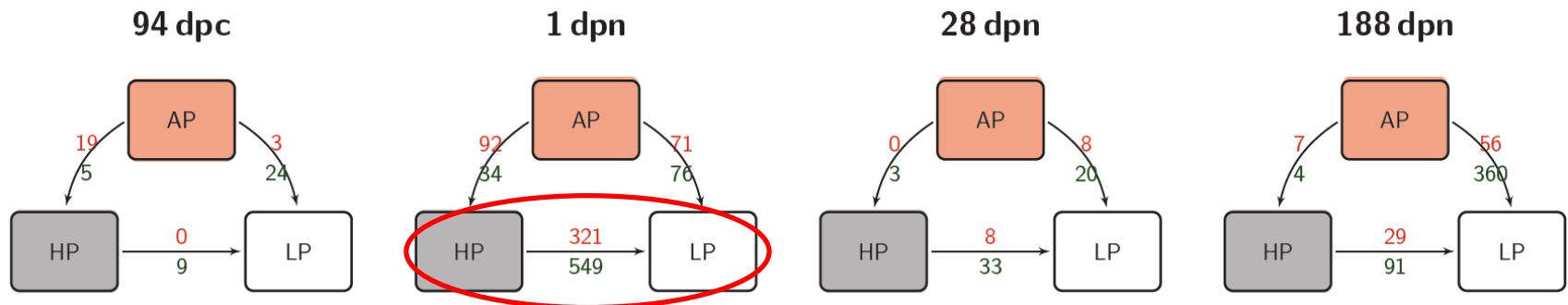
Item	AP	HP	LP	P^1			
				D ²	Sex	L ³	BW ⁴
No. of piglets (sample)	55	56	55				
Birth weight (g)	1368 \pm 40 ^a	1226 \pm 37 ^b	1235 \pm 37 ^b	0.02	0.14	0.02	–
Body weight day 1 (g)	1482 \pm 60 ^b	1327 \pm 51 ^{a,b}	1268 \pm 51 ^b	0.04	<0.01	<0.01	<0.0001
Hot carcass weight (g)	1050 \pm 43 ^a	943 \pm 37 ^{a,b}	898 \pm 36 ^b	0.05	<0.01	<0.01	<0.0001
Perirenal fat (g)	3.60 \pm 0.20 ^A	2.99 \pm 0.17 ^B	2.96 \pm 0.17 ^B	0.05	0.84	0.001	<0.0001
Omental fat (g)	6.89 \pm 0.40	5.99 \pm 0.34	5.95 \pm 0.34	0.18	0.04	0.53	<0.0001
Internal organs (g)	236.9 \pm 8.24 ^A	218.1 \pm 7.03 ^{A,B}	210.5 \pm 6.95 ^B	0.08	0.43	<0.01	<0.0001

Rehfeldt *et al.* 2012

Hierarchical order of tissue, stage, and diet



Tissue-specific effects dominate stage-specific effects which dominate diet-specific effects



Pathways in skeletal muscle tissue

Expression	Ontogenetic stage	Affected pathway	<i>P</i> -value	No. of genes involved	Genes involved in pathway
LP > HP	1 dpn	Mitochondria dysfunction	4.35E-09	14	COX6B2, COX7A2, COX8A, MAPK12, NDUFA7, NDUFB7, NDUFB11, NDUFS6, NDUFS7, NDUFS8, NDUFV2, PSENN, SURF1, UQCR11
LP < HP	1 dpn	Regulation of actin-based motility by Rho	2.39E-02	5	FNBP1, MYLK, PAK2, RHOU, WIPF1
LP < HP	1 dpn	Integrin signalling	7.64E-03	10	ACTN1, CAPN3, FNBP1, ITGA2, MYLK, PAK2, PARVA, RHOU, TTN, WIPF1
LP < HP	1 dpn	Protein kinase A signalling	2.59E-03	17	ADD3, AKAP8, AKAP9, AKAP12, AKAP2/PALM2-AKAP2, CDC27, GLI3, MTM1, MYH4, MYLK, PPP1CC, PRKAR1A, PRKAR2A, PTPLA, TCF4, TGFBR2, TTN
LP < HP	1 dpn	Mitotic roles of Polo-like kinases	2.30E-03	6	CDC27, HSP90B1, PLK2, PPP2R2A, PPP2R5E, SMC1A

