ART
&
Epigenetic Modification

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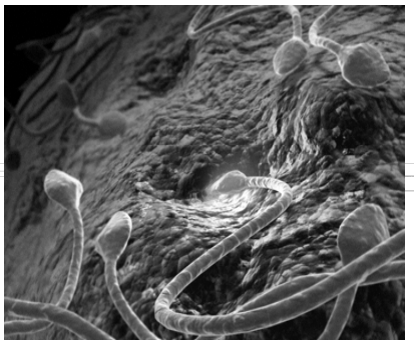
Early Concepts: Preformation vs. Epigenesis

- The question of how a zygote becomes an animal has been asked for centuries.
- As recently as the 18th century, the prevailing theory was a notion called **preformation –** the idea that the egg or sperm contains an embryo.
  - A preformed miniature infant, or "homunculus," that simply becomes larger during development.

Early Concepts: Preformation vs Epigenesis

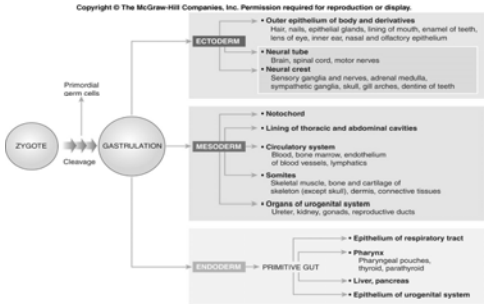
- Kaspar Friederich Wolff (1759) demonstrated there was no preformed chick in the early egg.
  - Undifferentiated granular material became arranged into layers.
  - The layers thickened, thinned, and folded to produce the embryo.

Fertilization: Beginning of life

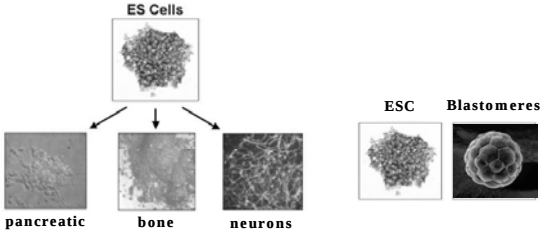


Organogenesis

Many different structures are derived from the three embryonic germ layers during organogenesis.



Different Cell Types



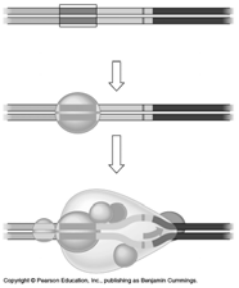
Same DNA
but
Different Gene Expression

DIFFERENTIATION

- Virtually all cells within a multicellular organism are genetically identical
- Differences between cells are due to differences in gene expression
  - Different subsets of genes are "on" and "off"
  - Different cell types make different proteins

GENE EXPRESSION

- Much of the regulation of gene expression occurs at the level of transcription
- Transcriptional regulation of gene expression is directed by
  - Maternal molecules in the cell's cytoplasm
  - Signals from other cells



The range of commitment options available to a cell

- Totipotent
- Pluripotent
- Multipotent
- Oligopotent
- Unipotent

Potency

Embryonic

Amniotic

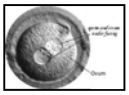
Adult

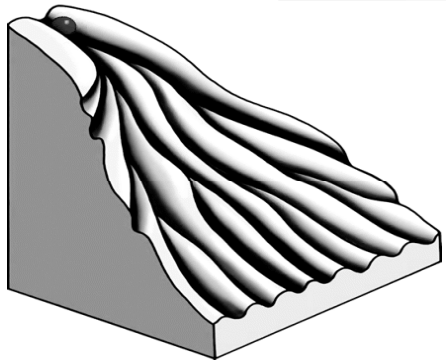
Molecular Reprogramming

Goal: Manipulate a somatic cell's potency

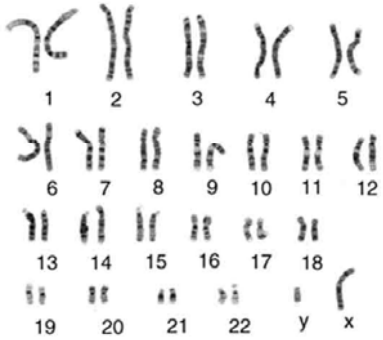
- Totipotent
- Pluripotent
- Multipotent
- Oligopotent
- Unipotent

