CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Edith Heard

Année 2012-2013 :
"Épigénétique, développement et hérédité"

25 Février, 2013

Cours IV
"L'empreinte parentale, une mémoire de l'origine maternelle ou paternelle des gènes"

Séminaire
Professor Anne Ferguson-Smith
(Cambridge University, UK)

COLLÈGE DE FRANCE 1530

Parental origin can matter

Hybrids of different species, but of the same genus, have very different phenotypes depending on parental origin

Male donkey x female horse
Mule

Male tiger x female lion
Tigon

Male horse x female donkey
Hinny

Male lion x female tiger
Liger

Parent-of-origin phenomena observed in a wide range of phyla from both the plant and animal kingdoms.

1. Paternal chromosome elimination in Sciara (Metz, 1938; Crouse et al., 1960)
2. Inactivation of the paternal genome by heterochromatization in the scale insects (coccids) (Nur, 1990)
3. Parental dominance in hybrid plants (Heslop-Harrison, 1990)
4. Parent-of-origin specific modification of position effect variegation in Drosophila (Spofford, 1976)
5. Phenotypic differences in progeny produced from interspecific crosses in fish (Whitt et al., 1977) and between donkey and horse
6. Preferential inactivation of the paternally derived X chromosome in marsupials and rodent extraembryonic tissues (Cooper et al, 1971; VandeBerg et al., 1987)
7. Parent-of-origin dependent switching of yeast mating types (Klar, 1987)
8. Allelic exclusion of immunoglobulin genes (Holliday, 1990)
9. Gamete-of-origin dependent modifications of transgene methylation and expression in mice (Swain et al., 1987; Reik et al., 1987; Sapienza et al., 1987) and transgenic zebrafish (Martin and McGowan, 1995)
10. Parent-of-origin specific gene expression in plants and mammals

Genomic Imprinting

• Genomic imprinting results in the expression of genes from only one of the two parental chromosomes – defies Mendel's laws of genetics!

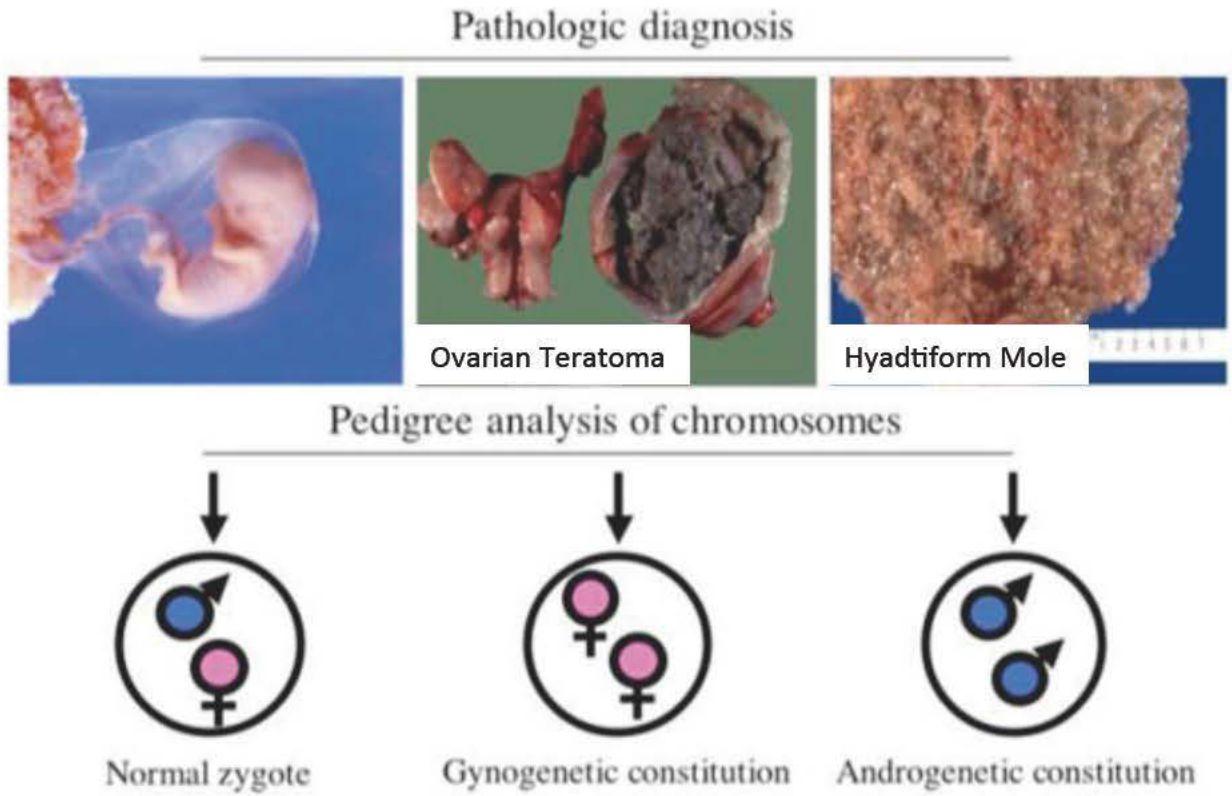
• Brought about by epigenetic instructions — imprints — that are laid down in the parental germ cells.

• Imprinting in mammals is thought to influence the transfer of nutrients to the fetus and the newborn from the mother.

•Imprinted genes often affect growth in the womb and behaviour after birth.

• Aberrant imprinting disturbs development and is the cause of various disease syndromes.

Evidence for Genomic Imprinting in Humans



"*In contrast to androgenetic ova producing only hydropic villi, parthenogenetic oocytes in the ovary produce several mature tissues. Remarkable differences in the end products of both types of conceptuses are of special interest with regard to the* ***possible physiologic difference between maternally and paternally derived genome*** *in the egg cytoplasm, influence of implantation site (ovary versus uterus), and interaction between mother and conceptus in early mammalian embryogenesis.*"

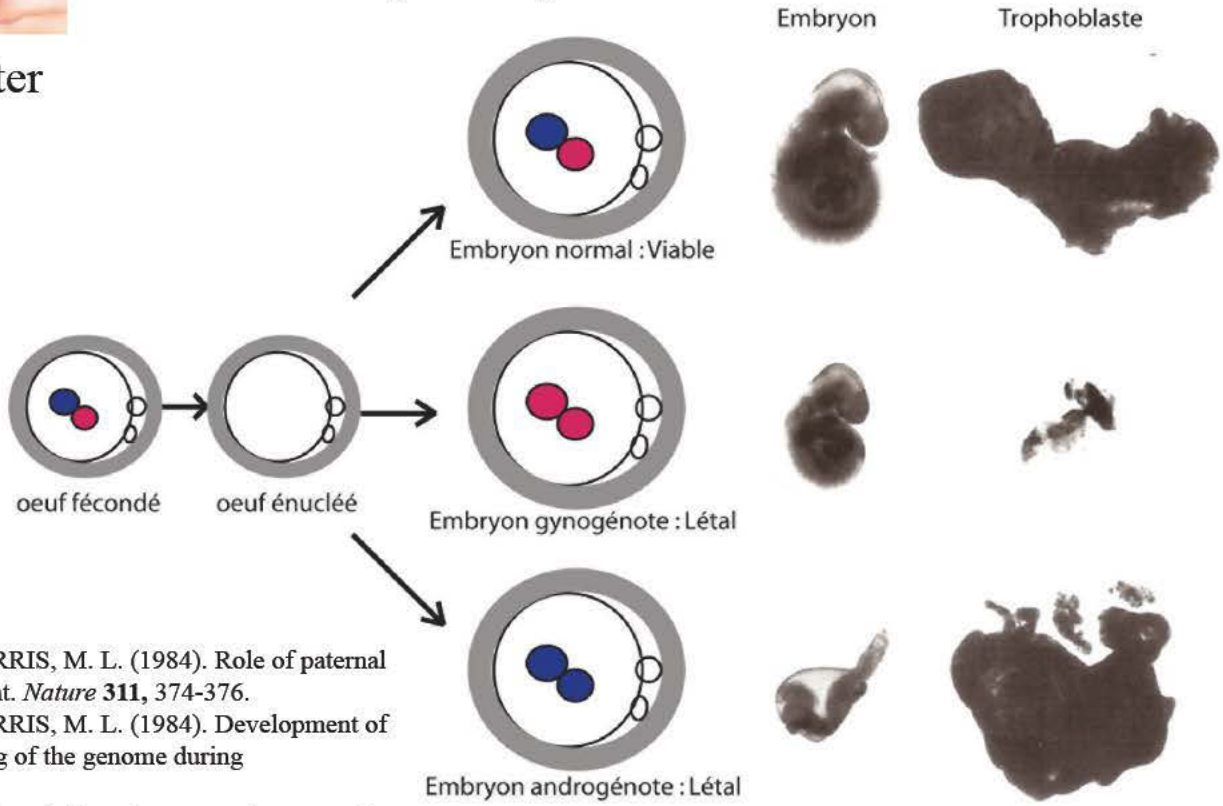
Wake, Takagi, and Sasaki (1978)

Evidence for Genomic Imprinting in Mice

Azim Surani Davor Solter

Nuclear transplantation experiments in mice by Azim Surani and Davor Solter :

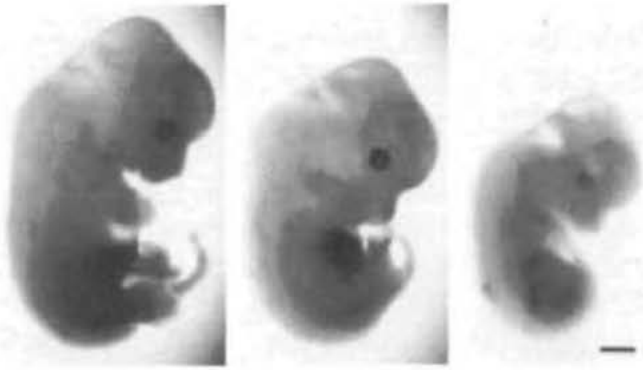
- Two male or two female pronuclei are incompatible with normal development
- Formal demonstration of the functional non-equivalence of mammalian parental genomes



BARTON, S. C , SURANI, M. A. AND NORRIS, M. L. (1984). Role of paternal and maternal genomes in mouse development. *Nature* **311,** 374-376.
SURANI, M. A., BARTON, S. C. AND NORRIS, M. L. (1984). Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. *Nature* 308, 548-550.
MCGRATH, J. AND SOLTER, D. (1984). Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* 37, 179-183.

Evidence for Genomic Imprinting in Mice

Development of chimaeras made with mixtures of normal cells and androgenetic (AG) or gynogenetic (GG) cells on day 13 of gestation.