Summary – Macronutrients in pigs

- High and low maternal protein:carbohydrate ratios showed lower birth weights of progeny
- Adaptive responses at mRNA level revealed:
 - Hierarchical order of tissues in response to dietary challenges
 - Liver tissue appeared more resilient to nutritional modulation than skeletal muscle



Transcriptional and epigenetic responses to maternal diets varying in methylation-related micronutrients

Species: Pig

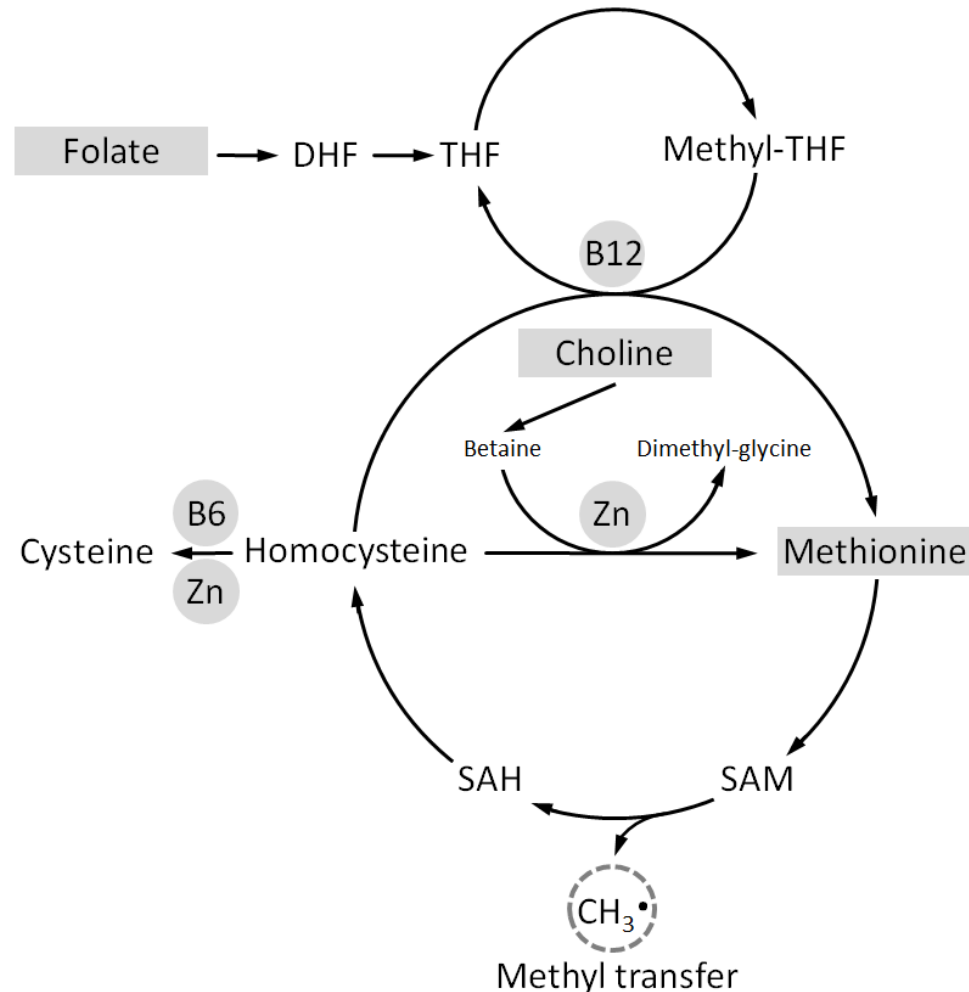
Environmental factor: Micronutrients

Hypothesis: Supplementing methylation-related micronutrients during pregnancy at higher-than-recommended doses may alter processes beyond preventing birth defects.



