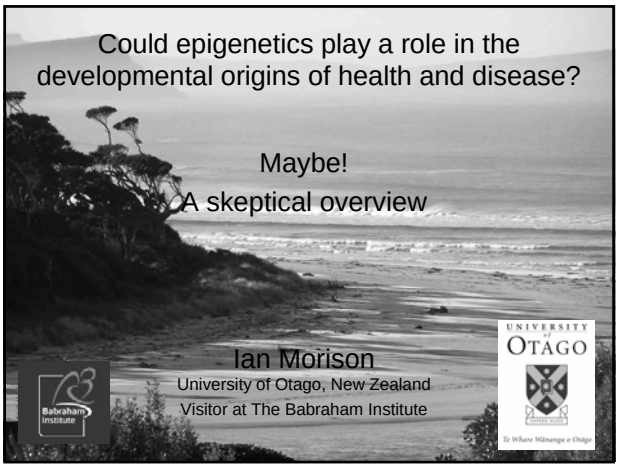
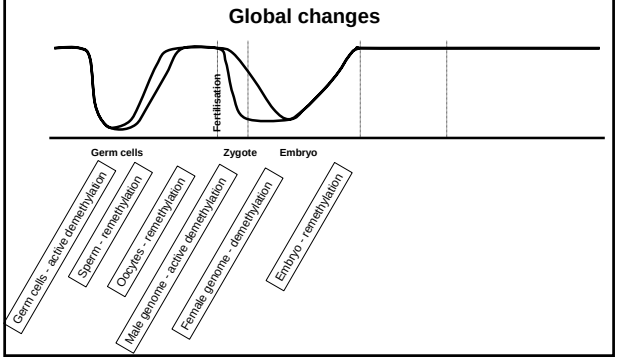
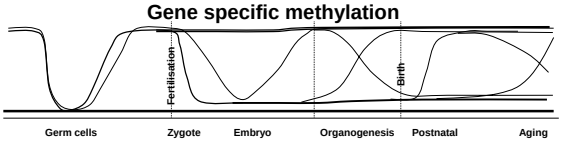


Dynamic methylation in development



Dynamic methylation in development



Normal changes

Germ cell development and early embryonic development

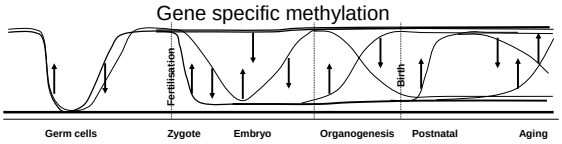
totipotency; pluripotency

Cell differentiation - gain and loss of methylation

Postnatal effects on specific genes

Aging may be associated with gain and loss of methylation?

Could epigenetic (methylation) plasticity contribute to variation?



Opportunities for modulation of methylation (normal vs. pathological)

Germ line (more or less)?

Less erasure in zygote / over-erasure in zygote?

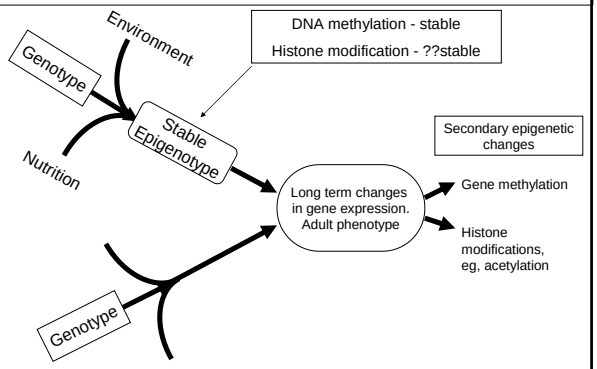
Increased methylation in preimplantation embryo?

Loss of imprinting?

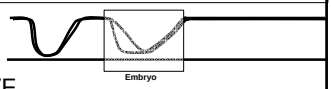
Altered methylation during organogenesis?

Altered age related methylation?