Potency





Ketton and Gould Theory, 1984



The Biology of the Cell

Epigenetic
&
Cell Differentiation

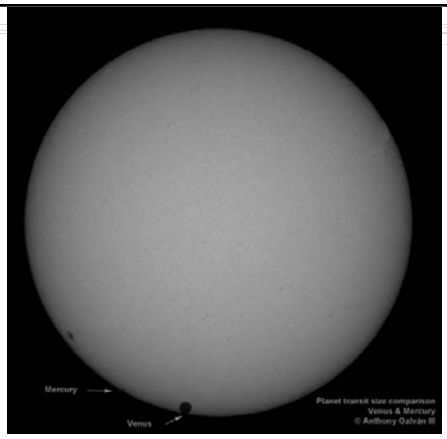
Starting at the beginning

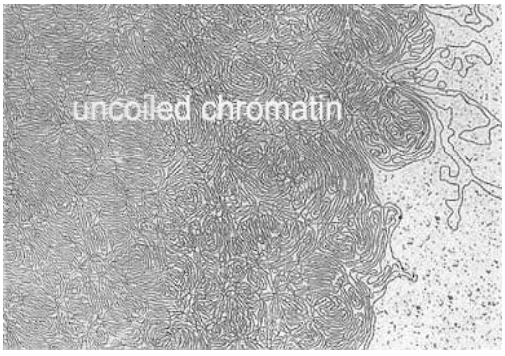
The conundrum

Human DNA: 2 m

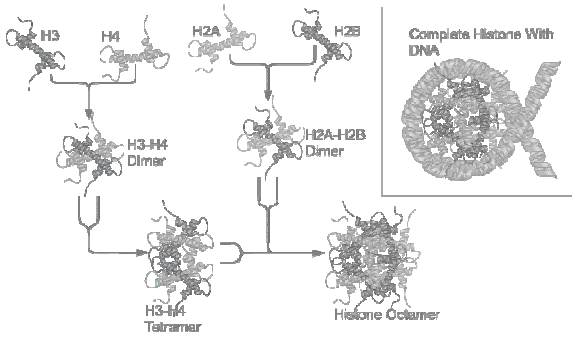
Human nucleus r = 10 µm

10,000 x compaction

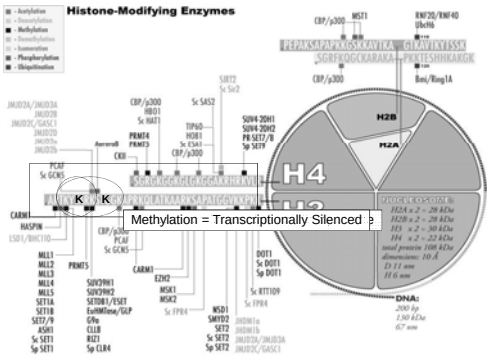




Histone



Histone Modifications



Epigenetics?

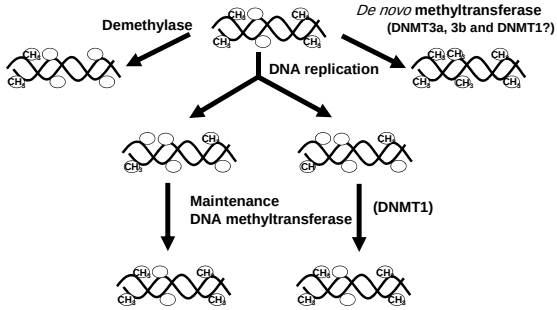
Study of changes in gene expression that occur without changes in the DNA code

gene expression patterns

- **inheritable through mitosis and meiosis**
- **changes during cell differentiation**
- **influence by environmental factors**

London 23/03/2007

DNA methylation reactions



Covalent modifications of the N-terminal tail of the canonical core histones

Anders H. Lund et al. Genes Dev. 2004; 18: 2315-2335

Cold Spring Harbor Laboratory Press

DNA methylation silences gene expression by two mechanisms

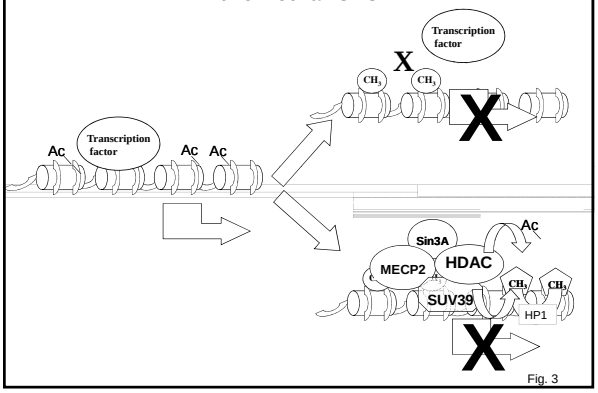
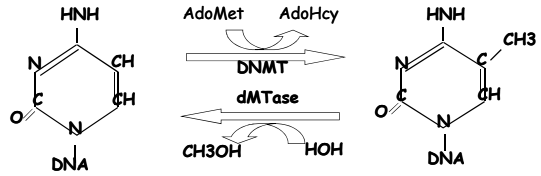


