The Role of Epigenetics and Nutrition in Development (and the joy in discovery)

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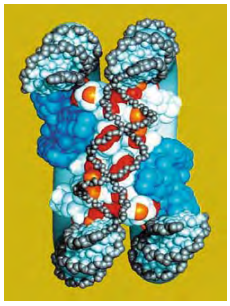
BCM Baylor College of Medicine

Genetics & Heritability *c.* 1987: What regulates gene expression?

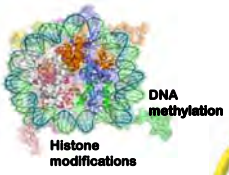


While my companion contemplated with a serious and satisfied spirit the magnificent appearances of things, I delighted in investigating their causes.

Curiosity, earnest research to learn the hidden laws of nature, gladness akin to rapture, as they unfolded to me, are among the earliest sensations I can remember.

Mary Wollstonecroft Shelley (1817)
Frankenstein, or the Modern Prometheus

Aagaard Lab: Discovering Mechanisms for Enhancing the Diversity of Our Genomic Inheritance



Epigenome

"second genome" (microbiome)

mtDNA

Science progresses best when observations force us to alter our preconceptions.

Vera Rubin

Theories crumble, but good observations never fade.

Harlow Shapley

As a consequence, geneticists described evolution simply as a change in gene frequencies in populations, totally ignoring the fact that evolution consists of the two simultaneous but quite separate phenomena of adaptation and diversification.

Ernst Mayr

Developmental Origins of Disease (The Observation)

Diverse Birth Phenotype, Common Later in Life Risks

Large for Gestational Age
(maternal obesity)

- Birth injury
- Risk of childhood obesity
- Among diabetic mothers:
  - Neonatal hypoglycemia
  - Risk of juvenile diabetes
- Maternal BMI >30 is an independent predictor of LGA

Small for Gestational Age
(maternal smoking)

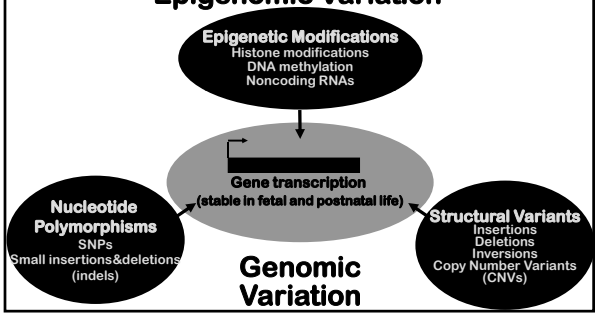
- Birth asphyxia
- Neonatal morbidity and mortality
- Lifelong risk of adult metabolic disease:
  - Neonatal hypoglycemia
  - Juvenile diabetes and obesity
  - Adult diabetes and obesity
  - Adult hypertension and cardiovascular disease

HAPO Study Cooperative Research Group: *NEJM*, 2008; *Diabetes*, 2009; *BJOG*, 2010
NICHD BPMU & Mild Gestational Diabetes *NEJM*, 2009; *Obstet Gynecol*, 2010; *J Met Fet*, 2010
Singh, *Obstet Gynecol*, 2010
Catalano, *Am J Clin Nutr*, 2009
Catalano, *Diabetes*, 2009
Catalano, *BJOG*, 2007

Southampton Women's Survey (Barker et al): *Lancet*, 1986; *Lancet*, 1989; *Nature 2004*
Southampton Second Generation (Hanson et al): *Science*, 2004; *NEJM*, 2008
Liggins Institute (Gluckman et al) *PNAS*, 2007
Nurses Health Study (Rich-Edwards et al) *JAMA*, 2008

Are their shared molecular mechanisms in fetal and early life?

Epigenomic Variation



Genomic Variation

Relevant Arenas for Studying Epigenomic Variation and Regulation of Fetal Growth

Our Currently Studied Models on the Developmental Origins of Adult Disease

Diverse interventions during development influence risk of adult metabolic disease:

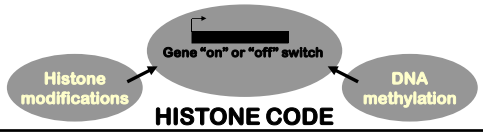
- Uteroplacental insufficiency
- Essential nutrient supplementation
- *In utero* tobacco exposure
- Maternal high fat diet exposure and obesity
- Comparative primate genomics

Primate Evolution

- Dramatic phenotypic variance over 30 million years
- Evolution occurs one generation at a time

What are 'epigenomic variations'? (don't let the science scare you)

- Chromatin can be semi-permanently modified
- Allows for changes in the gene switch without altering the DNA code
  - "Histone Code"
- Epigenetic modifications such as histone acetylation and methylation and DNA methylation are key components of the histone code:

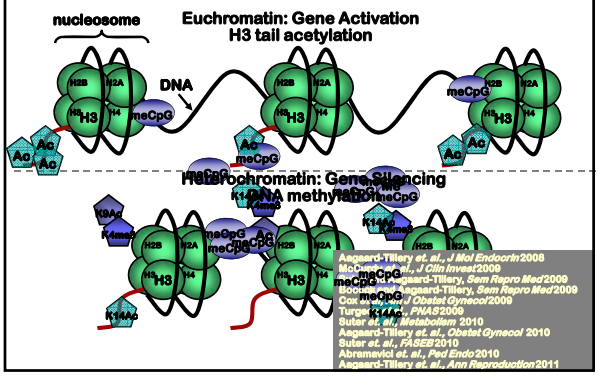


Genomic Variation is Encoded by DNA is Embedded in Chromatin altered *in utero* environment?

Nature uses only the longest threads to weave her patterns, so that each small piece of her fabric reveals the organization of the entire tapestry.

Richard P. Feynman

Nature's Tapestry: Genomic and Epigenomic Variations Alter Accessibility to DNA



Primate Model of Maternal Obesity

Macaque Consortium (ONPRC)

Maternal Obesity in the Macaque

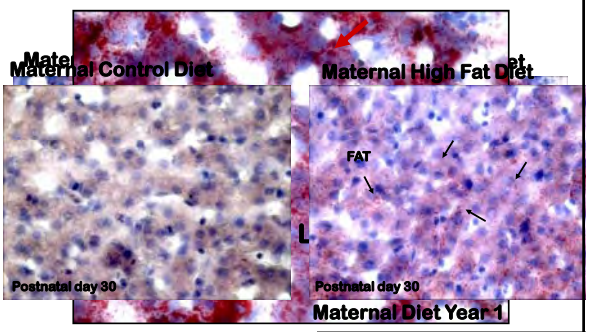
- ✓Hyperinsulinemic, hyperglycerolemic, and euglycemic
- ✓Increased leptin/body weight ratio
- ✓Normal serum lipid and triglyceride levels
- ✓Undergo cesarean at day 130 (term 167 day), with fetal necropsy and harvest of serum and tissue
- ✓20% of dams do not become obese despite up to 4 years on the high fat diet ("resistant" phenotype)

Aagaard-Tillery et. al., J Mol Endocrin 2008
McCurdy et. al., J Clin Invest 2009
Cox et. al., Am J Obstet Gynecol 2009
Suter et al., FASEB 2011

Any man who can drive safely while kissing a pretty girl is simply not giving the kiss the attention it deserves.

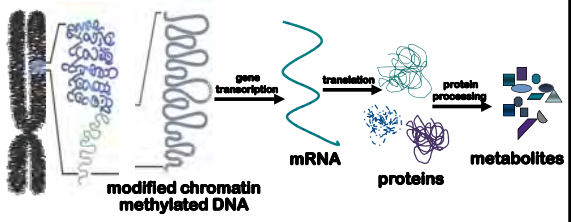
Albert Einstein

Fetal Liver: Non-alcohoholic Fatty Liver Persistent Postnatal NAFLD Disease



Aagaard-Tillery *et. al., J Mol Endocrin*, 2008
McCurdy *et. al., J Clin Invest*, 2009

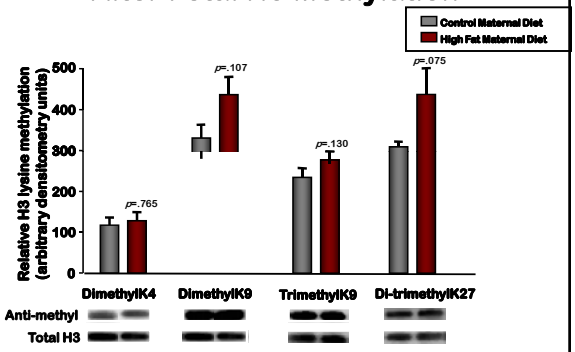
Molecular Pathogenesis: 'Omics' Science



Genome Epigenome Transcriptome Proteome Metabolome

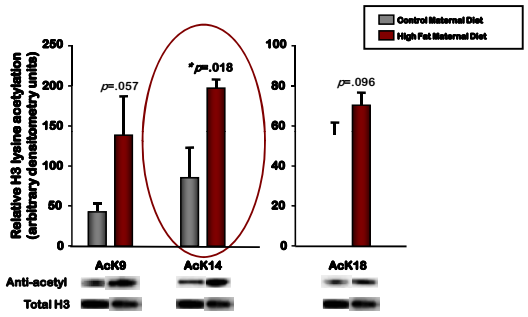
Genomics Epigenomics Transcriptomics Proteomics Metabolomics

Maternal Diet Does Not Significantly Alter Fetal H3 Methylation



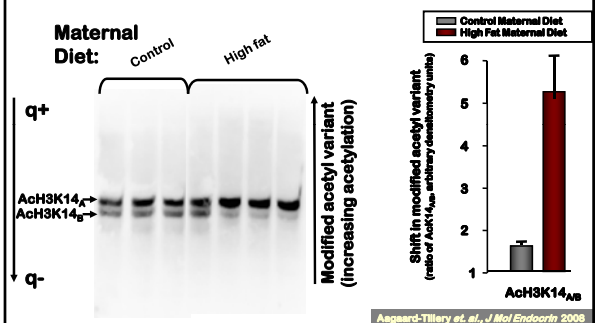
Aagaard-Tillery *et. al., J Mol Endocrin* 41: 91-102. 2008.