Goal - to identify stable epigenetic modifications



Post-fertilisation - pre-differentiation
ART

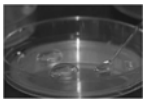


- Embryo culture and IVF
- Increase risk of Beckwith Wiedemann syndrome and Angelman syndrome
- Altered culture media (mice)
- Manipulation itself alters imprinting
- Large offspring syndrome (cows/sheep)

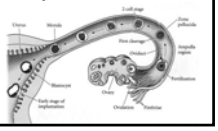
Very important issues for human and animal ART

Post-fertilisation - pre-differentiation
ART

- BUT
  - Can we generalise?
  - Rate of abnormalities in mice ~ 700 fold that of human
  - Large offspring syndrome associated with use of serum
  - Human abnormalities may be related to infertility
    - E.g., Doornbos ME et al Hum Reprod 2007 Rate of BWS/AS same in IVF as in infertile controls.
- AND
  - Even if human IVF does cause methylation abnormalities, **does this imply plasticity in natural conceptions**?



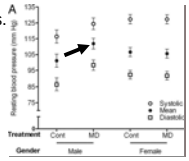
?



Post-fertilisation - pre-differentiation
In vivo manipulation - sheep (1)

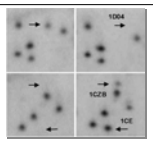
- "DNA methylation…. determined by maternal periconceptional B vitamin and methionine status" Sinclair KD et al PNAS 2007;104:19351
  - 'Methyl deficiency' - dietary deficiency of cobalt and sulphur levels, ⇓ capacity of the rumen to synthesize methionine and vitamin B12.
  - 'Deficient' and control day-6 blastocysts were then transferred to normally fed surrogate ewes.

Males only



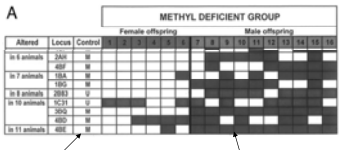
- Offspring heavier, fatter, insulin resistant, higher blood pressure.
- Changes in DNA methylation in 4% of genes.

Post-fertilisation - pre-differentiation
In vivo manipulation - sheep (2)



RLGS showed loss (and gain) of methylation in 4% of genes

Binary change ON/OFF!!



For some genes the changes were very consistent - in males

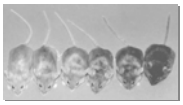
(best examples shown)

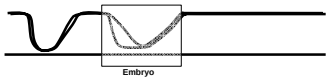
Methylated in controls, unmeth in deficient animals

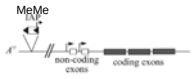
Sinclair KD et al PNAS 2007;104:19351

Post-fertilisation - pre-differentiation
Agouti^{vy} mice *in vivo*

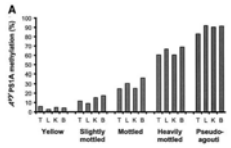
Abnormal Normal







Increasing methylation of IAP



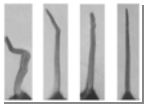
Variable phenotype, but all tissues show similar methylation.

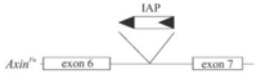
Therefore, level of methylation is set before differentiation into different lineages.

Rakyan V et al, Biochem J 2001; Waterland R et al, MCB 2003.

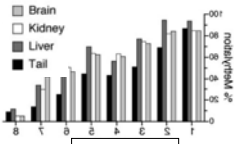
Post-fertilisation - pre-differentiation
Axin1^{Fused} mice *in vivo*

Abnormal Normal





Increasing methylation



Tail phenotype

Waterland R et al, Genesis 2006; Waterland R et al, MCB 2003.

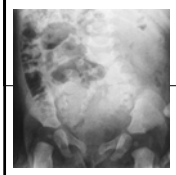
Agouti^{vy} and *Axin1*^{Fu} - manipulation by diet

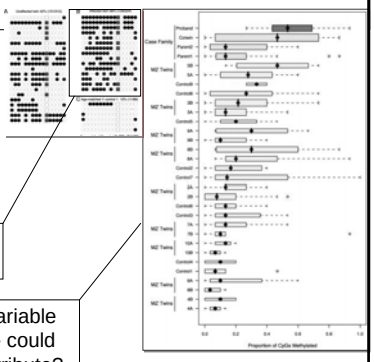
- High doses of folate, B12, betaine and choline (methyl donors) to pregnant mothers
  - normalises coat colour; normalises kinky tail
  - Increases methylation at IAP

Manipulation of an early embryo's "diet" changes the epigenotype

Wolff GL et al, 1998 Faseb J; Waterland R, MCB 2003

Human *AXIN1 - caudal duplication anomaly*





Twins discordant for caudal duplication anomaly. Oates NA 2006

Affected twin - high methylation of *AXIN1*

AXIN1 methylation variable between individuals - could the environment contribute?