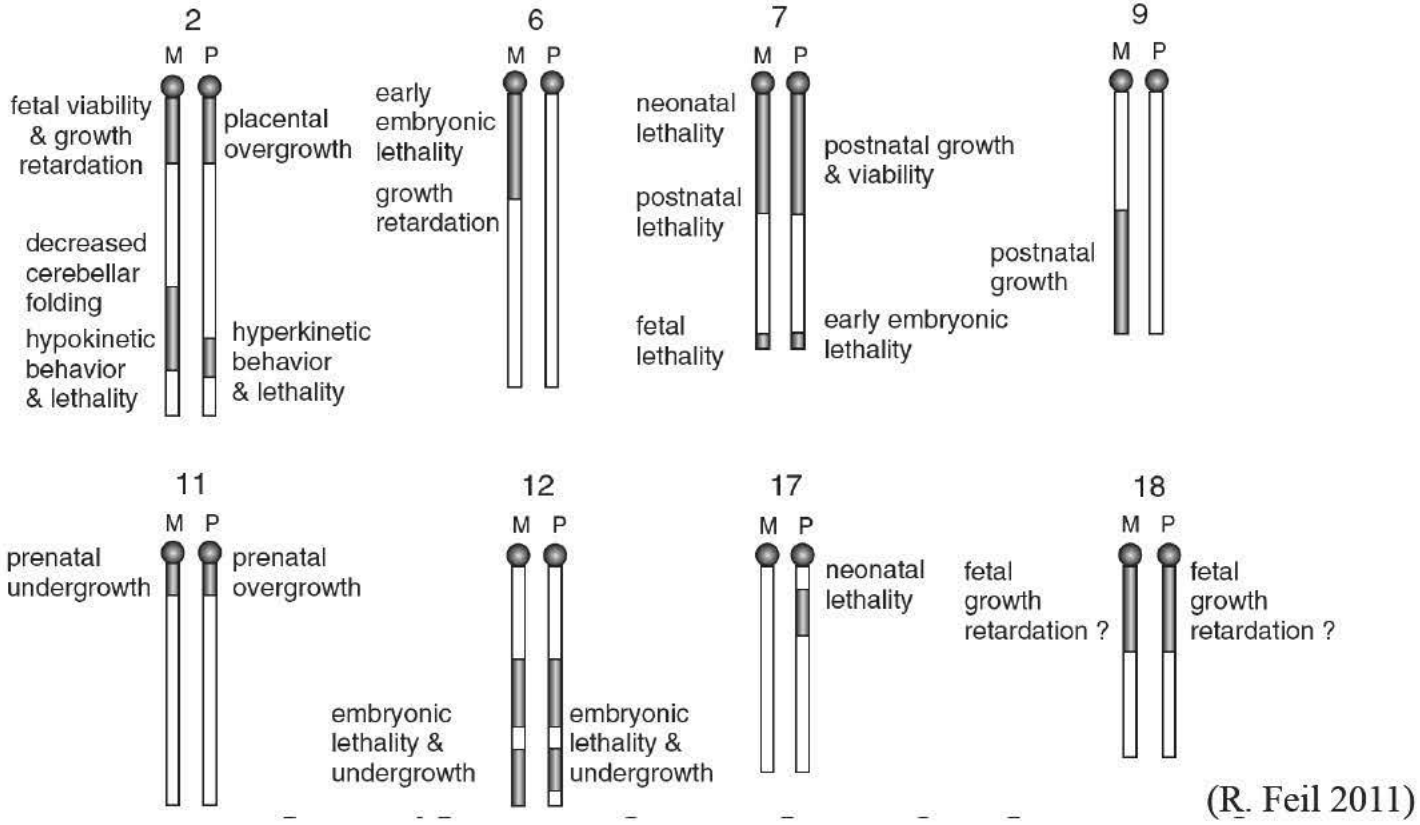
	AG/ICM→F	CONTROL	GG/ICM→F
Weight (mg)	138	92	50
A–P axis	10.3	8.9	7.6

Reciprocal phenotypes are observed:
gynogenotes are growth retarded while androgenotes show enhanced growth.

Later defects in mesoderm derivatives, skeleton, brain development…
(Barton et al, 1991; Fundele et al, 1990)

Evidence for Genomic Imprinting in Mice

Bruce Cattanach



(R. Feil 2011)

Thirteen sub-chromosomal regions exhibiting unipaternal disomy identified:

- Defects in **development**, **behaviour**, **growth**, **viability**
- Suggesting differential expression from the 2 parental chromosomes homologues
- Requirement for both maternal and paternal alleles for normal development.

Searle and Beechey (1978) Complementation tudies with mouse translocations Cytogenet Cell Genet 20, 282-303

Cattanach and Kirk (1985) Differential activity of matenral and paterbally derived chromosome regions in mice. Nature 315, 496-498.

Genomic Imprinting in Humans

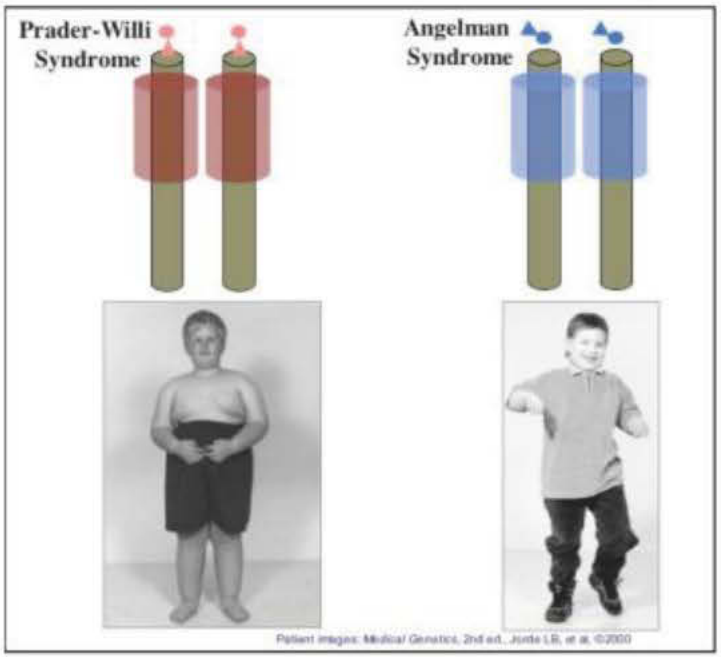
First human clinical syndromes recognized to result from imprinted loci were **Prader-Willi syndrome** and **Angelman syndrome** (Nicholls et al., 1989).

Identical genetic deletions as well as uniparental disomy for a domain on 15q result in very different clinical phenotypes depending on the parental origin of the deletion/disomy.

NATURE · VOL 342 · 16 NOVEMBER 1989

Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome

Robert D. Nicholls*†‡, Joan H. M. Knoll†, Merlin G. Butler§, Susan Karam‖ & Marc Lalande*†¶



Mild-moderate mental retardation

*Hypo*tonia

Obesity

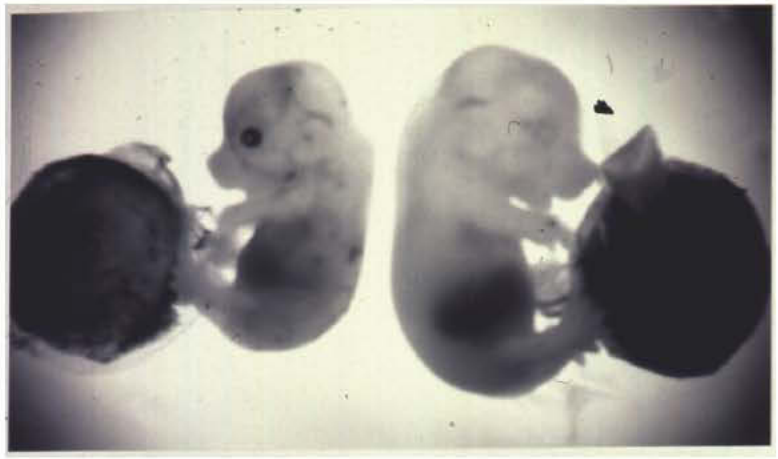
Severe mental retardation

*Hyper*tonia

Happy disposition

Discovery of Imprinted Genes

Paternal duplication of distal chromosome 7 causes growth enhancement

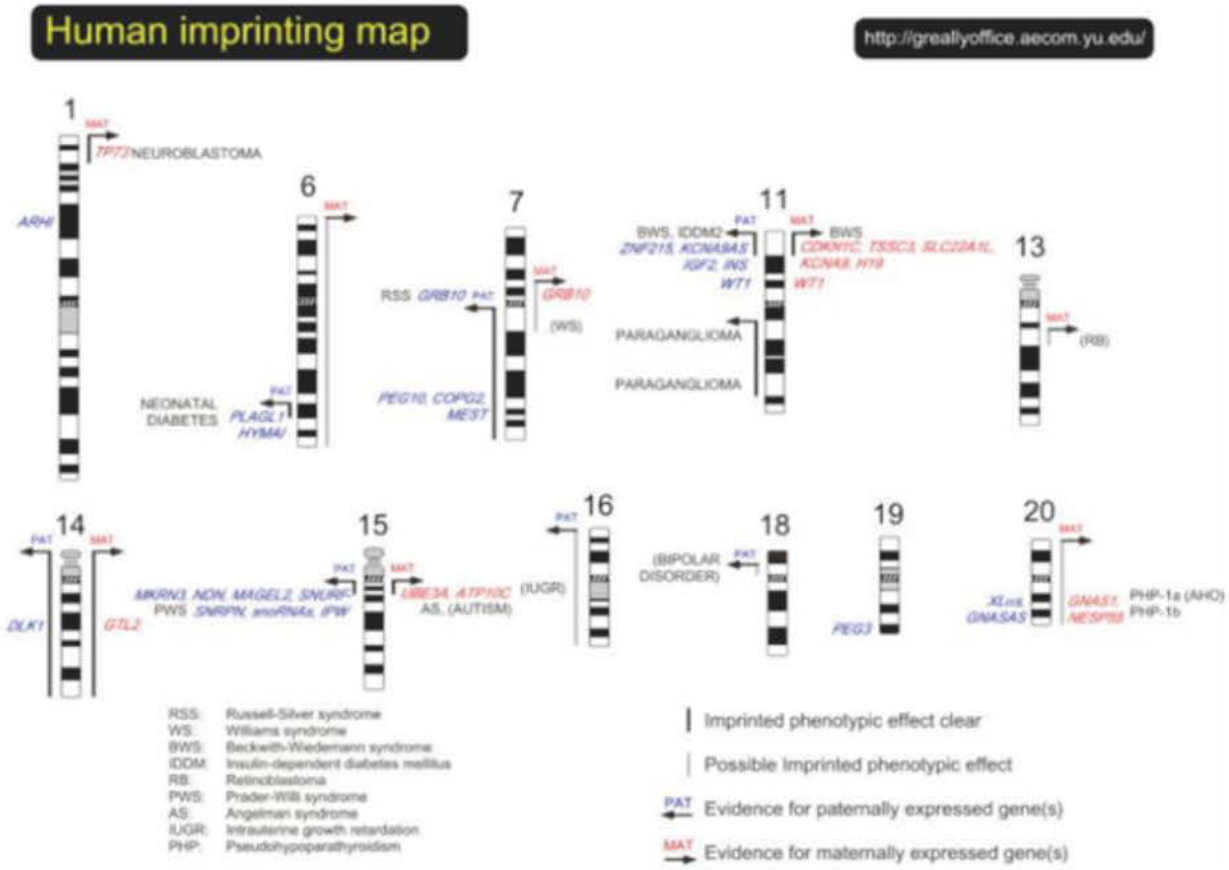


Courtesy of Azim Surani

In humans, paternal duplication of equivalent region results in Beckwith-Wiedemann

Clinical Features:
Macroglossia; high birth weight; hemihypertrophy; Wilm's tumour

Genomic Imprinting in Humans



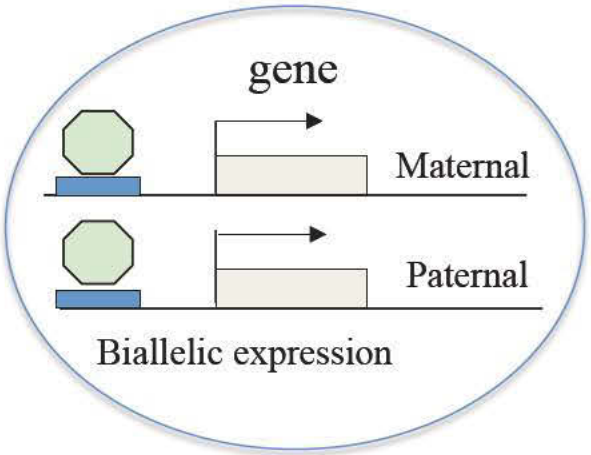
Studies on patients with imprinting disorders:

- Discovery that imprinted gene tend to be in clusters
- Identification of "Imprinting Control Regions" thanks to analysis of chromosomal microdeletions