Maternal Diet Alters Modification-Specific Fetal H3 Acetylation



Aagaard-Tillery *et. al., J Mol Endocrin* 41: 91-102. 2008.

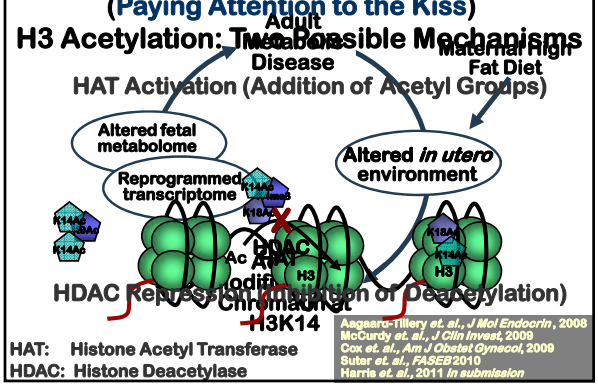
Maternal Diet Significantly and Specifically Modifies Fetal Hepatic H3 Acetylation at Lysine 14 (H3K14)



Aagaard-Tillery *et. al., J Mol Endocrin* 2008
Cox *et. al., Am J Obstet Gynecol*, 2009
Suter *et al., FASEB J*, 2011

How does the maternal diet modify fetal hepatic H3K14? (Paying Attention to the Kiss)

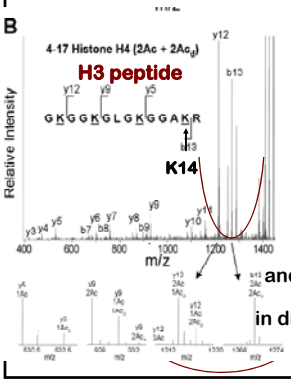
H3 Acetylation: Two Possible Mechanisms



HAT: Histone Acetyl Transferase
HDAC: Histone Deacetylase

Aagaard-Tillery *et. al., J Mol Endocrin*, 2008
McCurdy *et. al., J Clin Invest*, 2009
Cox *et. al., Am J Obstet Gynecol*, 2009
Suter *et. al., FASEB* 2010
Harris *et. al.*, 2011 *in submission*

Complementary Methods Characterizing Fetal Histone Modifications



Method 2: MOLDI-QqTOF Mass Spectrometry

✓ Well-developed proteomics methodology

✓ Qualitates and quantitates *in vitro* activity against an H3 peptide:

- m/z (x-axis) identifies the site of modification
- relative peak intensity (y-axis) quantifies the degree of modification (number of acetyl groups on a given Lysine[K])
- MOLDI-QqTOF confirmed our prior Western blot analysis

✓ Preferential fetal hepatic hyperacetylation on Lys 14 (H3K14ac2K14ac3, p<0.01)