AXIN1 methylation (blood)

In vivo manipulation of diet - BUTS

Sheep model is of dietary deficiency of cobalt and sulphur

Are methylation changes the cause or consequence of physiological changes?

Does the altered DNA methylation result from availability of methyl donors?

Mouse model uses "supraphysiologic methyl group supply" (Sinclair 2007)

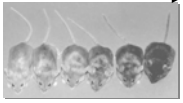
Genistein, soy-derived phyto-estrogen, has same effect as B12, folate on coat colour.

Bisphenol A (estrogenic compound) has the opposite effect.

- reduces methylation of the A^{vy} IAP element.

In vivo manipulation of Agouti^{vy} diet - BUTS

Increasing methylation of IAP



Yellow Obese

Brown Leaner

Prediction: Folate/B12 → leaner animals

BUT: supplementation worsened obesity - opposite

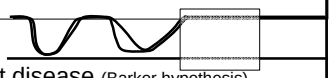
i.e. we don't understand the model - care is needed.

http://hgm2006.hugo-international.org/Presentations/S2/HGM2006_S2_04_Waterland.ppt

Early embryo - summary

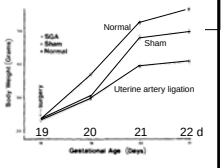
- Epigenetic changes will affect all lineages similarly.
- Epigenetic change can be induced, but the mechanism is unclear.
- Focussed studies with specific dietary or drug manipulations are needed.

Organogenesis



- Fetal origins of adult disease (Barker hypothesis)
  - Low birth weight - obesity, cardiovascular disease, diabetes, etc
- DNA methylation occurs as tissues differentiate
- Could epigenetic variation/plasticity contribute to:
  - Diabetes?
  - Hypertension?
  - IUGR / metabolic syndrome?
- Much hope - few data

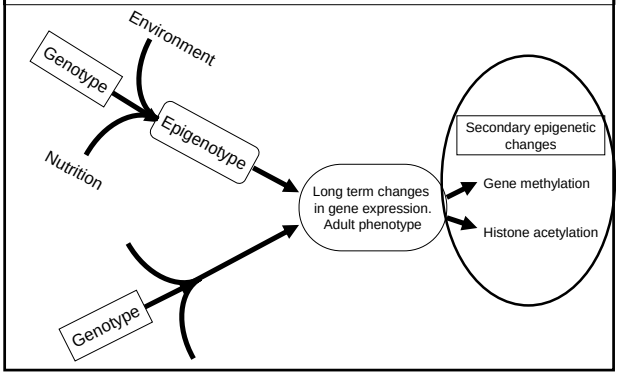
Rat IUGR - diabetes model



- Rat IUGR model
  - reduced pancreatic beta-cell mass
  - reduced insulin secretion
  - insulin resistance
  - type 2 diabetes in adults.
  - bilateral uterine artery ligation 3 days before birth
  - rapidly induces IUGR
- *Pdx1* expression is halved (it regulates pancreatic development)
  - Epigenetic changes at *Pdx1* promoter - histone deacetylation, changes in histone methylation and increasing DNA methylation with age.

Ogata ES Metabolism 1986; Simmons RA Rev Endocr Metab Disord 2007;8:95

Primary or secondary change

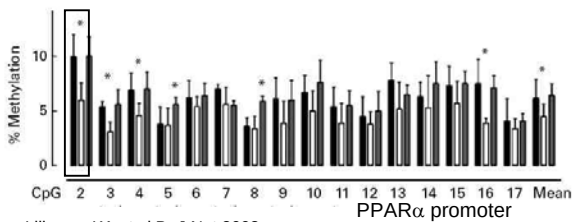


Rat low protein (high carb) model

- Pregnant rats - LP/HC diet during gestation
- Upregulation of glucocorticoid receptor and PPARα occurs with disturbed metabolic control.
- Many publications - only one useful so far.

Rat low protein (50%) (high carbohydrate) model best results

- Liver - PPARα promoter methylation (by pyrosequencing)
- Reduction in average methylation from **6.1% to 4.5%** (black bars to white bars) in 34 day offspring.