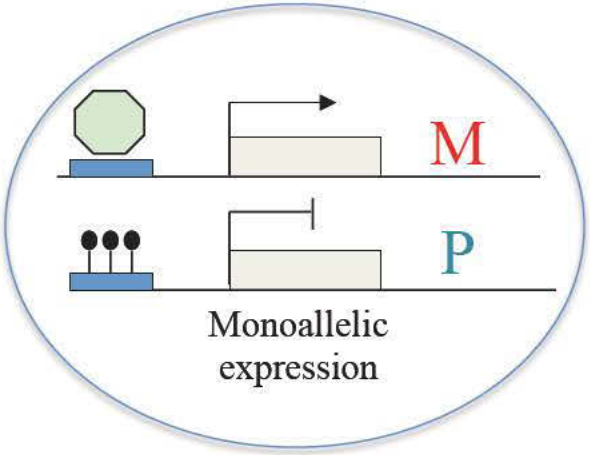
Genomic Imprinting

Expression of an allele that differs based on the sex of the parent that transmitted the allele

Both alleles of a gene expressed



One allele of a gene expressed



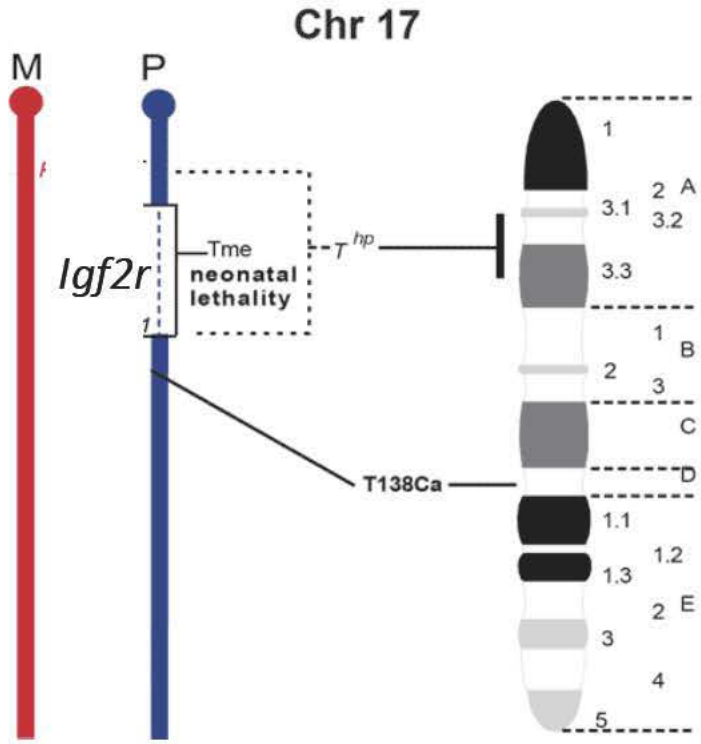
Epialleles:
Epigenetic differences
Eg X inactivation, imprinting…

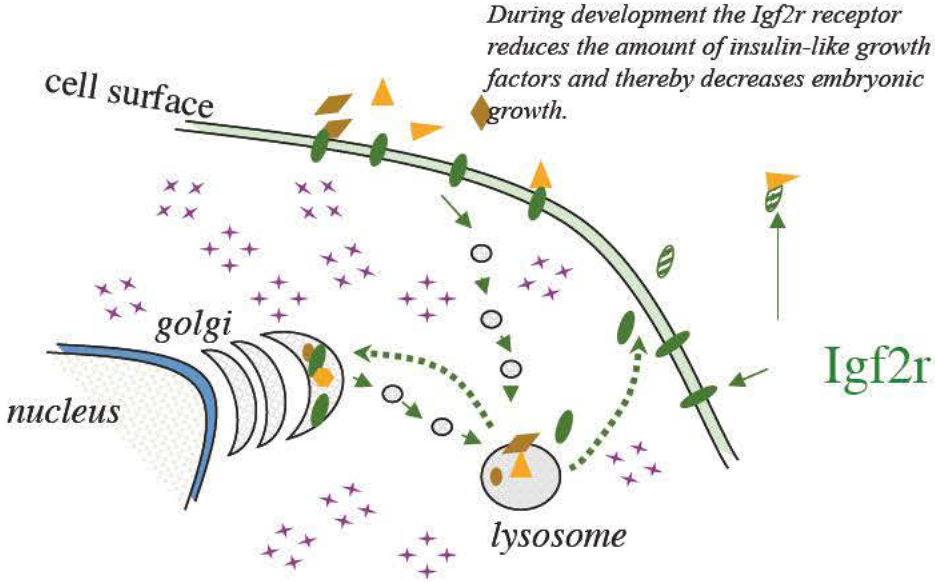
Discovery of Imprinted Genes

Imprinted (maternally expressed) *Igf2r* gene on chromosome 17

Johnson (1974) identified a maternal-effect mutant *Tme*: maternal but not paternal copy of part of chromosome 17 is essential for embryonic development

Identification of a maternally expressed imprinted gene mapping to *Tme* locus, *Igf2r* (insulin-like growth factor type 2 receptor), also known as the cation-independent mannose-6-phosphate receptor - a scavenger receptor for the *Igf2* growth hormone.





Courtesy of D. Barlow

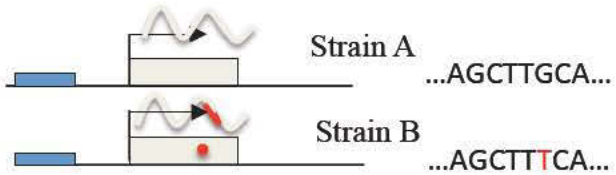
Barlow DP, Stoger R,Herrmann BG, Saito K, Schweifer N. 1991. The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature* 349:84–87

Discovery of Imprinted Genes

Paternally expressed *Igf2* and maternally expressed *H19*: reciprocally imprinted genes on distal chromosome 7

In 1991: *Igf2* gene (insulin-like growth factor type 2, which signals through the *Igf1* receptor) identified as a paternally expressed imprinted gene (De Chiara et al, 1991, Ferguson Smith et al, 1991).

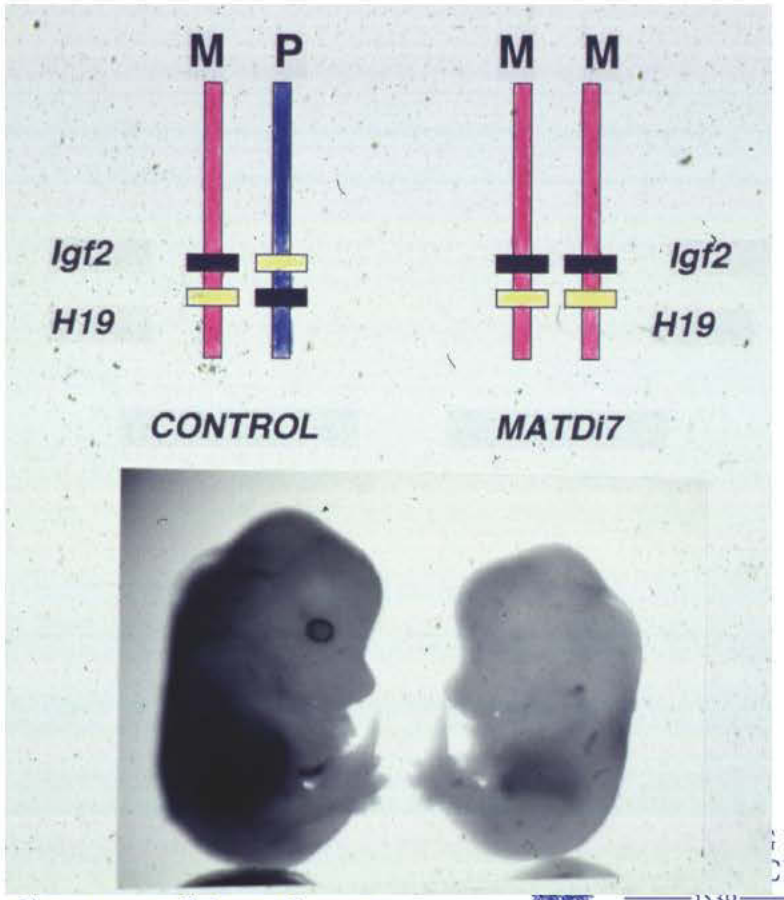
Discovery of *H19* (fetal hepatic cDNA clone 19), a maternally expressed imprinted gene closely linked to *Igf2,* that produces a non protein-coding RNA (Bartolomei et al, 1991, Zemel et al, 1992).



Crossing different mouse strains that have highly polymorphic genomes, enables distinction of paternal and maternal alleles (at both DNA and RNA levels) using restriction fragment length polymorphisms (RFLPs).

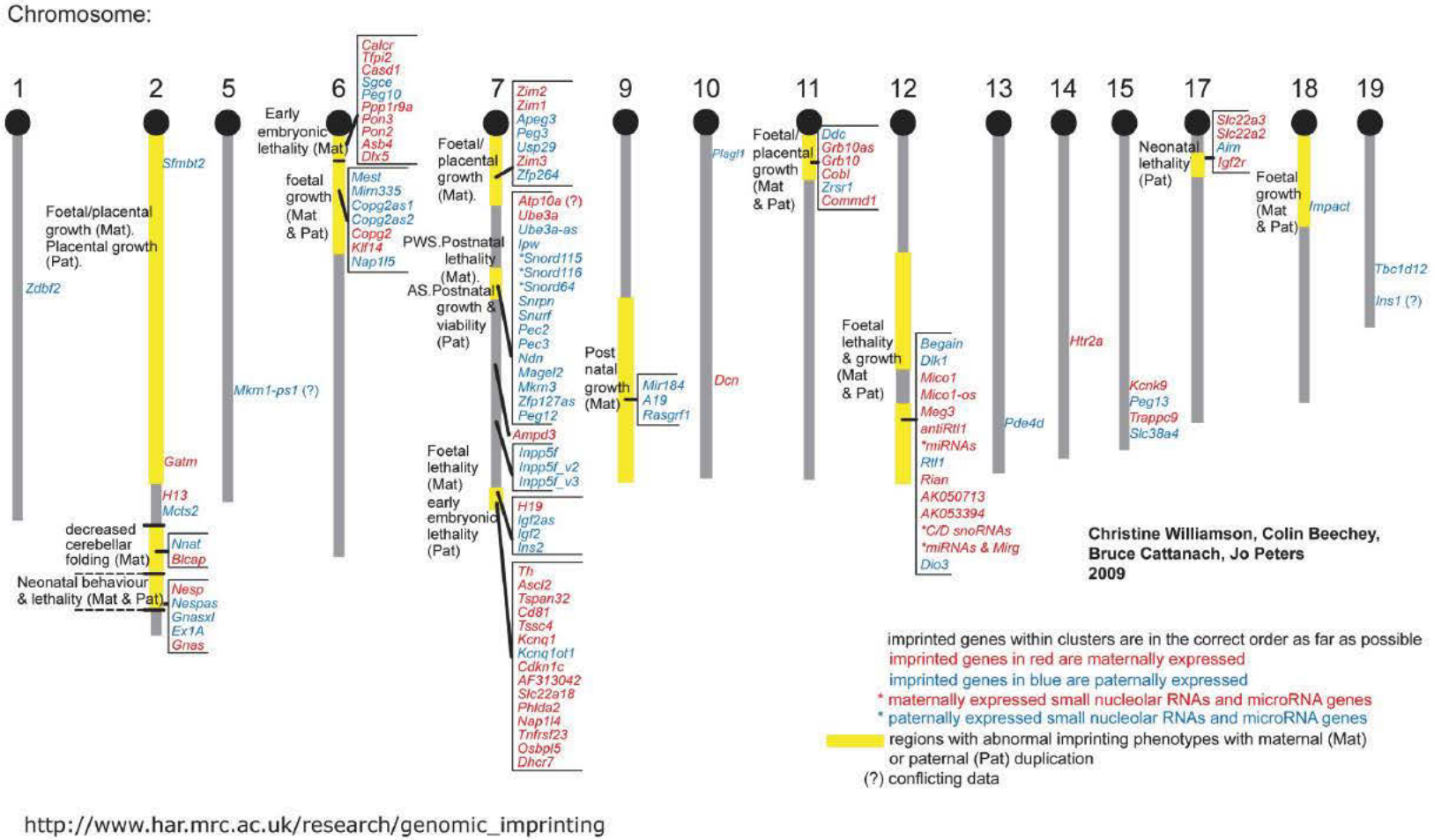
⇒ Determine whether only one (pat or mat) or both alleles are expressed in different tissues of progeny (F1)