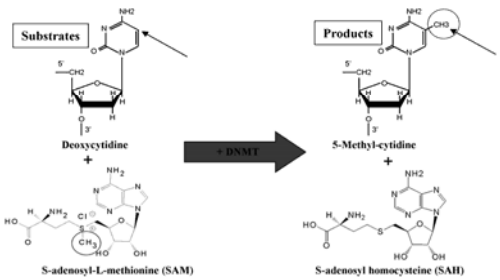
Fig. 3

The environment is dynamic, the chromatin is dynamic, would DNA methylation remain static through out life?



The reversible methylation reaction

DNA Methylation

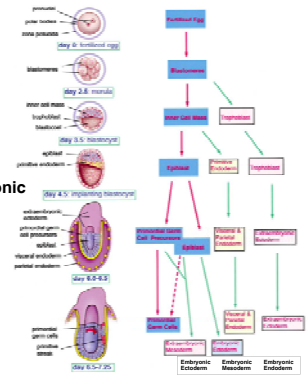


Epigenetics

the Good, the Bad and the Ugly

The Good

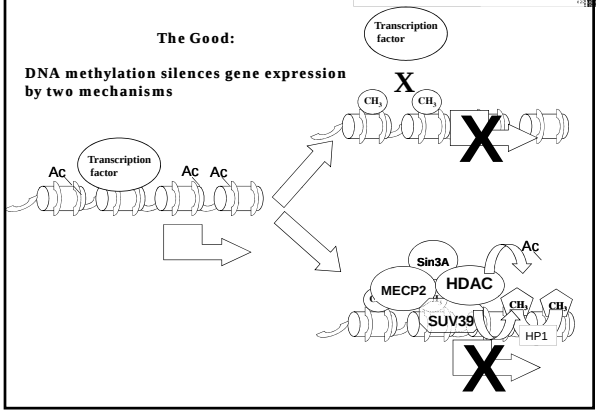
Blue = embryonic
white = extraembryonic



Developmental Cell 2: 537 (2002) Zhao and Garbers, Male Germ Cell Specification and Differentiation

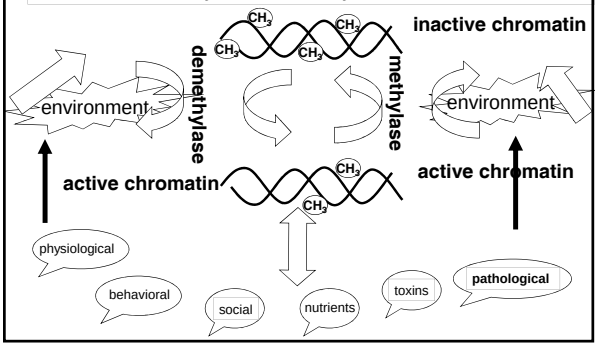
The Good:

DNA methylation silences gene expression by two mechanisms

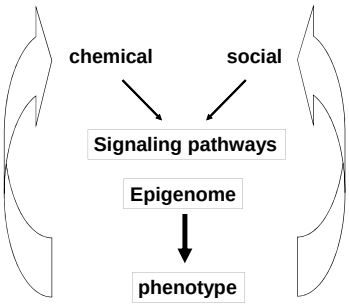


The Good

The gene expression pattern is a **dynamic** equilibrium between methylase and demethylase activities



Bi-sided sward: life-long dynamic epigenome?



Maternal behavior

Nest bout

Nursing

Grooming and licking

pup licking and grooming (LG) and arched-back nursing (ABN)

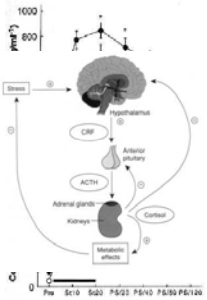
low-LG-ABN mothers

high-LG-ABN mothers.