traction & Western blot

and accepted approach

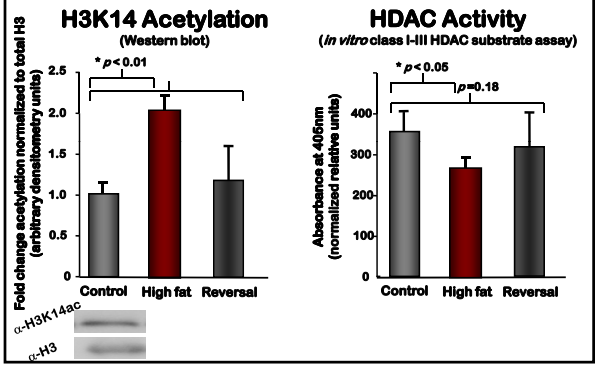
on-specific Ha antibodies

in discerning histone

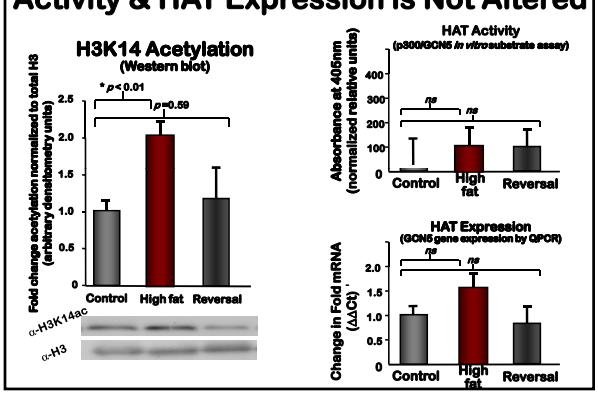
HAT/HDAC

Aagaard-Tillery *et. al., J Mol Endocrin* 2008

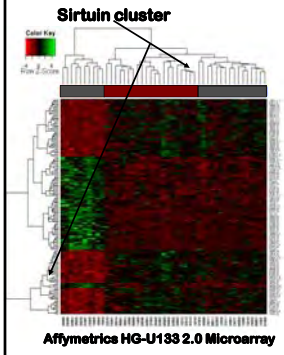
Histone Deacetylase (HDAC) Activity *in vitro* Mirrors Fetal H3K14 Acetylation



Fetal Histone Acetyltransferase (HAT) Activity & HAT Expression is Not Altered



Which fetal HDAC is altered by a maternal high fat diet?

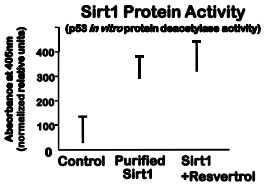


Sirtuins: Highly Conserved Protein Deacetylases

- ✓ Ubiquitous NAD+-dependent enzymes that deacetylate lysine residues.
- ✓ *C. elegans* : increased *Sir2* promotes lifespan longevity in response to caloric restriction.
- ✓ Mammalian systems:
  - Sirt1-7 protein deacetylase activity regulates mitochondrial function and energy homeostasis.
  - *Sirt1* overexpression protects against high fat diet-induced NAFLD in adult transgenic mice.
  - Pharmacologic SIRT1 activators (resveratrol) are in clinical trial for the treatment of metabolic syndrome.
- ✓ Sirt1 retains histone deacetylase activity, although not well described.

McCurdy *et. al., J Clin Invest*, 2009

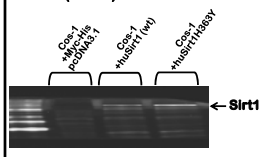
Is SIRT1 both a protein *and* a histone deacetylase?



SIRT1 is a K14 Histone Deacetylase

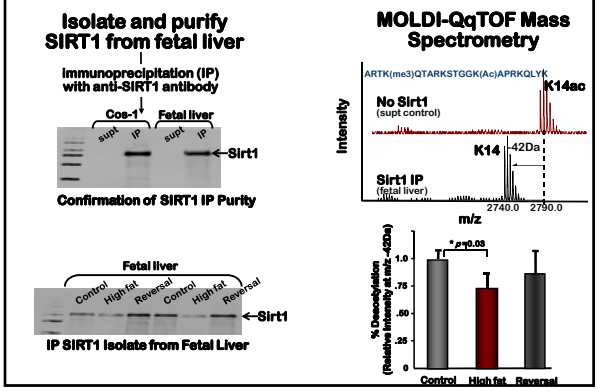
Pharmacologically Activated (resveratrol) or Genetically Deactivated (H363Y) Sirt1

Cos-1 (kidney epithelial) transfected with human Sirt1 control (wt) or mutated (H363Y) cDNA

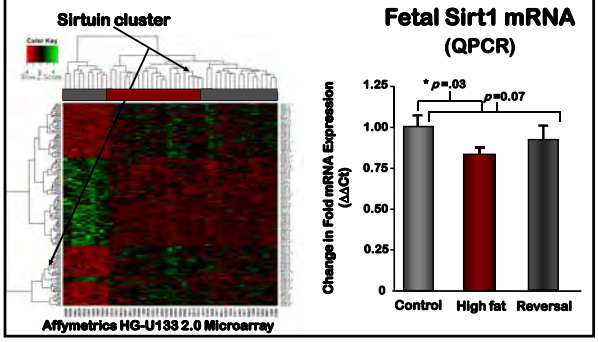


Confirmation of SIRT1 transfection (myc-his tag)

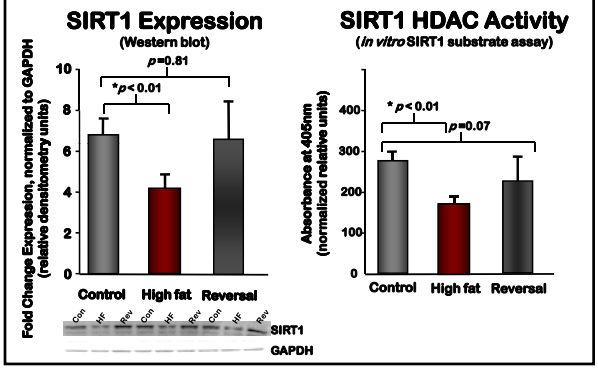
Is *fetal* SIRT1 K14 histone deacetylase activity altered by the maternal diet?