Maternal duplication of distal chromosome 7 causes reduced growth, similarly to deletion of paternal Igf2 allele



Courtesy of Azim Surani

Imprinted Genes tend to be Clustered



http://www.har.mrc.ac.uk/research/genomic_imprinting

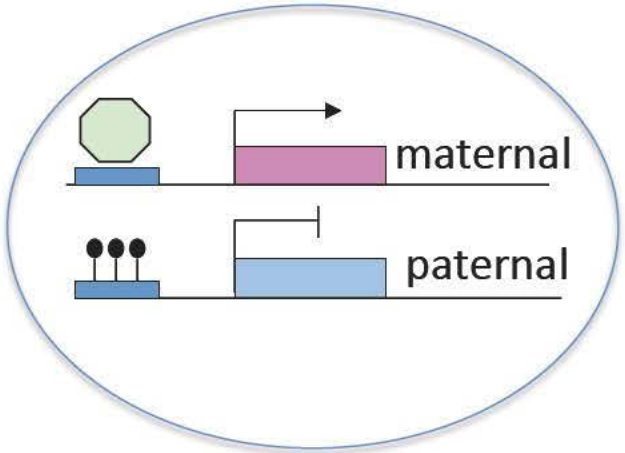
So far, 16 clusters of imprinted genes identified in mouse and humans as well as some imprinted "loner" genes

Total of 100-140 imprinted genes

Imprinting Mechanisms

Imprinting Mechanisms

What are the epigenetic mechanisms allowing parent-of-origin specific information to be passed from parent to embryo and to be maintained in the adult but erased and reset in its germ line?



The underlying marks must:

- control transcription
- be inherited somatically
- be established in the germ line
- be erased in next generation

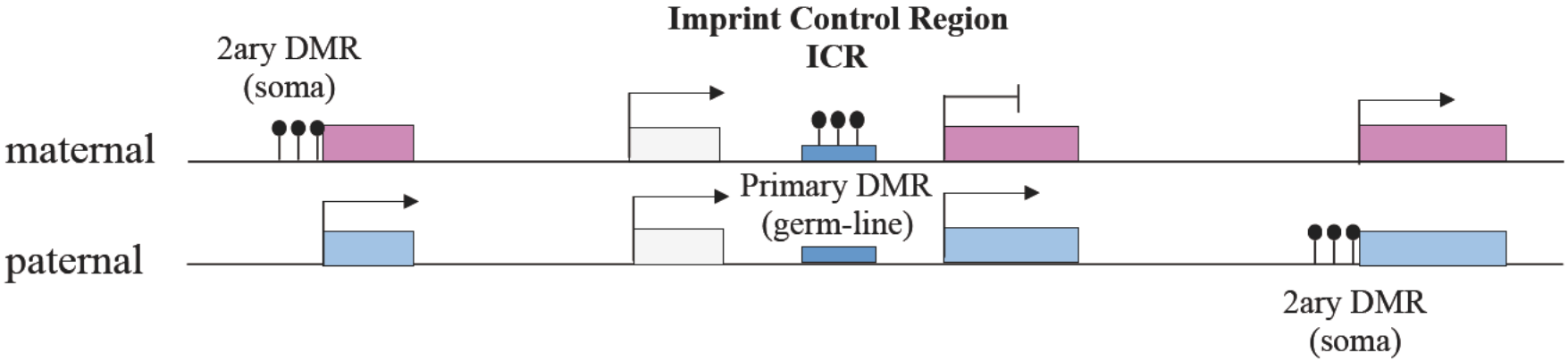
Imprints can sometimes also be overridden or ignored in certain tissues

Imprinting Mechanisms

DNA methylation: a strong candidate as it is a stable epigenetic mark, that can be propagated over cell divisions and can influence transcription (Holliday, Riggs 1975)

CpG islands at imprinted loci are differentially DNA methylated (DMRs)

Differential methylation is found in regions defined genetically as critical for imprinting control (Sutcliffe et al, 1994; Wutz et al, 1997; Thorvaldsen et al, 1993; Smilinich, 1999; Lin et al, 2003; Williamson et al, 2006)



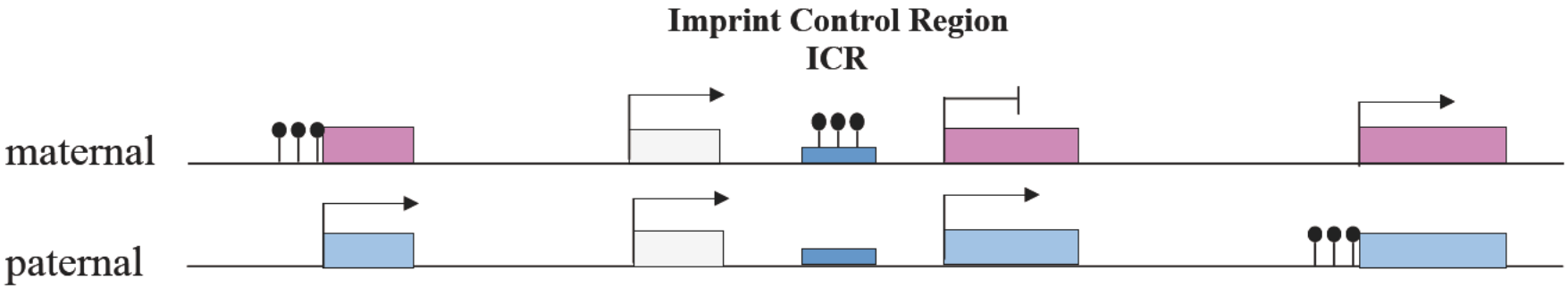
DNA methylation plays a key role in imprinting as imprinted gene expression is disrupted in *Dnmt1* KO embryos.

Li E, Beard C, Jaenisch R. (1993) Role for DNA methylation in genomic imprinting. *Nature*, 366, 362-365.

The *de novo* methyltransferase Dnmt3a and its cofactor Dnmt3l are required for the establishment of germ line imprints (Bourc'his et al, 2001; Kaneda et al, 2004)

Imprinting Mechanisms

At least two modes of imprinting control: Insulator mediated and non-coding RNA mediated

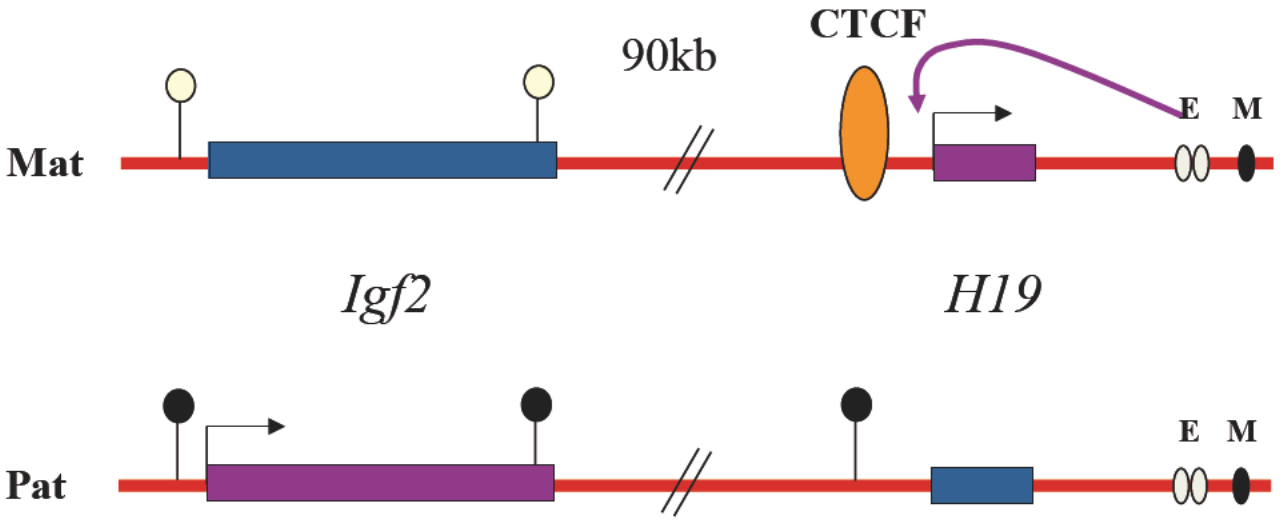


ICRs contain Germ-line DMRs
Most are maternal (ie established in the oocyte) but a few are paternal

Imprinting Mechanisms

1. Insulator mediated control of imprinted gene expression

DMR = Differentially Methylated Region
CTCF = CCCTC-binding factor - can only bind unmethylated DNA



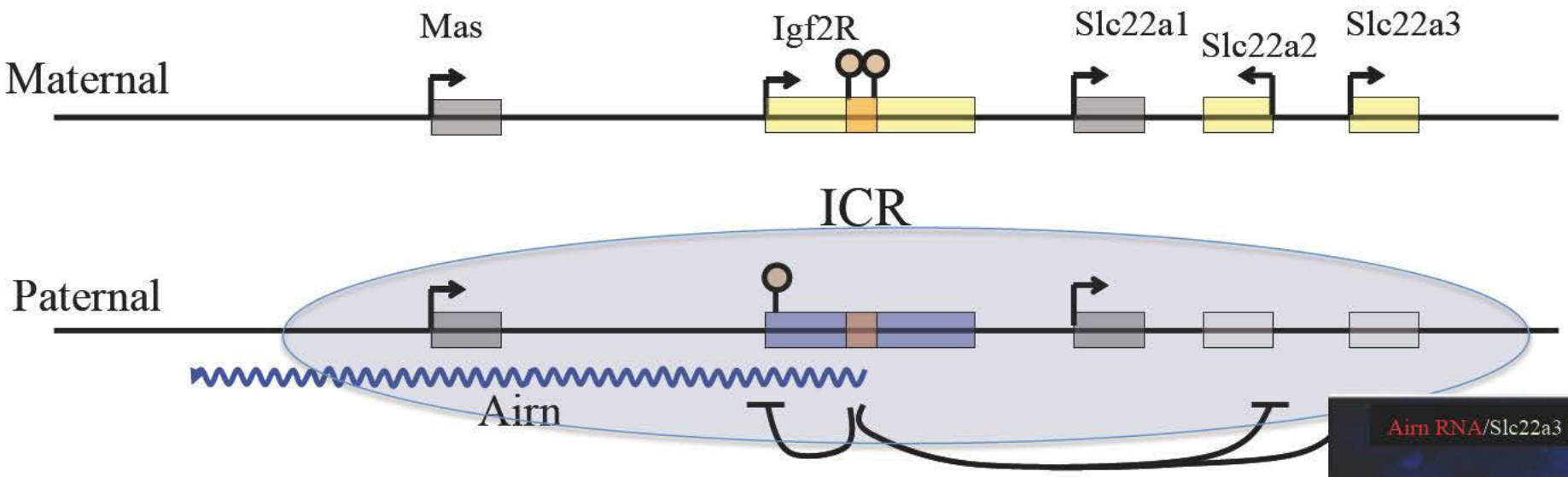
S. Tilghman
M. Bartolomei
W. Reik
A. Surani
L. Dandolo

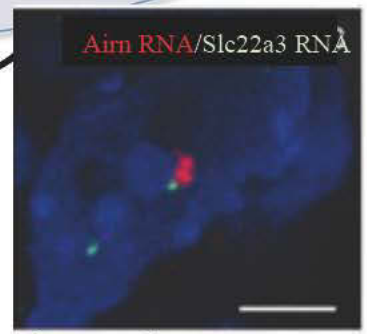
Role of the non-coding H19 RNA?