Offsprings sensibility to stress

high-LG-ABN mothers.

Modest responses to stress

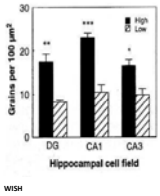


low-LG-ABN mothers.

high responses to stress

Liu D et al science 277 1997

GR mRNA expression in the hippocampus of the offspring



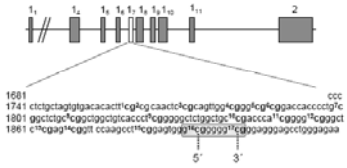
WISH

High hippocampus GR expression in offspring of high-LG-ABN mothers

Liu D et al science 277 1997

GR promoter mapping

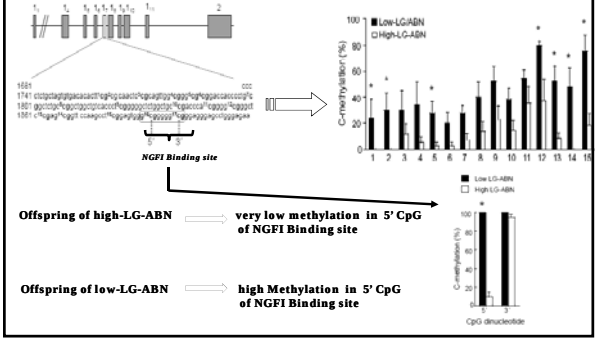
GR EXON 1_7 promoter mapping



Presence of CpG

GR promoter mapping

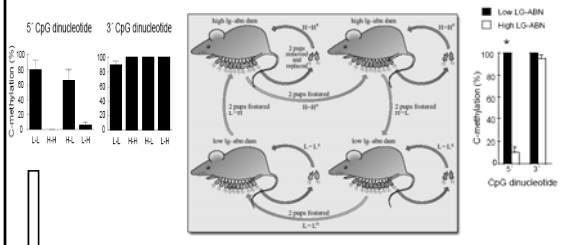
GR EXON 1_7 promoter sodium bisulfite mapping



Offspring of high-LG-ABN ⇨ **very low methylation in 5' CpG of NGFI Binding site**

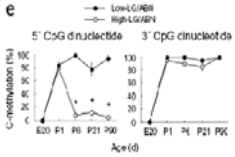
Offspring of low-LG-ABN ⇨ **high Methylation in 5' CpG of NGFI Binding site**

Cross –fostering and epigenetic marking



DNA methylation pattern is establish through a behavioral mode of programming without germ line transmission

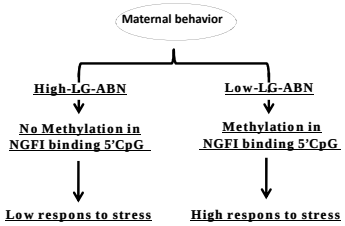
Timing of maternal effect on DNA methylation



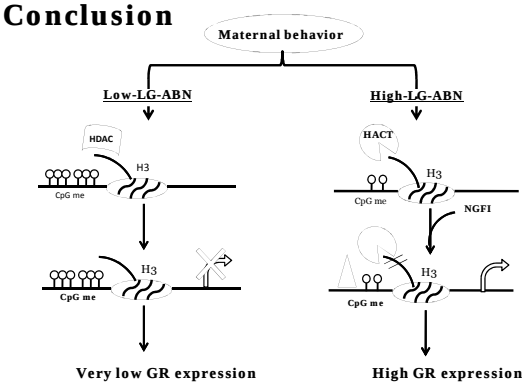
DNA methylation pattern is established during the first week Of life and remains consistent through to adulthood

SUMMARY

Maternal behavior can lead to differential methylation which occurs the first week of life and is maintained during the life