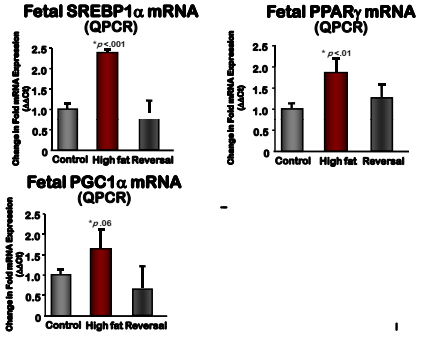
Fetal Hepatic *Sirt1* Gene Expression is Altered by the Maternal Diet



Maternal Diet Alters SIRT1 Protein Levels and *in vitro* HDAC Activity



Transcription Factors Dysregulated in NAFLD and (protein)deacetylated by SIRT1 are Altered by Maternal Diet



Implications

SIRT1

Given significant literature establishing Sirtuins as critical mediators of lifespan longevity with caloric-restriction from *C. elegans* to mammals, our data on fetal SIRT1 in primates suggests that the Sirtuins are evolutionary conserved means of regulating and reprogramming fetal metabolism.

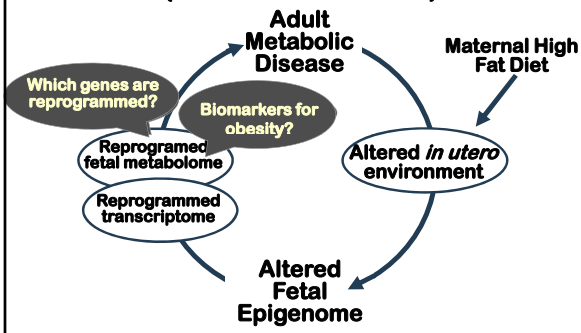
Conclusions

- A high fat maternal diet rewrites the fetal histone code (epigenome) on the H3 tail at lysine 14 (H3K14) via altered histone deacetylation.
- Employment of biochemical methods and MOLDI-QqTOF mass spectrometry enabled our novel characterization of SIRT1 as a fetal histone lysine deacetylase which specifically and preferentially modifies H3K14.

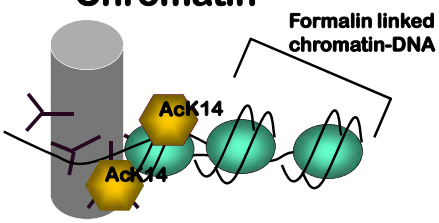
A kiss is a lovely trick designed by nature to stop speech when words become superfluous.

Ingrid Bergman

Epigenomics Studies ("nature's kiss")



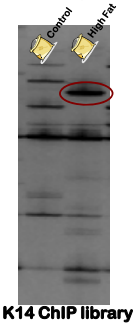
Isolation of Fetal DNA Associated with Modified Chromatin



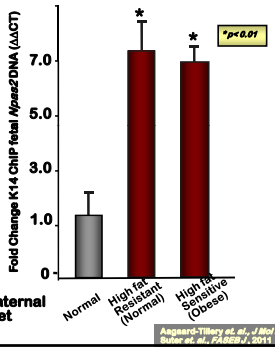
Anti-acetyl H3 K14 ChIP antibody

Aagaard-Tillery *et. al., J Mol Endocrin*, 2008
Suter *et al., Metabolism*, 2010
Suter *et. al., FASEB*, 2011

Maternal Diet, Not Obesity, Alters Fetal *Npas2*-H3K14 Association (Acetyl K14 ChIP Library)



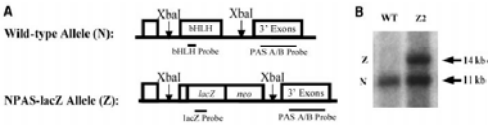
K14 ChIP library (ChIP Seq)



Aagaard-Tillery *et. al., J Mol Endocrin*, 2008
Suter *et. al., FASEB J*, 2011

Why is epigenetically reprogrammed *Npas2* of potential interest?

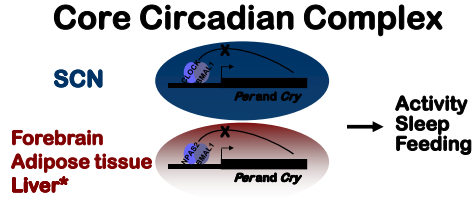
Npas2-l- **Murine Transgenic**
Steven McKnight, UT Southwestern



- Distinct phenotype
  - *Ad lib* feeding: trend towards obesity
  - Restricted feeding: mortal misadaptation
  - Our primate model: fetal *Npas2* is increased under maternal high fat diet

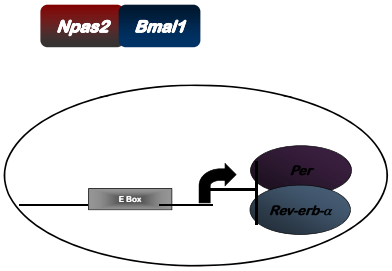
Aagaard-Tillery *et. al., J Mol Endocrin*, 2008
Aagaard-Tillery *et. al., SGI*, 2010
Mendez-Figueroa *et al.*, 2011 *in submission*