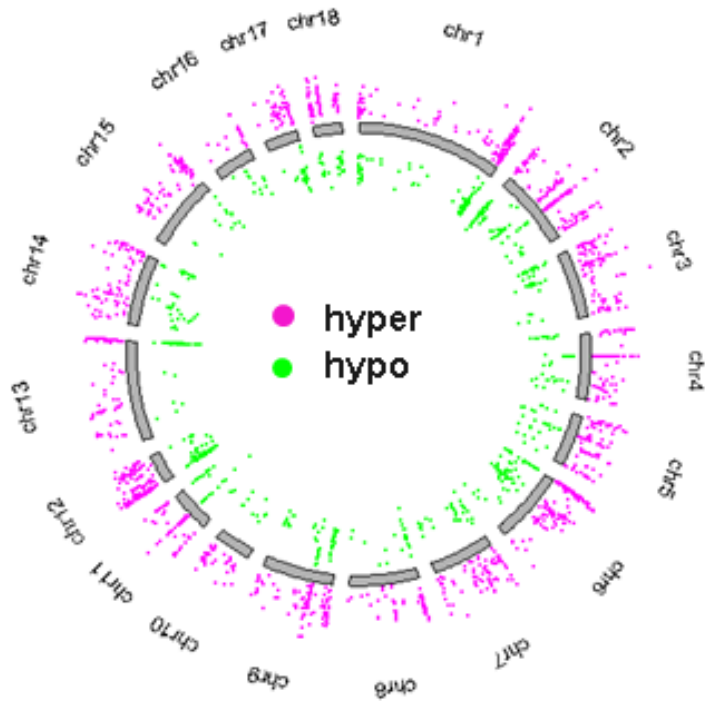
One-carbon cycle targeted by maternal diet

Micronutrient	CON	MET
Methionine, mg/kg	2050.0	4700.0
Choline, mg/kg	500.0	2230.0
Folic acid, mg/kg	3.0	92.2
Vitamin B6, mg/kg	3.0	1180.0
Vitamin B12, $\mu\text{g}/\text{kg}$	31.0	5930.0
Zinc, mg/kg	21.8	149.0



Candidate genes associated with MET diet

Genome-wide methylated CpGs



11,000 CpG with 10x coverage

Integration of differentially **methylated genes** and differentially **expressed genes (DEG)** pointed to altered **lipid metabolism**

Differentially methylated genes

IRX4, ZNF33B, CERS1, DTD1, FKBP14, IMPAD1, KCNG1, BAHCC1, KANK4, SYPL2, TMCC3, TMEM165

DEG	Function	logFC	logCPM
<i>Cyp4F2</i>	synthesis of cholesterol, steroids and other lipids	-1.86	6.89
<i>LDHB</i>	fatty acid metabolism	2.18	7.73
<i>PON3</i>	associates with high-density lipoprotein (HDL)	-3.68	5.56

$p < 0.01$; FDR < 0.10

Summary – Micronutrients in pigs

- Supplementation of methylation-related micronutrients showed effects at the phenotypic & transcriptional & epigenetic level
- Phenotypes mediated by methylation-related micronutrient intake during pregnancy should be further discussed with regards to acute (foetal) and persistent (postnatal) aspects of health and growth

Transcriptional responses in muscle tissue following variable incubation temperature

Species: Chicken

Environmental factor: Egg incubation temperature

Hypothesis: miRNAs regulate dynamic transcriptional changes of broiler embryos in response to thermal interventions during incubation.