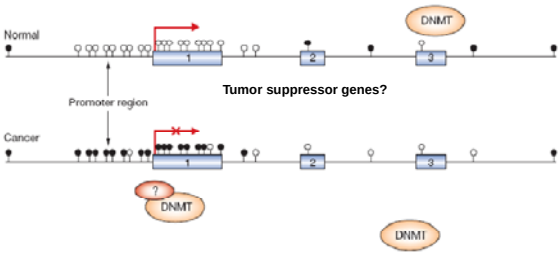


Conclusion

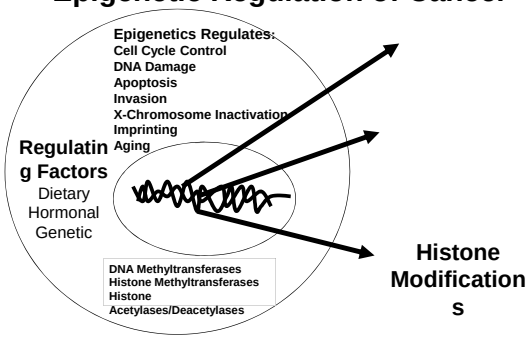


The Ugly: Cancer

Nature Clin Pract Oncol 2005;2:S4-S11



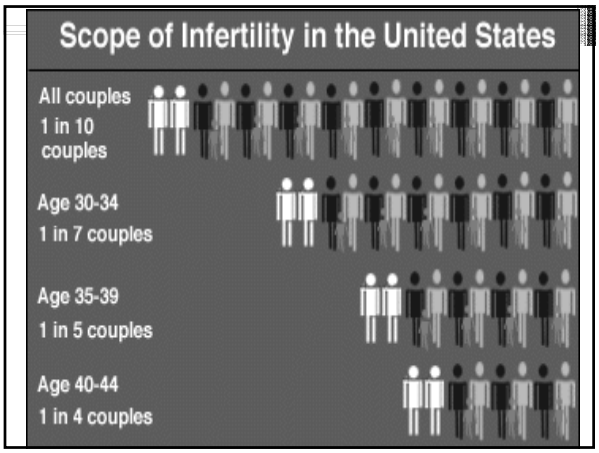
Epigenetic Regulation of Cancer



Verma M, Srivastava S. Lancet Oncol. (2002) 3:755-63.

Assisted Reproductive Techniques (ART) & Epigenetics

Why ART?



World Population Statistics

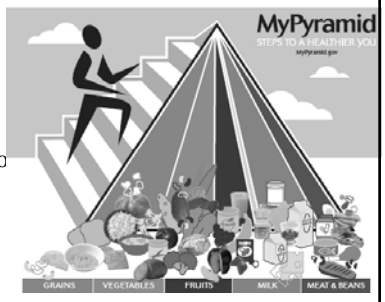
What is exponential growth?

1987	5 billion
1999	6 billion
?	7 billion

The United Nations projects that our world population could reach 7 billion somewhere between 2011 and 2015.

Your job as a world citizen:

- To work towards providing the right food, nutritionally, to all people in order to help them lead healthier lives.



Can you meet the needs of a human without animals?
If you don't have some of these areas available in your diet, what effect do you think it has?

LIVESTOCK PRODUCTS

• **Milk**	**33.230 million tons**
• **Beef**	**1.237 million tons**
• **Mutton**	**0.827 million tons**
• **Poultry meat**	**0.514 million tons**
• **Eggs**	**9.618 billions**
• **Wool**	**41.2 thousand tons**
• **Hair**	**25.0 thousand tons**
• **Skins and hides nos.**	**57.6 million**

Economic Survey (2006-07)

IMPACT OF BIOTECHNOLOGIES

Assisted Reproductive Technologies/Cloning

Embryo Transfer: 40,000-50,000 Calves/Year

IVM/IVF: 4,000 Calves in 1996

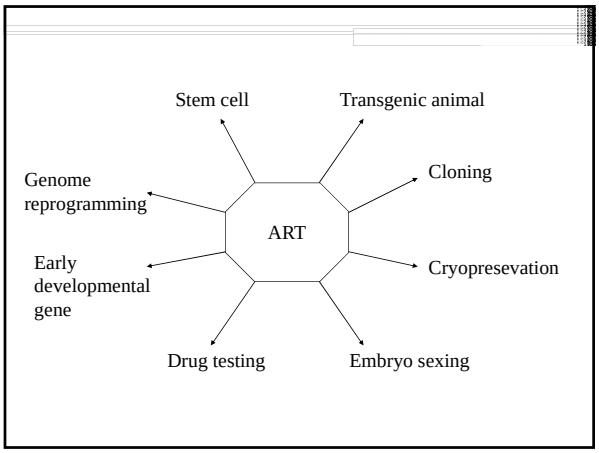
Blastomere Nuclear Transfer Cloning
(61 males and 126 females)