Clock Family Genes Comprise the Core Circadian Complex



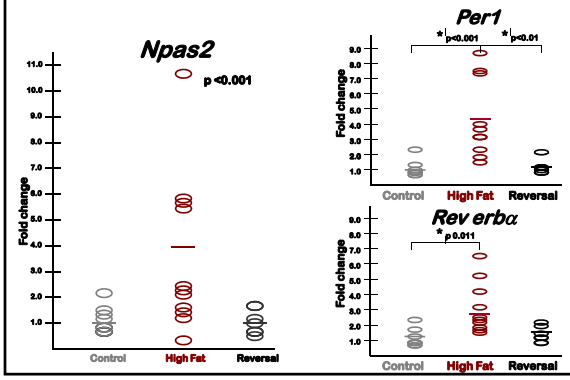
- **Central Clocks**: Temporal coordination of metabolism
  - Coupled to the light cycle (SCN)
  - Responds to stimuli and feeding (forebrain)
  - Entrains peripheral oscillators
- **Peripheral Clocks**: Coordinate behavior and peripheral metabolism
  - Amplify or dampen central signals
  - Facilitates local adaptive responses
  - Integral role in feeding behavior
- Transcriptional oscillation enables canonical regulation

Transcriptional Oscillation Allows for Phase-Antiphase Integrity of *Npas2/Clock* Circadian Complex

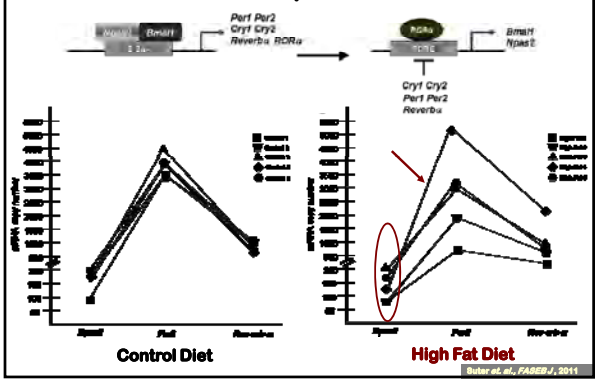


Hepatocyte

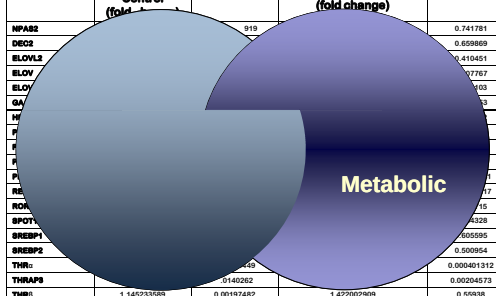
Maternal Diet Regulates Fetal *Npas2*, *Per1*, and *Rev erb* α Expression



High Fat Diet Exposure Disrupts Circadian Gene Amplitude In Fetal Liver



Expression Profiling of Circadian Regulators of Metabolism

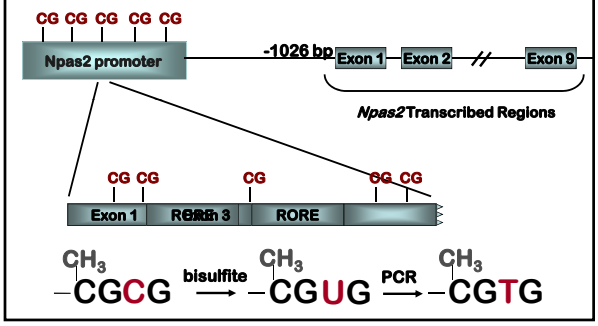


Gene	"Diet Effect" High Fat vs Control (fold change)	*p*-value	"Obesity Effect" Reversal vs Control (fold change)	*p*-value
NPAS2	[illegible]	[illegible]919	[illegible]	0.741781
DEC2	[illegible]	[illegible]	[illegible]	0.659869
ELOVL2	[illegible]	[illegible]	[illegible]	[illegible].410451
ELOV[illegible]	[illegible]	[illegible]	[illegible]	[illegible]77767
ELO[illegible]	[illegible]	[illegible]	[illegible]	[illegible]03
GA[illegible]	[illegible]	[illegible]	[illegible]	[illegible]3
H[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
P[illegible]	[illegible]	[illegible]	[illegible]	[illegible]1
RE[illegible]	[illegible]	[illegible]	[illegible]	[illegible]17
ROR[illegible]	[illegible]	[illegible]	[illegible]	[illegible]15
SPOT[illegible]	[illegible]	[illegible]	[illegible]	[illegible]328
SREBP1	[illegible]	[illegible]	[illegible]	[illegible]605595
SREBP2	[illegible]	[illegible]	[illegible]	0.500954
THRα	[illegible]	[illegible]49	[illegible]	0.000401312
THRAP3	[illegible]	[illegible]0140262	[illegible]	0.00204573
THRβ	1.145233589	0.00197482	1.422002909	0.55938
TIM2	0.741951309	0.0149646	1.451373248	0.0411848
TRAP220	0.71731947	0.00833976	0.97296018	0.539078