- No role: decoy for Igf2 enhancer usage?
- Cis- or trans-acting RNA regulator?
  - Produces a miRNA
- Most ancestral imprinted ncRNA

Imprinting Mechanisms

2. Non-coded RNA mediated control of imprinted gene expression





Antisense 108 kb Airn transcription inhibits Igf2r transcription

Airn long range cis-silencing mechanism (chromatin changes?)

Role(s) for non-coding RNA?

- Airn transcription, but not its spliced or unspliced lncRNA products, silences the *Igf2r* promoter (Latos et al, 2012, *Science* 338,1469-1472)
- Airn RNA accumulates and targets histone H3K9 methylation to chromatin in *cis*, in extraembryonic lineages (Nagano et al, 2008, *Science* 322, 1717-1720)

Imprinting Mechanisms

Non-coded RNAs are produced by the ICRs of several imprinted clusters

Transcriptional interference, recruitment of chromatin marks, altered chromosome structure…or simple bystanders?

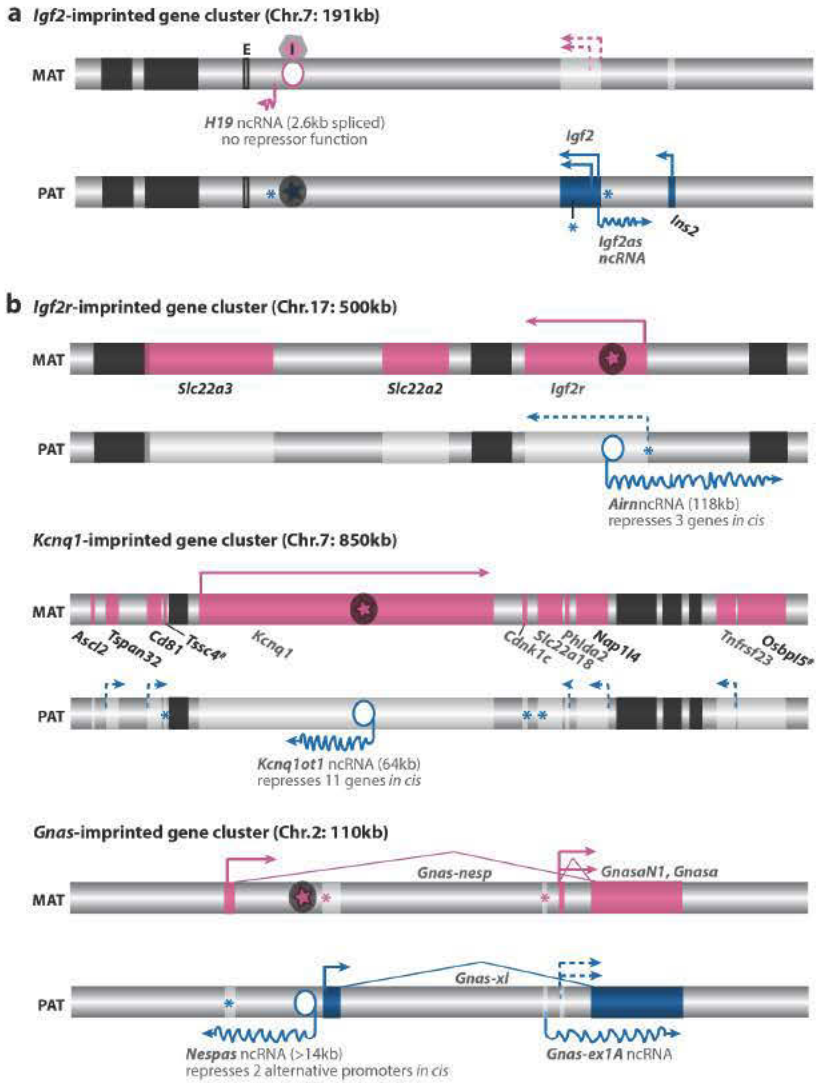
Four of these ncRNAs are linked to ICRs and have been tested for a functional role in imprinting

H19 - Igf2

Igf2R

Kcnq1

Gnas



D. Barlow
Ann. Rev Genetics 2011

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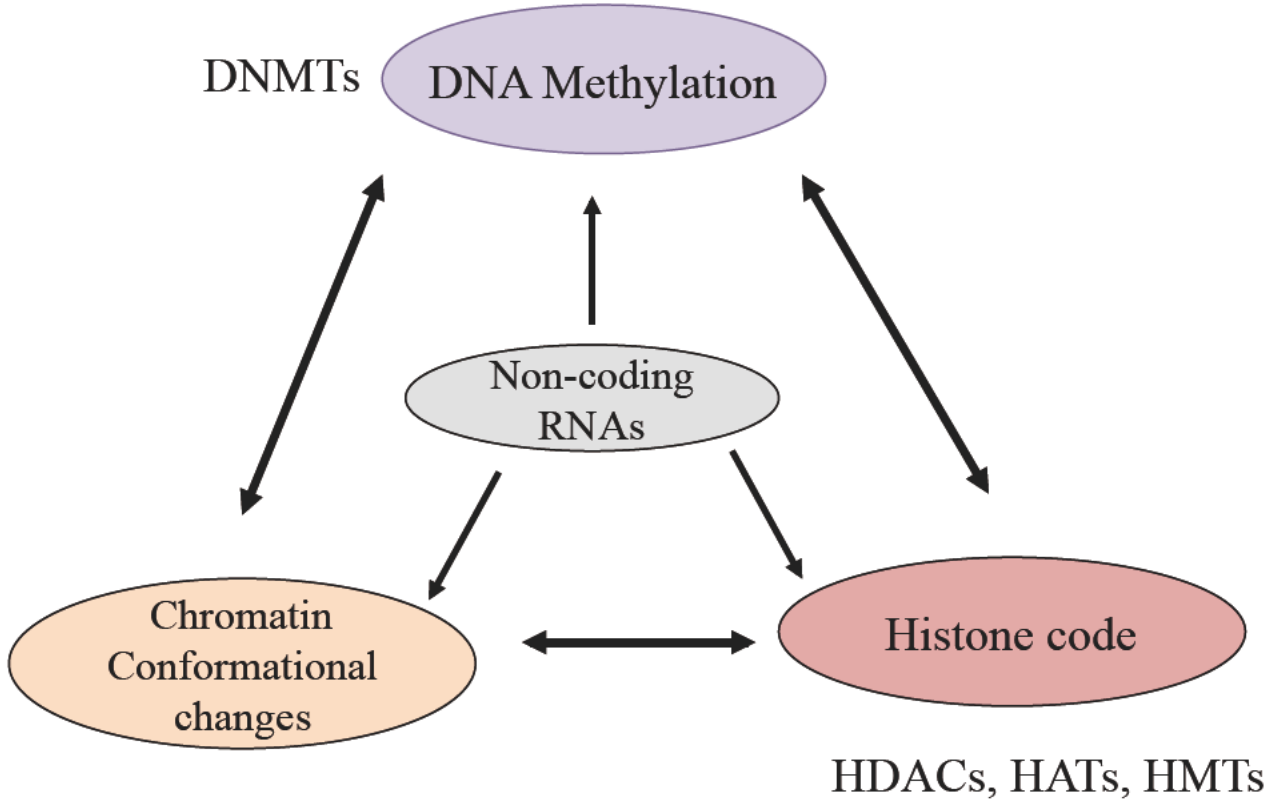
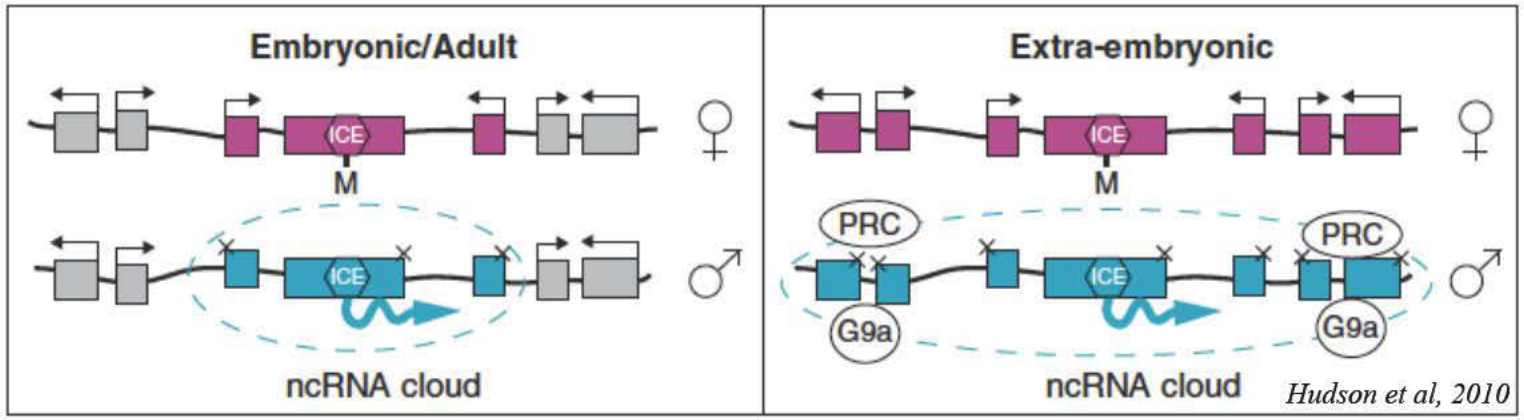
Imprinting Mechanisms

• DNA methylation is the instructive mark for germ line imprinting at *most* loci.
• Non-coding RNAs and other marks also play a role in establishing and maintaining imprinted gene expression at various embryonic stages and in different lineages.



Genes showing imprinted expression only in extra-embryonic tissues may be regulated by different epigenetic mechanisms compared with genes showing imprinted expression in extraembryonic tissues and in embryonic/adult tissues.

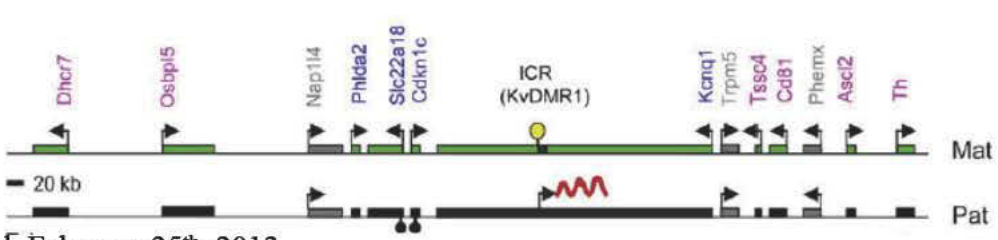
Imprinting Mechanisms

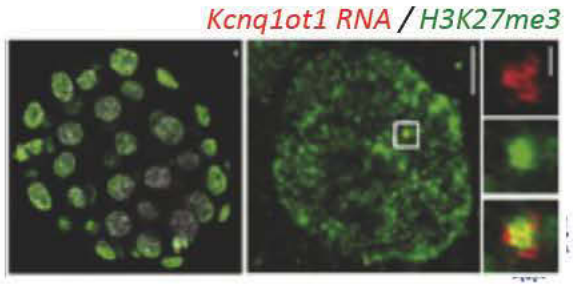


Histone modifications (H3K27me3, H3K9me2, H21K119u1) deposited by Polycomb complexes (PRC) and G9a HMTase, are involved in maintaining imprinted gene repression independently of DNA methylation at the *Kcnq1* and *Igf2r* clusters in extraembryonic tissues.

