Mammalian Genomic Imprinting and Cancer

"Mom's and Dad's genes are NOT created equal"

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Oncology for Scientists I

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Objectives

- Define Epigenetics & Genomic Imprinting
- Discovery
- Wha is the “imprint”
- Lifecycle of an Imprint
  - DMRs and ICEs
- 2 main mechanisms of imprinting
  - Insulator and long non-coding RNA
- Genomic Imprinting and Cancert

Epigenetics

- The study of mitotically or meiotically heritable changes in gene expression or cellular phenotype, caused by mechanisms other than changes in the underlying DNA sequence

Genomic Imprinting

Definition…

- "An epigenetic process leading to parental-specific expression of one to two percent of mammalian genes" – Barlow, 2011

- "An epigenetic process that can involve DNA methylation and histone modulation in order to achieve monoallelic gene expression without altering the genetic sequence" – Wikipedia

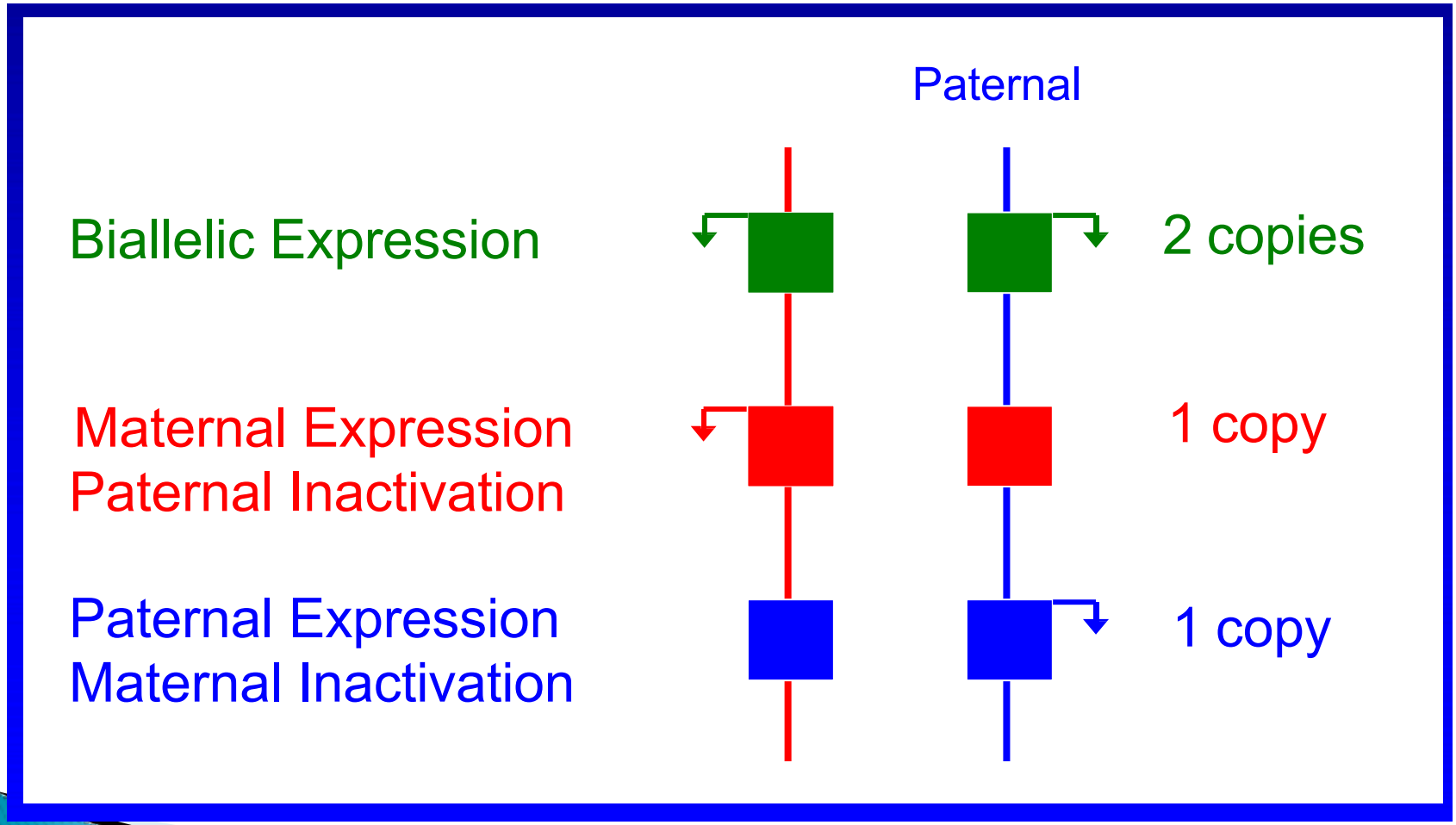
- "Parental-specific gene expression in diploid cells." – Barlow and Bartolomei 2014