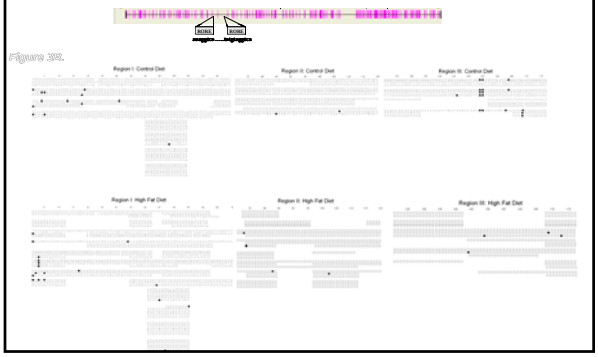
Epigenetic Regulation of Gene Expression in a Canonical Pathway

- Predicted role for altered DNA methylation in critical promoter regions.
- Potential for differential occupancy of transcription factors in the promoter region because of modified histones.

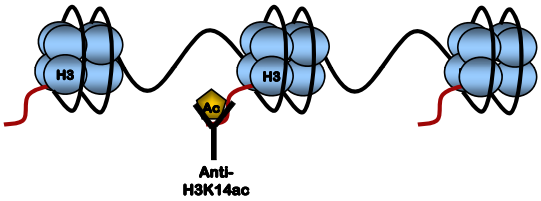
Primate *Npas2* Promoter: CpG Sites Surround Conserved RORE Transcription Element



Npas2 Promoter Methylation Does Not Vary with Maternal Diet Exposure

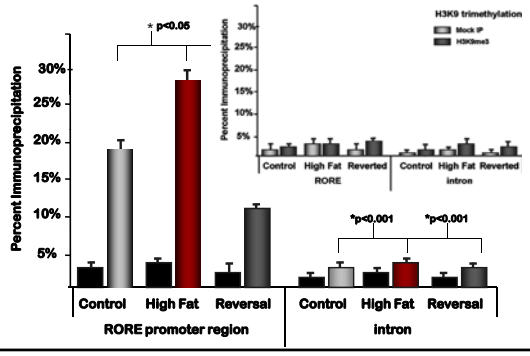


ChIP for Determining Gene Specific Promoter Occupancy (Deciphering the "Histone Code")

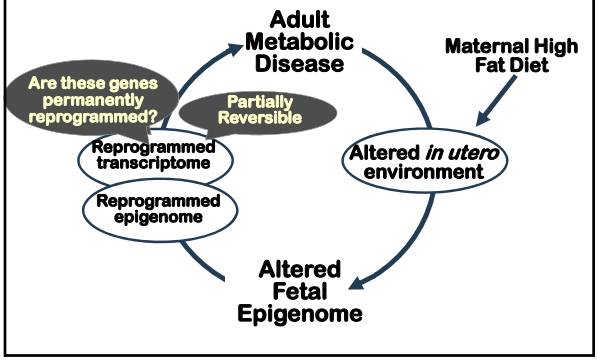


Use promoter region-specific ChIP-PCR to determine if maternal diet alters the fetal *Npas2*-specific histone code (*i.e.,* differences in promoter occupancy by H3K14ac)

Maternal Diet Alters *Npas2* Promoter Occupancy via H3K14 Acetylation

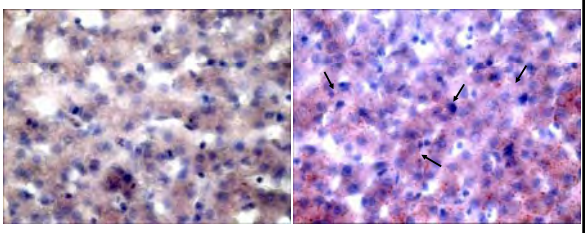


Epigenomics and Reprogramming in Fetal Life Studies
Next Questions



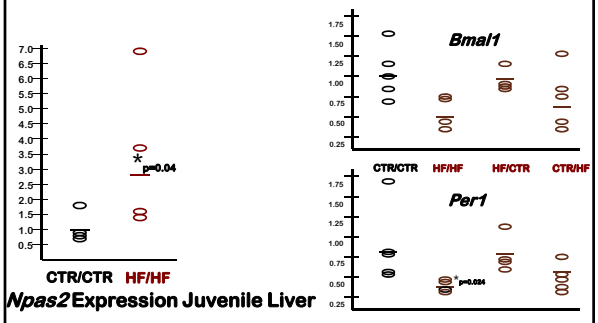
Persistent Non-alcohoholic Fatty Liver Disease