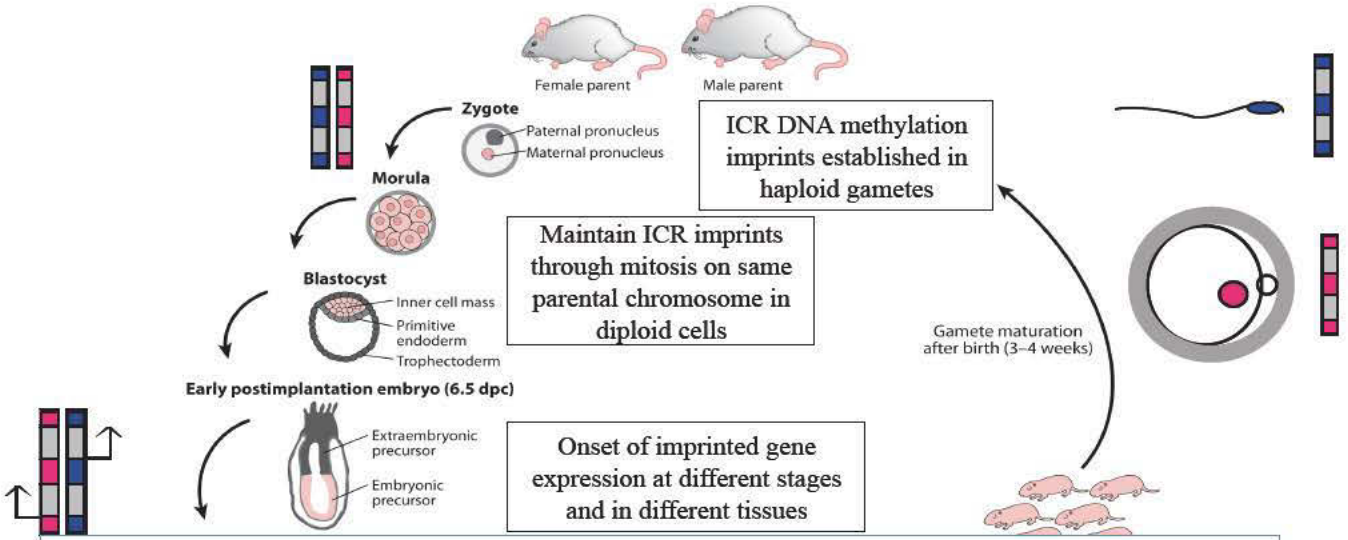
Non-coding RNAs (Kcnq1ot1, Airn) are implicated in recruiting these repressive complexes to extraembryonic-specific imprinted genes. The ncRNA forms a cloud that is larger in extraembryonic tissues and often covers the genes that are silenced.

(Lewis et al, 2004; Umlauf et al, 2004; Terranova et al, 2008, Nagano et al, 2008)

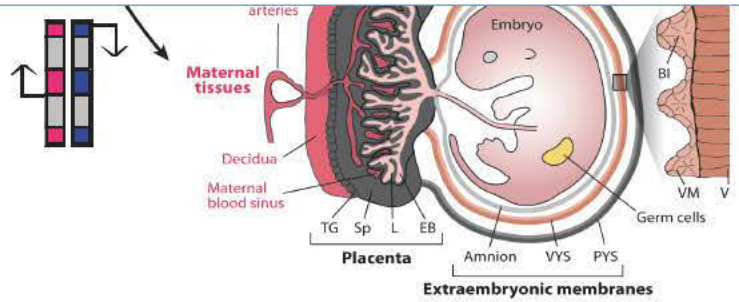




Developmental Dynamics of Imprinting



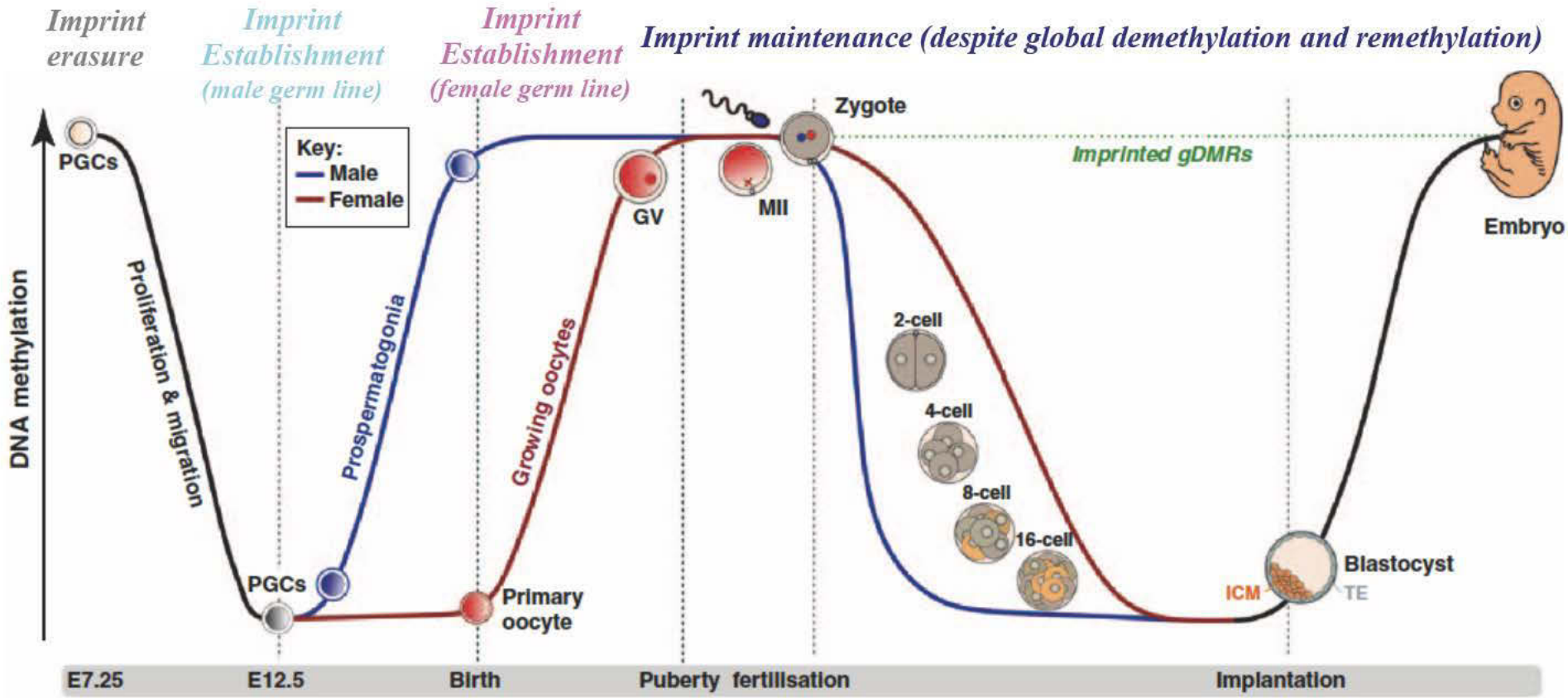
How are germ-line imprints established at ICRs?
How are they maintained in the face of global demethylation?
How do unmethylated ICRs resist somatic DNA methylation?
How do certain genes maintain imprinting in some tissues while others lose imprinting in a cell-type specific manner?



Barlow DP. 2011.
Annu. Rev. Genet. 45:379–403

Erasure of DNA methylation imprints in germ cells by 12.5 dpc

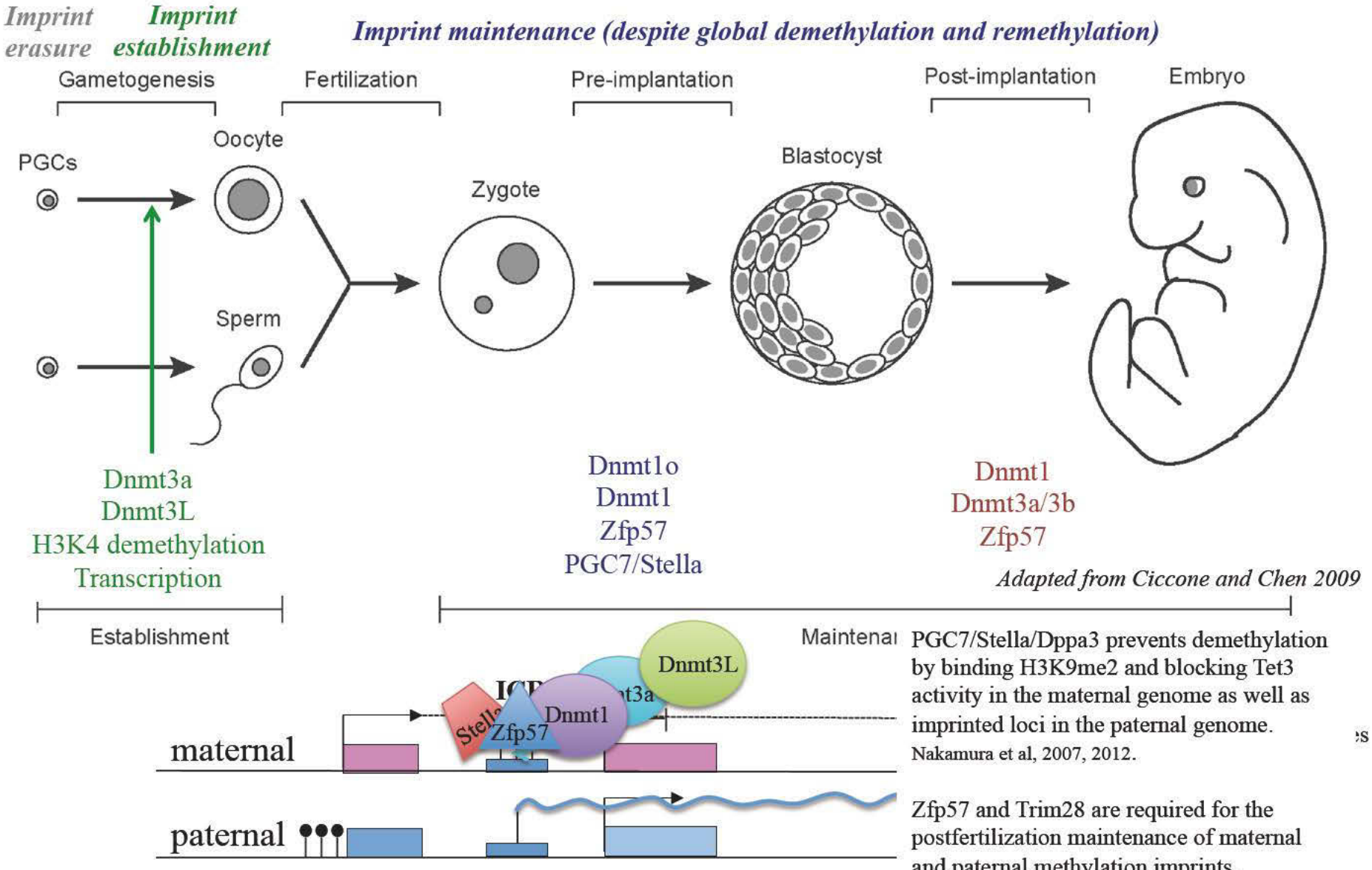
Developmental Dynamics of DNA Methylation



The mammalian *epigenome* shows dynamic changes in DNA methylation during development. How do imprints "resist" this?

How do imprints avoid inappropriate loss of methylation on one allele, or inappropriate gain of methylation on the other?

Developmental Dynamics of Imprinting



PGC7/Stella/Dppa3 prevents demethylation by binding H3K9me2 and blocking Tet3 activity in the maternal genome as well as imprinted loci in the paternal genome.
Nakamura et al, 2007, 2012.

Zfp57 and Trim28 are required for the postfertilization maintenance of maternal and paternal methylation imprints.
Li et al, 2008; Messerschmidt et al, 2012.