Experimental design



1. Early Treatment



2. Late Treatment

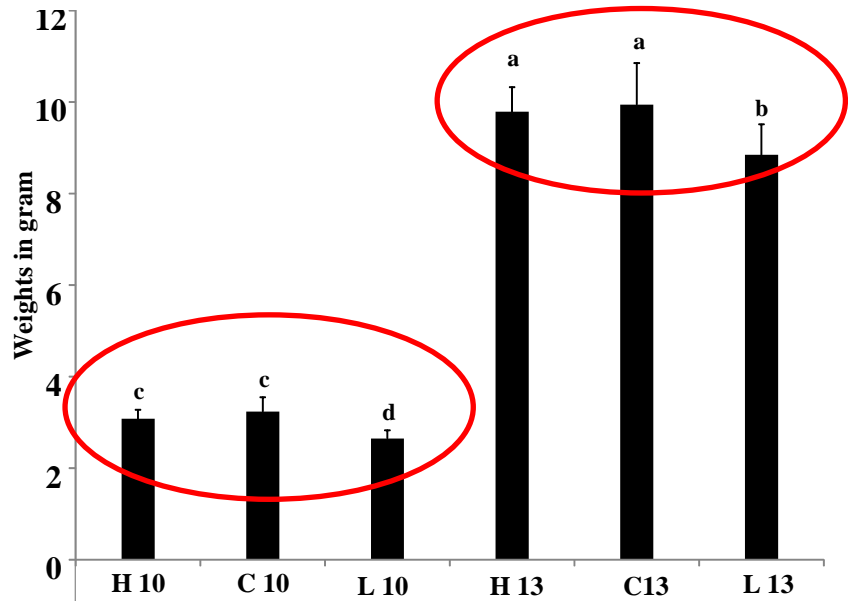


Embryo Samples n = 48

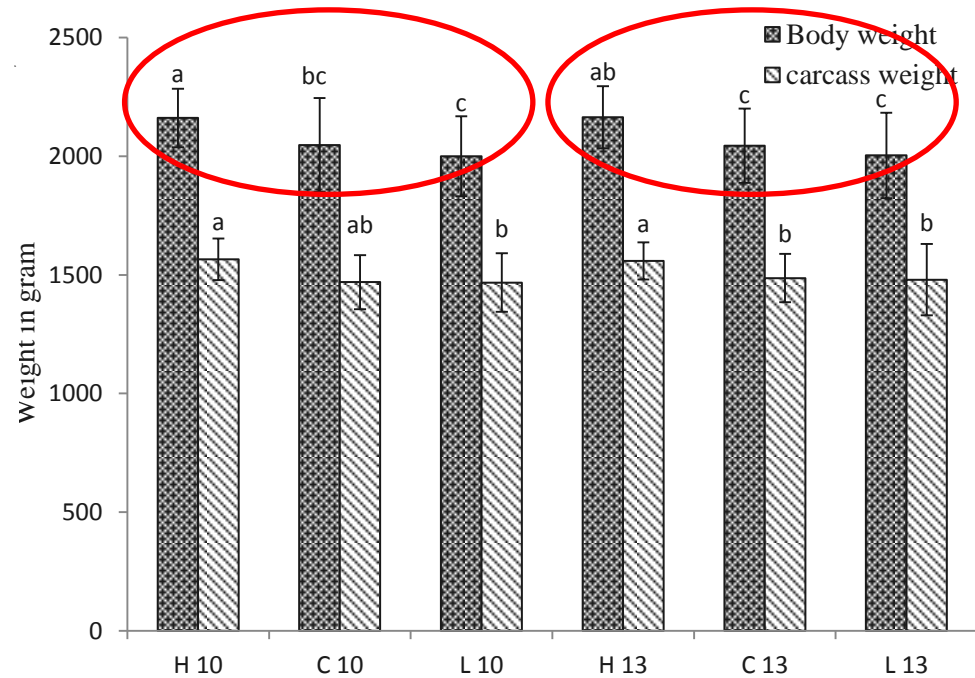
Day35 Samples n = 40



Embryonic & adult weights



- Body weight at embryonic days 10 & 13



- Body & carcass weights at adult day 35

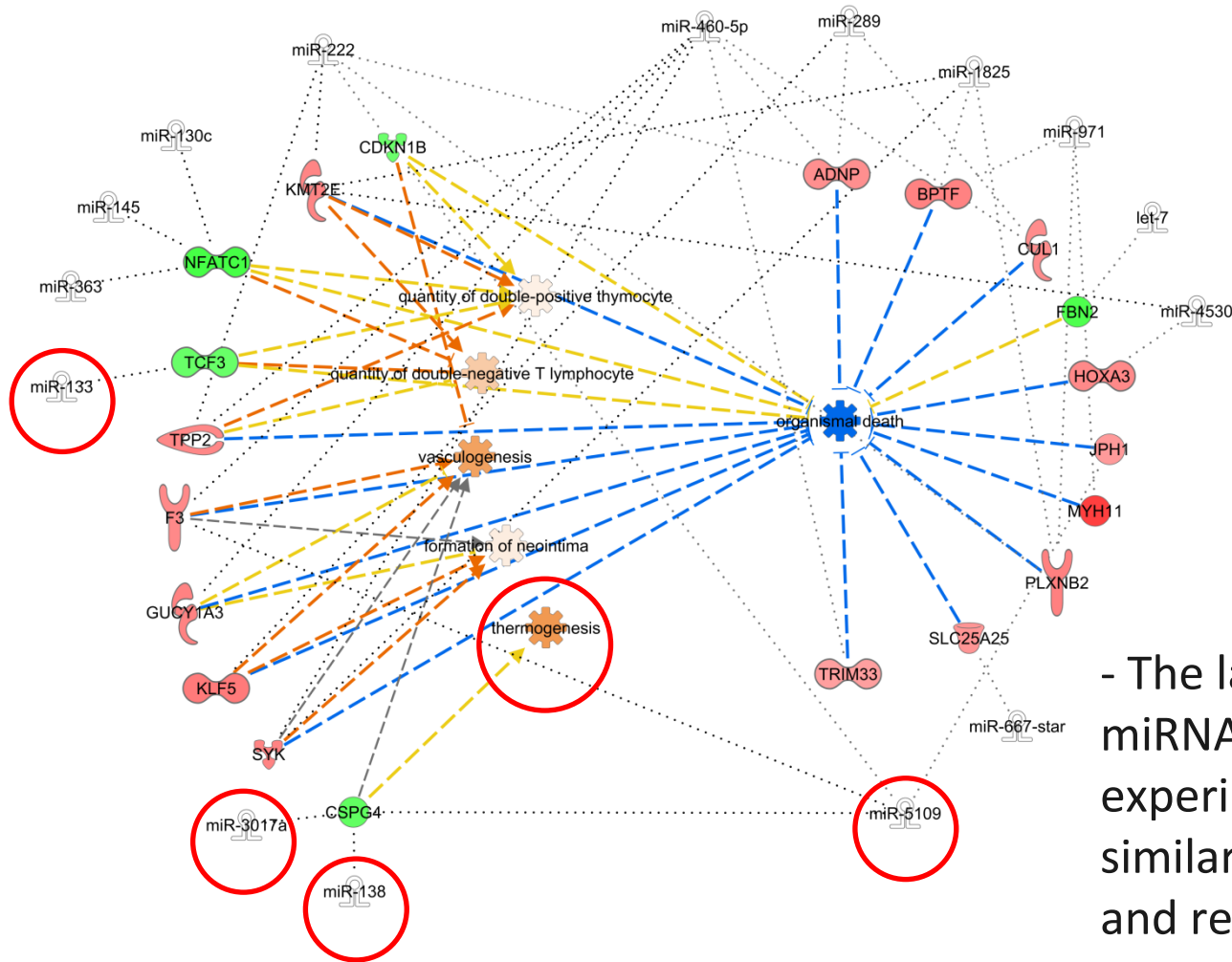
Differentially expressed miRNAs and mRNAs

Functional miRNAs and potential target genes for selected treatment conditions

Stage	Treatment	Tissue	miRNA-mRNA pair	Unique Target	Unique miRNA
Embryo	H10-C10	Hind	444	200	38
		Breast	941	421	40
	L13-C13	Hind	104	49	25
		Breast	394	168	50
D35	L10-C10	Hind	10	8	7
		Breast	19	10	1
	L13-C13	Hind	2	1	2
		Breast	7	6	5

→ Results indicate a major regulatory role of miRNAs to acute responses under modified environmental conditions.

miRNA related network in breast muscle



- The large repertoires of shifted miRNA–mRNA pairs in various experimental groups fine-tune similar biofunctions, biodiversity and resilience.

Summary – Egg incubation temperature

- Phenotypic effects
 - Lower incubation temperature decreased embryonic weight
 - Higher incubation temperature increased adult body weight
- miRNA pattern
 - Major regulatory role of miRNAs in acute responses
 - Long-term miRNA responses are minor



Overall summary

Environmental factors showed effects at the phenotypic & transcriptional & epigenetic level

→ High potential for adaptation and resilience in mammals and birds

Differentially methylated CpG, differentially expressed mRNAs & miRNA–mRNA pairs showed treatment-condition-specificity in terms of timing, environmental factor, tissue, breed & developmental stage

→ No common molecular path or gatekeeper mediating transcriptional responses and epigenetic processes





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Protein : carbohydrate - ratio

HP	AP	LP
1:1.3	1:5.0	1:10.4