Important Terms:

- Haploid: contains only one set of chromosomes
- Diploid : contains 2 sets of chromosomes

- Biallelic expression: a gene is expressed from both the maternal and paternal alleles. You have 2 copies of the gene
  - Most genes
- Monoallelic expression: A gene is expressed from only one of the parental alleles. You have only one copy of the gene
  - Imprinted genes
- Differentially Methylated Region (DMR): Methylated on one parental allele and not the other.

Imprinting Defies Typical Mendelian Genetics



Imprinted Genes:

- Present in Placental mammals
- Absent in Egg laying mammals

- Involved in growth regulation and development
  - MAT expressed genes: Growth suppressors (TSGs?)
  - PAT expressed genes: Growth promoters (Oncogenes?)
- Imprint status varies between tissues, developmental stages and species

Why Imprinting?

- Hypothesis 1: response to "parental conflict"
  - Embryonic growth is dependent on mother but influenced by both mother and father
    - PAT growth promoters – maximize fitness of embryos with their own genes (embryos may have different fathers)
    - MAT growth suppressors – equal distribution of maternal resources
  - **Thus, predict paternally expressed genes would promote growth, maternally expressed genes should slow it down**
  - **Prediction mostly hold true**
    - Example- Igf2 (paternally expressed)-if defective=40% reduction in growth

Parent Offspring Conflict Hypothesis (Haig hypothesis)

Example- The Igf2 gene and its receptor Igf2r

- Igf2 (paternally expressed)-if defective=40% reduction in growth
- Igf2r (Igf2 receptor)- if defective=increase growth
- $Igf2^{-}/Igf2r^{-}$ = normal

Another test- Ask if imprinting fails to occur in a monogomous species

The Beach mouse is entirely monogomous

….but imprinting *still* occurs, contrary to model

Why Imprinting?

- Hypothesis 2: “Trophoblast defense”
  - Protects mother from spontaneous oocyte activation – imprinting silences genes on maternal chr. that promote placental development

Important Point:

- Genomic Imprinting affects BOTH male and female offspring and is therefore a consequence of parental inheritance, NOT OF SEX.
  - An imprinted gene that is active on the maternally inherited chromosome will be active on that chromosome and silent on the paternal chromosome in males AND females

"Mom's and Dad's genes are NOT created equal"