Imprint Control Regions

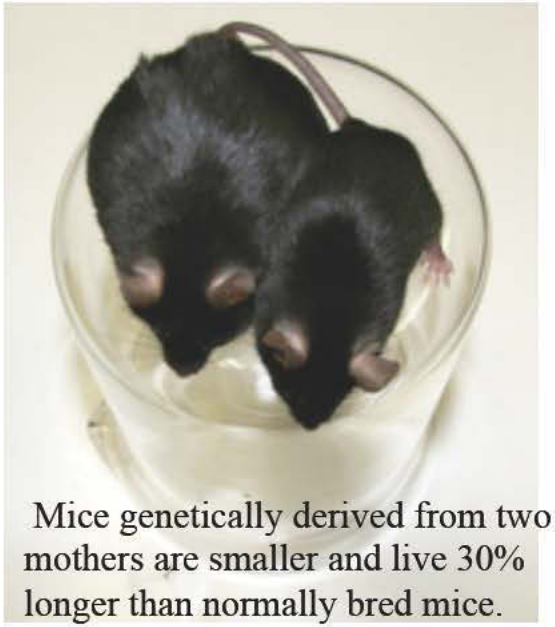
Most Germ-line DMRs are Maternal (ie established in the oocyte)
Maternal DMRs are usually in promoters or introns of genes (protein-coding or ncRNAs)
Paternal DMRs are usually intragenic

Table 1. *Locations of germline DMRs in imprinted loci*

Locus (DMR)	Chromosome	DMR methylation	DMR location	Reference
Gnas (Nespas/Gnasxl)	2	Maternal	Intron	Coombes et al. 2003
Gnas (1A)	2	Maternal	Intron	Liu et al. 2000
Mcts2	2	Maternal	Intron	Wood et al. 2007
Peg10	6	Maternal	Promoter	Ono et al. 2003
Peg1	6	Maternal	Intron[a]	Lucifero et al. 2002
Nap1l5	6	Maternal	Intron	Wood et al. 2007
Peg3	7	Maternal	Intron	Kim et al. 2003
Snrpn	7	Maternal	Intron	Mapendano et al. 2006
Kcnq1 (KvDMR)	7	Maternal	Intron	Yatsuki et al. 2002
Inpp5f	7	Maternal	Intron	Wood et al. 2007
Zac1	10	Maternal	Intron	This study
Grb10	11	Maternal	Intron	Arnaud et al. 2003
U2af1-rs1	11	Maternal	Intron	Wood et al. 2007
Peg13	15	Maternal	Intron	Ruf et al. 2007
Slc38a4				
Igf2r (Air)				
Impact	18	Maternal	Intron	Okamura et al. 2000
H19	7	Paternal	Intergenic	Tremblay et al. 1997
Rasgrf1	9	Paternal	Intergenic	Shibata et al. 1998
Dlk1-Gtl2 (IG-DMR)	12	Paternal	Intergenic	Takada et al. 2002

Deletion of two paternal DMRs (*H19* and *Dlk1-Gtl2*) is sufficient to allow viable parthenogenesis!

Imprinting is the only barrier to successful parthenogenetic development in mice



Mice genetically derived from two mothers are smaller and live 30% longer than normally bred mice.

Bi-maternal embryos produced by construction of oocytes from fully grown oocytes and nongrowing oocytes that contain double deletions in the H19 differentially methylated region (DMR) and the Dlk1-Dio3 intergenic germline-derived DMR, efficiently produce viable female mice that can reproduce normally!

⇒ Conclusive evidence that imprinted genes regulated by these two paternally methylated imprinting-control regions are the only paternal barrier that prevents the normal development of bi-maternal mouse fetuses to term.

Kono et al (2004) Birth of parthenogenetic mice that can develop to adulthood. Nature. 428:860-864.
Kawahara et al (2007) High-frequency generation of viable mice from engineered bi-maternal embryos. Nat. Biotechnol. 25, 1045-50.

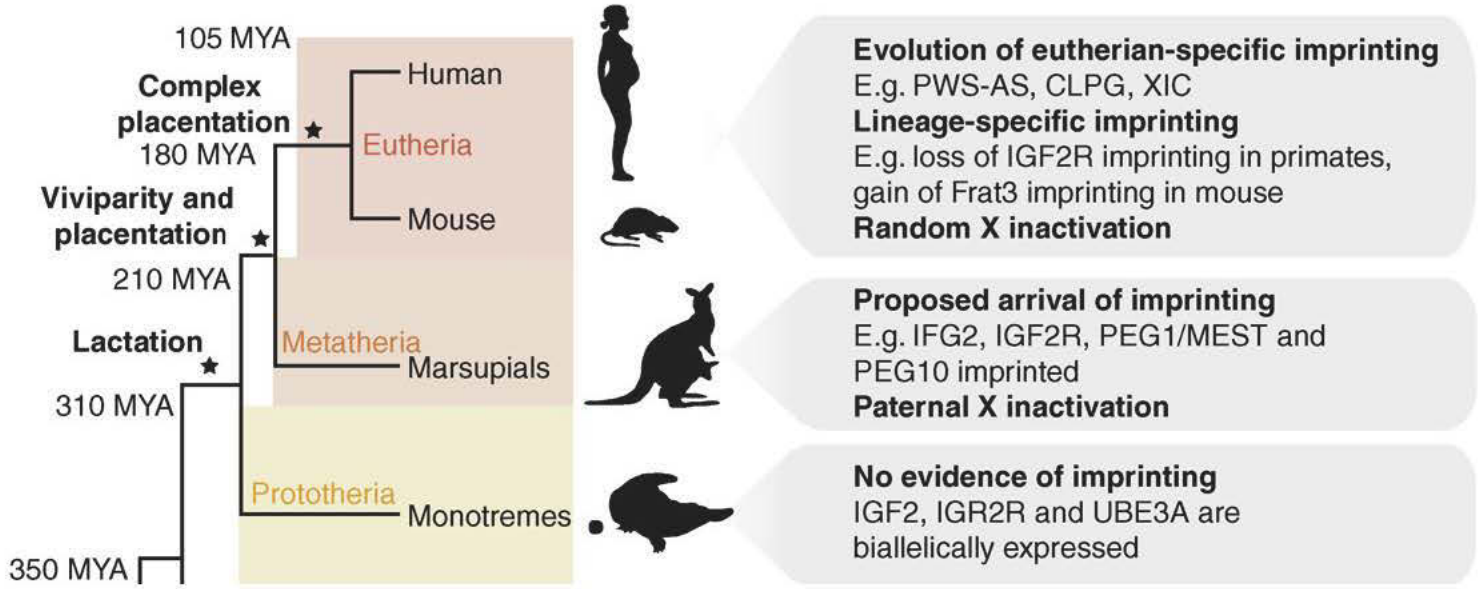
Why have Imprinting?

Numerous examples of parthenogenetic amphibians, fish, reptiles and birds exist.

Thus within vertebrates,
genomic imprinting seems to be mammal-specific.

How did genomic imprinting arise in mammals,
and *why* was it selected for?
(given the apparent cost: abandoning the advantages of diploidy!)

WHEN did Imprinting Evolve in Mammals?



Did genomic imprinting evolve just once - with viviparity?
Unlikely – instead imprinting evolution seems to have been a **stepwise, adaptive process, with each gene/cluster independently becoming imprinted as the need arose:**

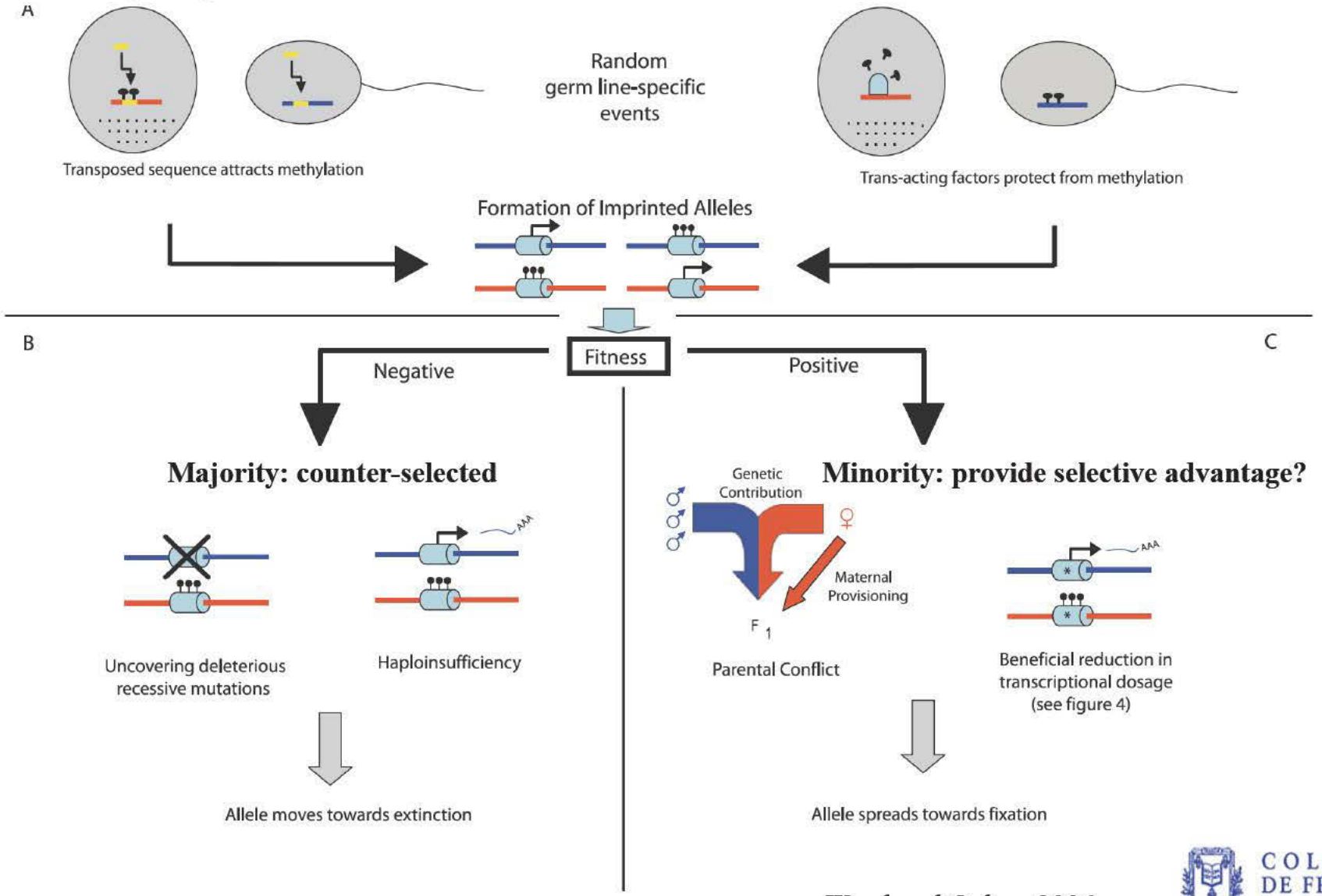
- H19-IGF2 is the most "ancient" imprinted locus in therians.

Smits et al (2008) Nat Genet. 40, 971-976.

- Other imprinted loci are eutherian-specific, or even rodent specific…

HOW did Imprinting Evolve in Mammals?