Maternal Control Diet | Maternal High-Fat Diet



Aagaard-Tillery et. al., J Mol Endocrin 2008
McCurdy et. al., J Clin Invest 2009
Cox et. al., Am J Obstet Gynecol 2009

Modifiable Prenatal Changes in *Npas2* Expression with Postnatal Diet Modification



Suter et al., FASEB J 2011

Conclusions

- A high fat maternal diet rewrites the fetal histone code (epigenome) on the H3 tail at lysine 14 (H3K14) via altered histone deacetylation.
- Exposure to a maternal high fat diet *in utero* per se significantly reprograms the expression of fetal hepatic *Npas2.*
- Although the *Npas2* promoter remains largely unmethylated, differential *Npas2* promoter occupancy of acetylation of fetal histone H3 at lysine 14 (H3K14ac) occurs in response to maternal high fat diet exposure.

Summary

Our primate studies suggest that the fetal histone code is functionally altered following *in utero* exposure to a maternal high fat diet, and (modifiably) reprograms the developmental:

- ✓ Epigenome. Modified chromatin at H3K14
- ✓ Transcriptome. Role in genome-wide transcriptional regulation
- ✓ Metabolome. Free fatty acid and lipid metabolites

Summary

Taken together, our emerging data suggests that fetal epigenetic signatures are altered by *in utero* exposures.

Full interrogation of these observations will be instrumental in efforts aimed at deciphering the effect of the gestational milieu on the fetal epigenome, transcriptome and metabolome in relation to metabolic disorders in later life.

Any intelligent fool can make things bigger and more complex... It takes a touch of genius - and a lot of courage to move in the opposite direction.

Albert Einstein

Be obscure clearly.

E. B. White

Opportunities for Advanced Understandings of Gene Regulation

Developmental Origins: 1000 Days Movement and the Common Good on a Global Scale

http://www.thousanddays.org/

THE HUNGER PROJECT

With early foundations already laid at least 25 years ago, the paradigm of developmental origins of human disease and organ dysfunction has been slow to develop. The delayed recognition undoubtedly resulted in major costs to society due to the lack of intervention that could have been reasonably justified long ago. Now that this paradigm is gaining ground, its full prevention implications must be recognised. In addition, this experience suggests that undue scepticism and demands for endless replication should be countered by prudent and responsible inference that paves the way for more rapid recognition of early warning signs when human health and the environment are at risk.

Phillipe Grandjean Late Insights into Early Origins of Disease, *Basic Clin Pharmacol Toxicol* 2008; 102: 94-99

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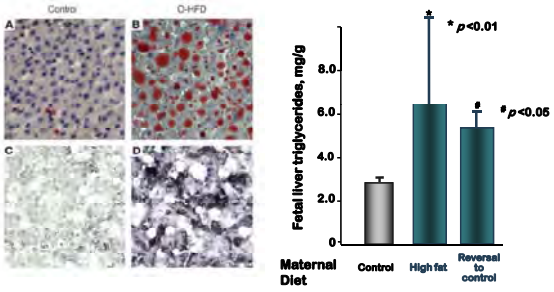
Fetal Hepatic Pathology Under Conditions of Maternal Obesity

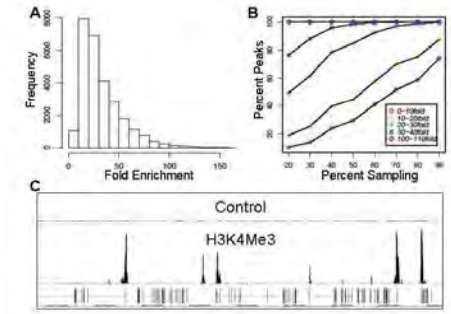


McCurdy et al. J Clin Invest doi10.1172. 2009.

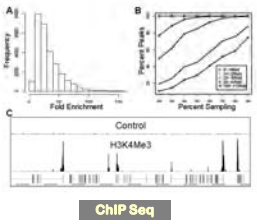
Fetal Epigenomic Profiling…Works in Progress

Antibody	Biological Role	Supplier
H3K4-trimethyl	**Gene activation**	**Abcam**
H3K9-trimethyl	Gene repression	Abcam
H3K27-trimethyl	Gene repression	Upstate
H3K9,14-acetyl	Gene activation	Upstate
H4Ac(K5,8,12,16)	Gene activation	Upstate
H2A.Z	Promoters	Abcam
H3.3	Gene activation	Abcam

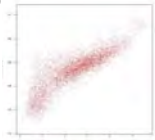
Fetal Epigenomic Profiling…Progress



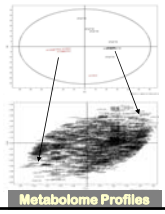
Fetal Epigenomic Profiling…Next Step



ChIP Seq



RNA Seq



Metabolome Profiles