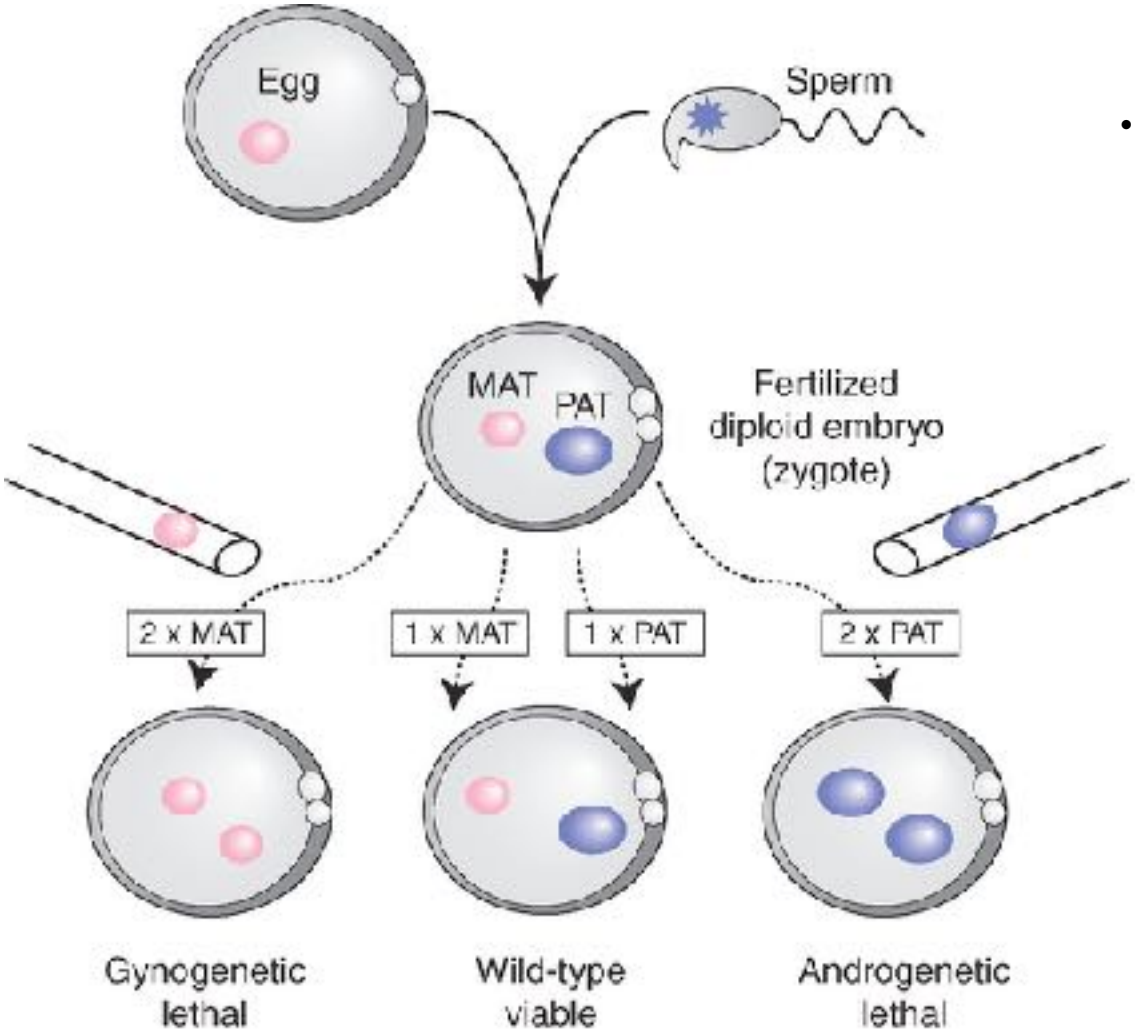
Discovery of Genomically Imprinted Genes

"Hairpin-Tail" Mouse

- Large deletion of chromosome 17
  - Initially used to prove that organisms heterozygous at a given locus are phenotypically identical irrespective of which gamete contributes which allele to the genotype (Johnson 1974)
- Deletion from MAT →→larger size and midgestation lethality
- Deletion from PAT→→ viable and fertile mice
  - Must be a difference between the Maternal and Paternal chromosome

Discovery… Nuclear Transfer Experiments (1980's Solter and Surani)