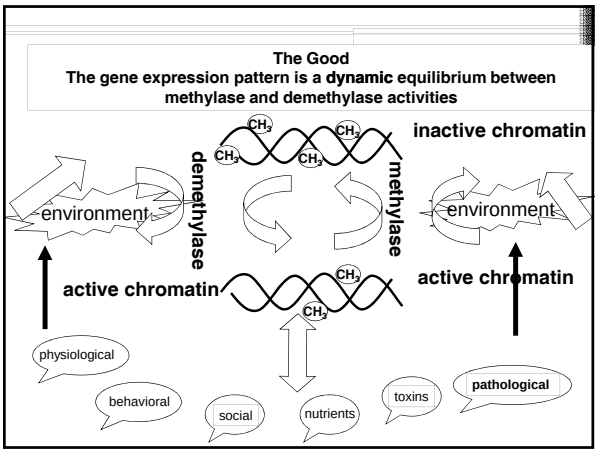
Embryo Splitting (754 males and 1,472 females)

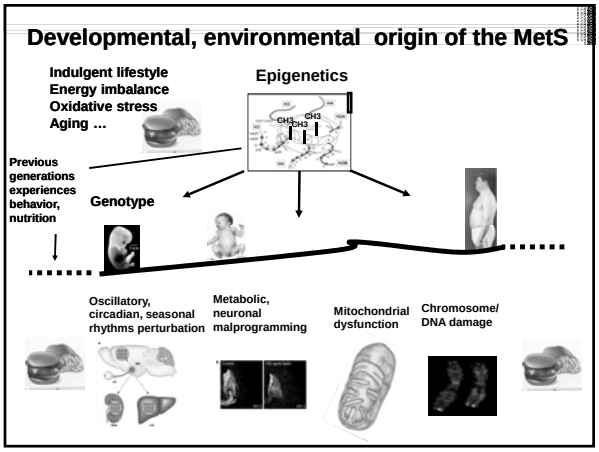
Reference: NAAB 1996 and Holstein Associate 2001

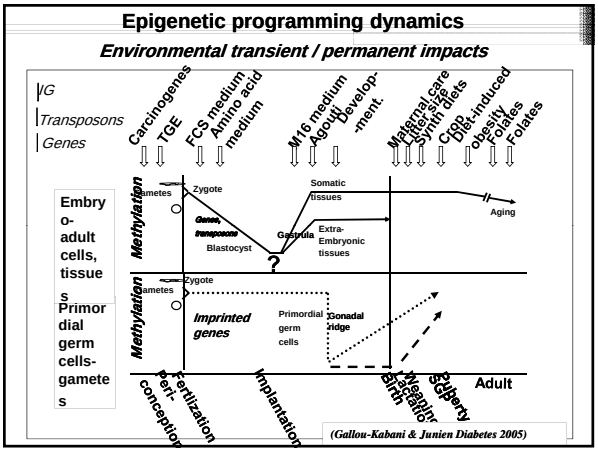
Biotechnologies, Animal Production, and Animal Health 1945 to 1995

- Milk Production Up 3-Fold
- Eggs/Year Up 134 to 254
- Broilers Days to 1.8 kg: Reduced from 84 to 43 Days and one-half the feed
- Leaner and Faster Growing Pigs
- Leaner and More Efficient Beef Cattle
- Overall Improvement in Animal Health


Stem cell
Transgenic animal
Genome reprogramming
Cloning
ART
Early developmental gene
Cryopresevation
Drug testing
Embryo sexing


The Good
The gene expression pattern is a dynamic equilibrium between methylase and demethylase activities
inactive chromatin
demethylase
methylase
environment
environment
active chromatin
active chromatin
physiological
behavioral
social
nutrients
toxins
pathological


Developmental, environmental origin of the MetS
Indulgent lifestyle
Energy imbalance
Oxidative stress
Aging ...
Epigenetics
Previous generations experiences behavior, nutrition
Genotype
Oscillatory, circadian, seasonal rhythms perturbation
Metabolic, neuronal malprogramming
Mitochondrial dysfunction
Chromosome/ DNA damage


Epigenetic programming dynamics
Environmental transient / permanent impacts
VG
Transposons
Genes
Carcinogenes
TGE
FCS medium
Amino acid medium
M16 medium
Agouti
Develop-ment
Maternal care
Folates
Folates
Methylation
Embryo-adult cells, tissues
Primordial germ cells-gametes
Zygote
Somatic tissues
Aging
Blastocyst
Gastrula
Extra-Embryonic tissues
Primordial germ cells
Gonadal ridge
Imprinted genes
Adult
Fertilization
Implantation
Puberty
(Gallou-Kabani & Junien Diabetes 2005)


Gone, but, Not Forgotten