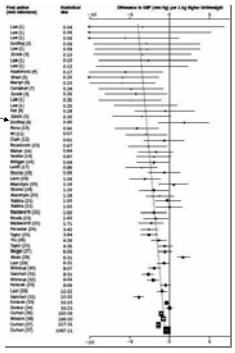
Lillycrop KA et al Br J Nut 2008

Rat low protein (50%) (high carbohydrate) model

- Is this change physiologically significant?
- Does it explain 10 fold increase in PPARα expression?
- Could it reflect changes in cellular composition of the liver?

Blood pressure?

- IUGR may cause hypertension
  - Controversial - good studies show very little difference (see Huxley R et al Lancet 2002)
- Hypertension associated with nephron number (Keller G NEJM 2003)
  - Therefore blood pressure may be controlled by anatomic size, not epigenetic memory



Blood pressure?

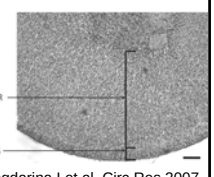
- Rat low protein model

(Hypertension, upregulated renin angiotensin system)

Angiotensin II receptor, type 1b

Reduction in methylation from 22% to 7% in whole adrenals (bisulphite cloning).

But *Agtr1b* expression predominantly from the adrenal cortex, which constitutes only a small minority of adrenal cells



Methylation

Expression

Bogdarina I et al, Circ Res 2007

Organogenesis summary

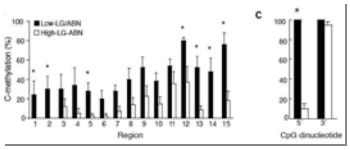
- Organ development is an obvious target for epigenetic modulation, but …
- Published data are poor.
- Need a combination of qualitative (bisulphite sequencing) and quantitative data (pyrosequencing, sequenom, etc) in pure tissues.

Post-natal

- Behavioural modification changes methylation of offspring
  - Rat model - stressed (low licking and grooming) vs. non-stressed (high LG) mothers.

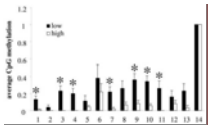
Glucocorticoid receptor promoter 1_7

Weaver I et al Nat Neurosci 2004

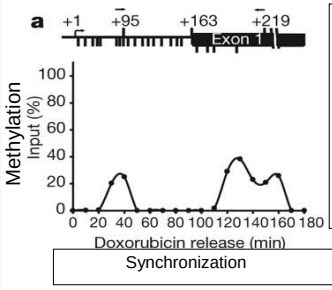


Estrogen receptor-α 1b promoter

Champagne F et al Endo 2006



But: Estrogen receptor-α methylation is transient and cyclical



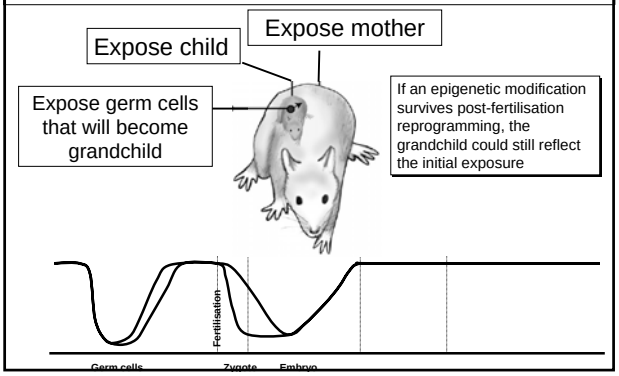
Synchronization

ER-α methylation responds to the endocrine environment.

It seems unlikely that methylation provides a longterm signal.

Kangaspeska et al Nature 2008

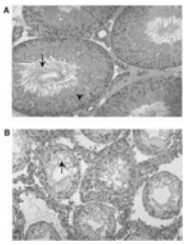
Transgenerational epigenetic effects



Transgenerational effects

- Best examples: Endocrine disruptors
- E.g., vinclozolin exposure during embryonic gonadal sex determination induces adult onset male fertility and spermatogenic defect for multiple generations (i.e. F1–F4)
- DNA methylation changes in several genes (inconsistent)
- If epigenetic change is primary how is it maintained during reprogramming?

Anway MD et al, Science 2005;308:1466



F3 male

Summary

- Early embryogenesis
  - susceptible to environmentally-induced epigenetic change.
  - Mechanisms need to be clarified.
  - Refined models needed.
- Organogenesis - possible but unproven
- Post-natal - fascinating possibilities
- Transgenerational
  - (more care with nomenclature).
  - Erasure mechanisms suggest that primary epigenetic signals are unlikely.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