Barlow DP. (1993) Methylation and imprinting: from host defense to gene regulation? *Science* 260:309–10

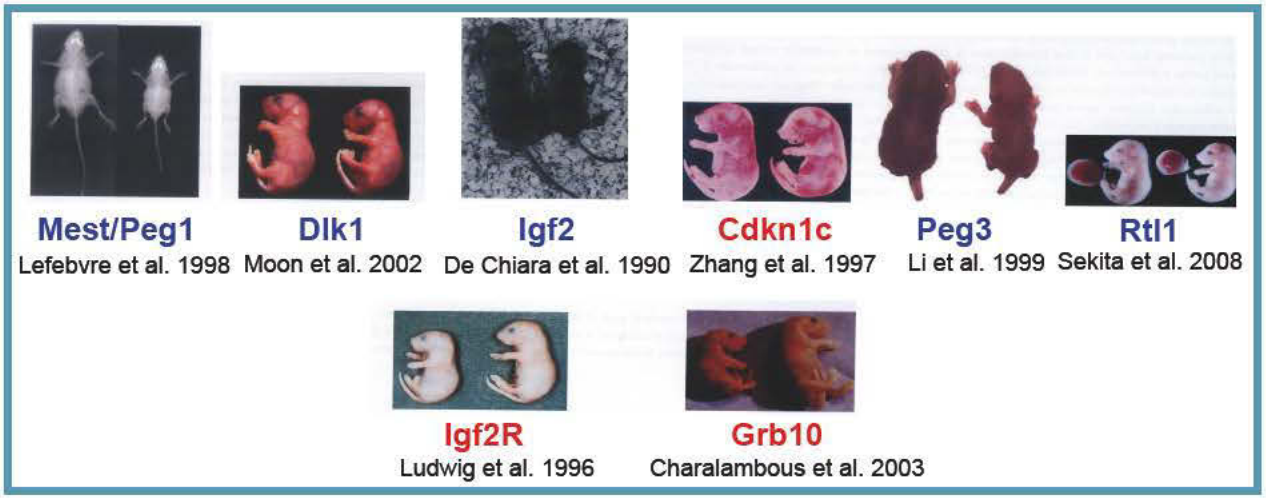


Wood and Oakey, 2006

WHY did Imprinting Evolve in Mammals?

Imprinted Genes Control:

- Embryonic Growth
- Placenta development
- Transfer of nutrients
- Animal behaviour



Parental conflict theory?

Parental conflict theory

Males and females do not have the same interests when they reproduce.

In polyandrous species (where females mate with more than one male), the offspring in a litter do not always have the same father….

The mother's interest is to have multiple equal (smaller) offspring. A father's interest is to have big offspring that can outcompete other males' offspring in the womb.

Genomic imprinting may have evolved from such a inter-parental tug-of-war over the resources allocated to the fetus by the mother during intrauterine gestation in viviparous mammals:

- Paternal epigenotypes drive expression of pro-growth genes
- Maternal epigenotypes suppress growth

The potential for conflicts between polygamous viviparous mammals is highlighted by the killing of lion young by non-paternal males

Moore, T. & Haig, D. (1991) Genomic imprinting in mammalian development: a parental tug-of-war. *Trends Genet.* 7, 45–49.

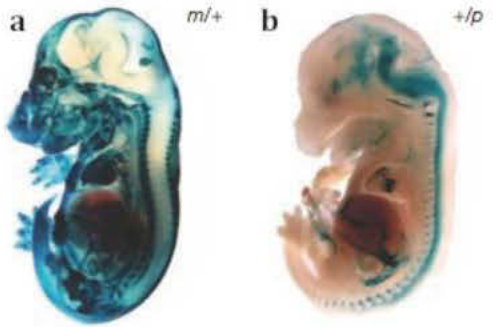
Imprinting and Behaviour

Postnatal behaviour may be influenced by imprinted genes

• Large number of neurological and psychiatric disorders with parent-of-origin effects: Eg PWS and AS (classical imprinting diseases), as well as in autism, bipolar affective disorder, epilepsy, schizophrenia, Tourette syndrome and Turner syndrome…

• Mice that have paternal disomy for chromosome 2 are hyperkinetic while those with maternal disomy are hypokinetic

• Imprinted *Grb10* gene is important in mouse foetal and brain development and influences adult social behaviour



Opposite imprinting of parental alleles in different locations of the body.

a. maternal *Grb10* is expressed in most regions of the body.
b. paternal *Grb10* is expressed only in the brain and central nervous system

Garfield et al. (2011). Distinct physiological and behavioural functions for parental alleles of imprinted Grb10.*Nature* 469, 534.

Imprinting and Behaviour

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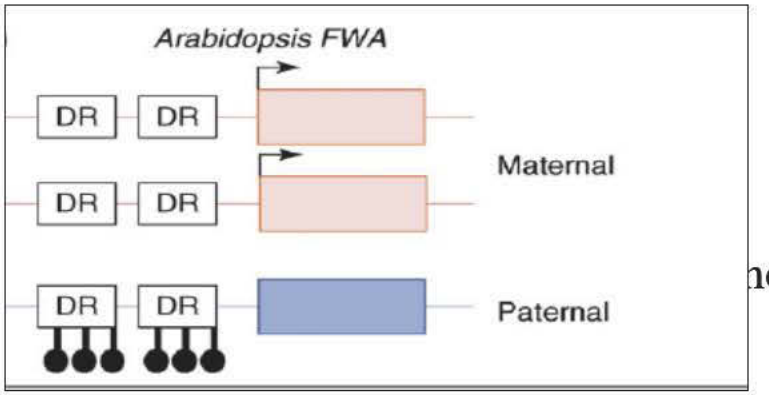
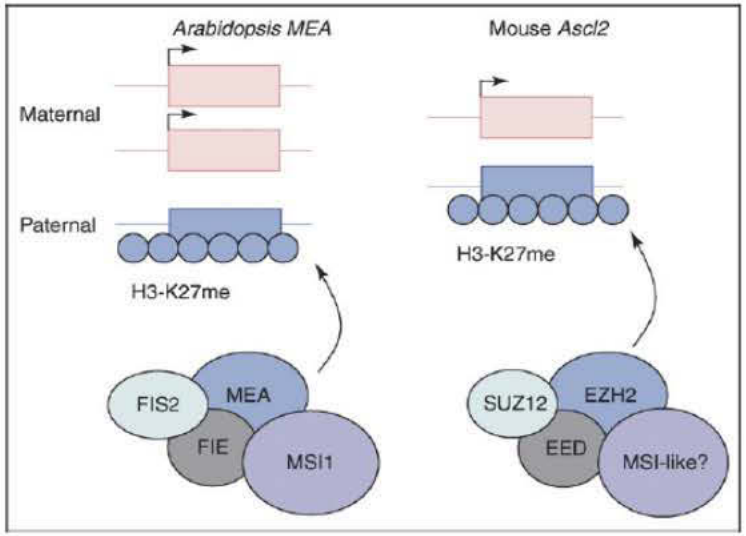
• Knockouts of *Grf1* and *Ube3a* (the mouse homologue of the human AS gene) have defects in contextual learning and memory (among others)

• *Peg1* and *Peg3* have a role in maternal behaviour such that mothers that lack these molecules neglect and do not feed their offspring

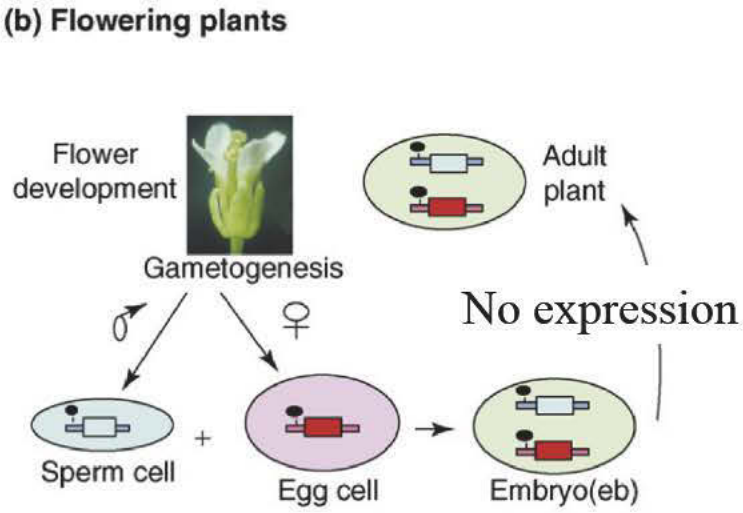
Genomic Imprinting in Plants

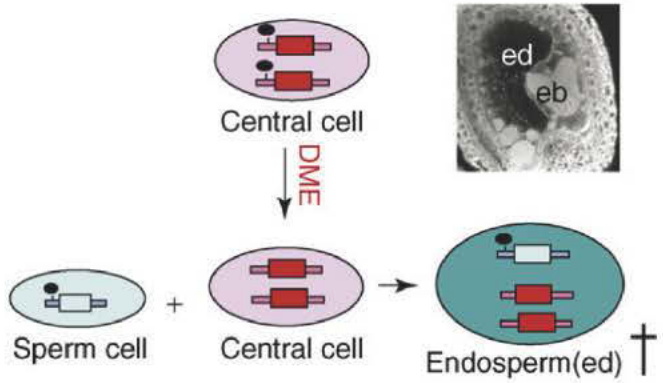
Imprinting evolved independently in flowering plants





DNA Methylation and sometimes Polycomb group proteins ensure silencing of the paternal alleles of imprinted genes.





Imprinted expression

E.

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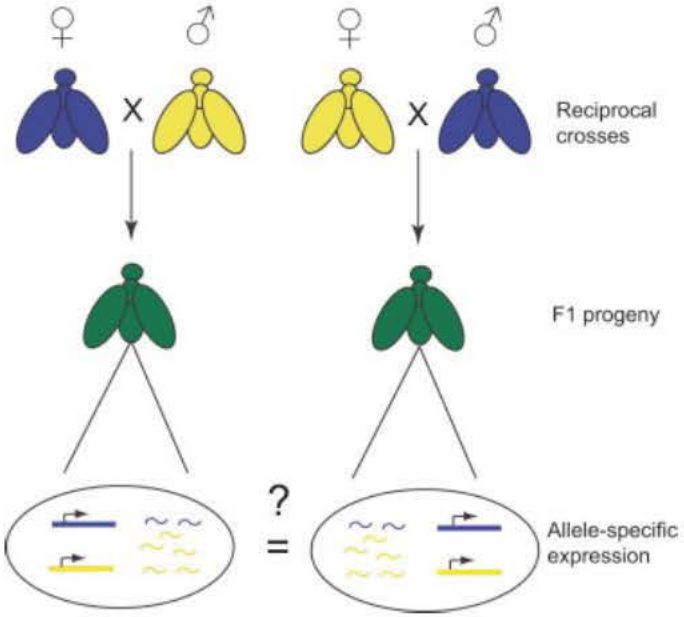
Genomic Imprinting in Drosophila?

Gynogenetic and androgenetic D. melanogaster are viable, ⇒**imprinting is not essential** in this species?

(Fuyama, 1984; Komma and Endow, 1995).

D. melanogaster can form parent-of-origin-specific imprints that affect gene activity, however, the prevalence of imprinted genes in their native genomic context within the D. melanogaster genome remains unclear (Menon and Meller, 2010).



RNA-seq to compare allele-specific expression between pools of 7- to 10-day-old adult female progeny from reciprocal crosses.

► RNA-seq identified 119 potentially imprinted genes in D. melanogaster
► These potentially imprinted genes were significantly clustered in the genome
► Polymorphisms and intrinsic noise caused imprinting-like RNA-seq data
► **Conclusion: D. melanogaster genes in their native context are not imprinted**

Coolon et al (2012) Genomic Imprinting Absent in *Drosophila melanogaster* Adult Females. *Cell Reports* 2 69-75.

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Genomic Imprinting: outstanding questions

- How and when during germline development are old imprints removed and new ones introduced?
- What are the molecular mechanisms (DNA demethylating activities, Dnmts, transcription, DNA binding factors, chromatin factors, RNAi pathways)
- How does epigenetic information spread across imprinting clusters – when? where?
- How are imprints maintained when there is genome-wide active and passive demethylation in the early embryo?
- How is tissue-specific imprinting and cell-type specific "escape" from imprinting achieved?
- How do imprinted genes and imprinting control elements evolve?
- How exactly do imprinted genes affect extraembryonic and embryonic development, and the nutritional exchange with the mother?
- In addition to growth and behaviour, are there other developmental processes and mechanisms in which imprinted genes play a role?