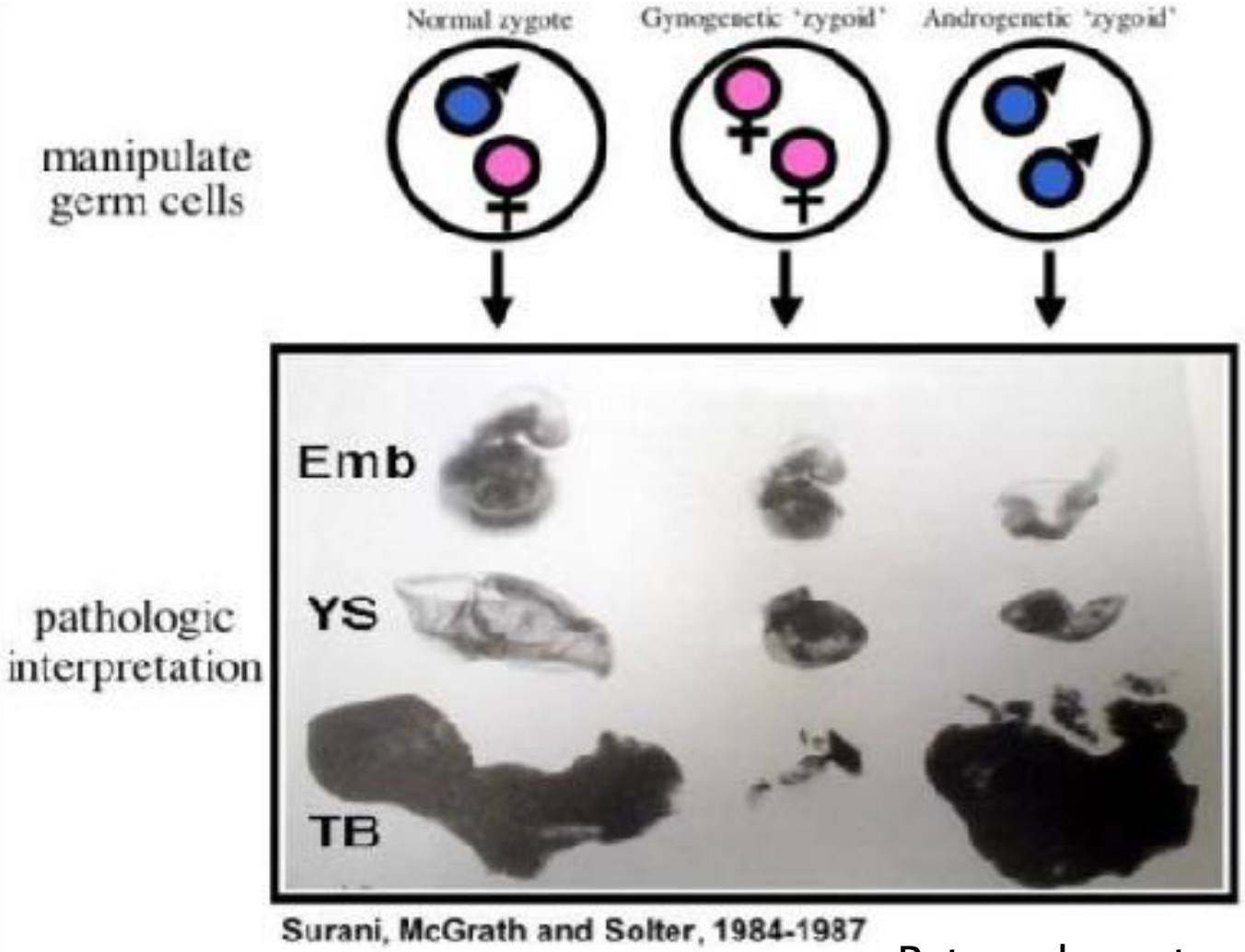
A maternal and paternal genome are needed for mammalian reproduction.



- 1st used to show that nuclei from fertilized hairpin-tail mutants could not be rescued when transferred into a wild type host egg.
  - Proof that embryonic genome, not oocye cytoplasm carried the hairpin tail defect

Barlow D P , and Bartolomei M S Cold Spring Harb Perspect Biol 2014;6:a018382

The two parental genomes provide reciprocal functions

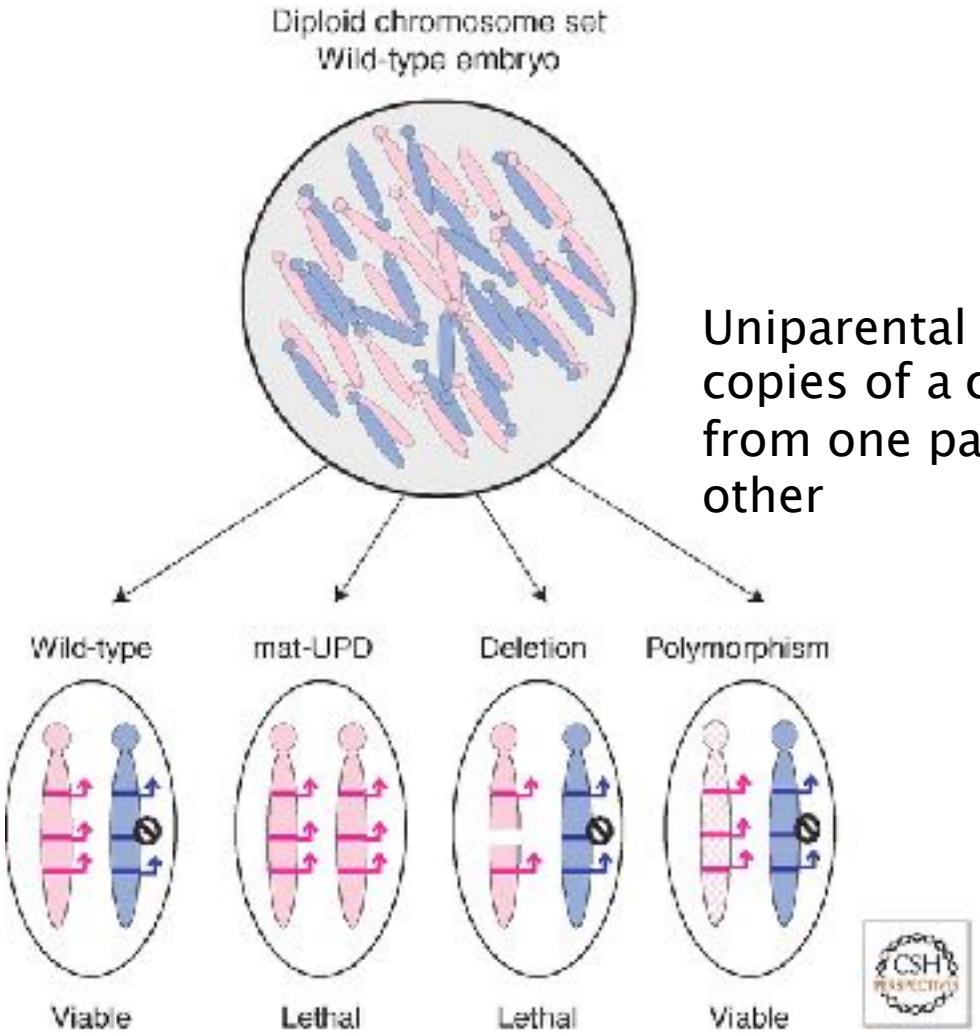


Surani, McGrath and Solter, 1984-1987

Paternal – extraembryonic

Maternal – embryonic

Mouse models to study genomic imprinting that allow the maternal and paternal chromosome to be distinguished.



Uniparental Disomy (UPD): 2 copies of a chromosome from one parent, none from other

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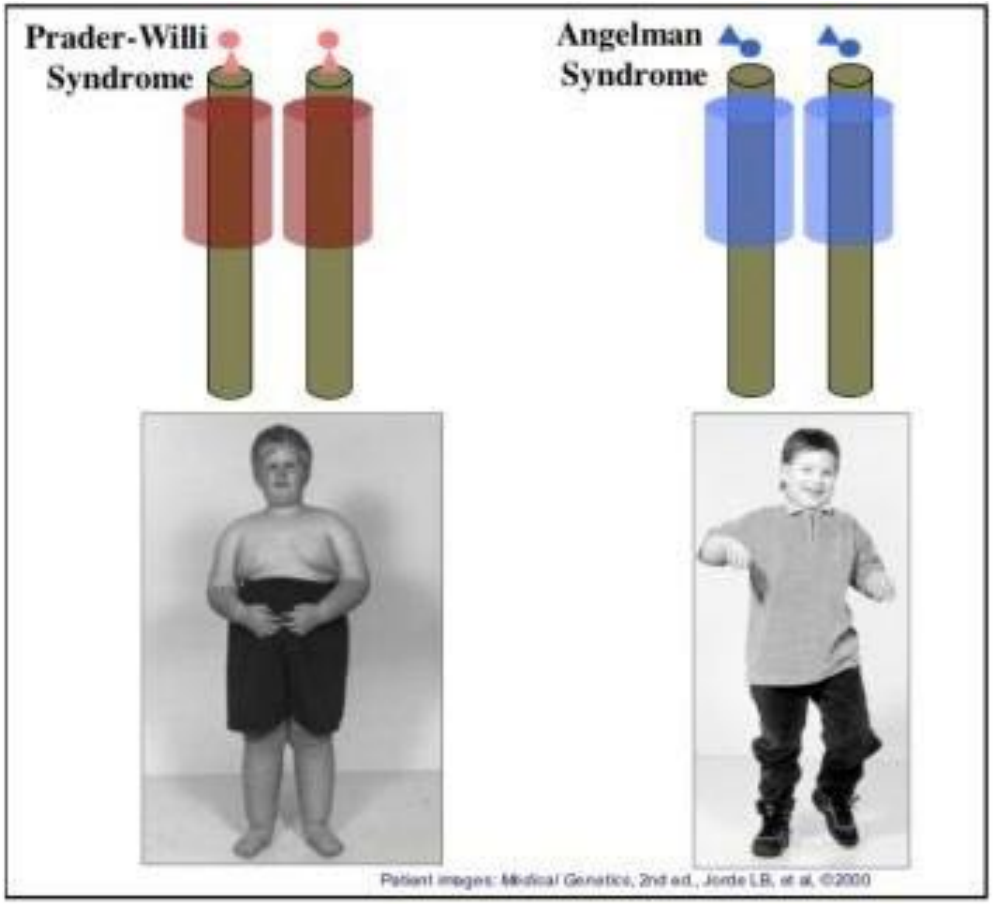
Prader-Willi (PWS) and Angelman Syndromes (AS)

- Disruption of identical genes on chr.15 leads to 2 different disorders depending on which parental chromosome

the disruption is inherited from

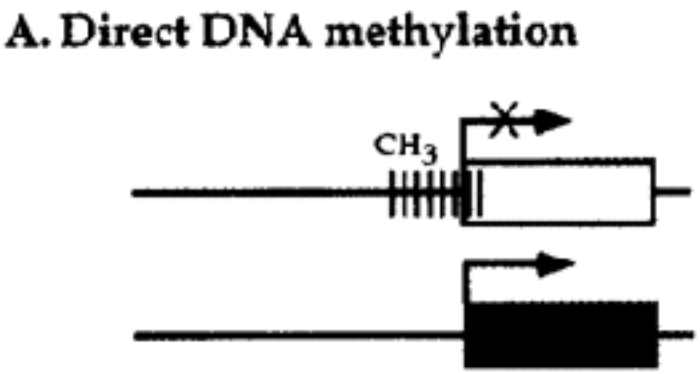
- PWS: genes lost on the PAT chromosome
- AS: genes lost on MAT chromosome



How are imprinted genes silenced?

- DNA methylation
  - differentially methylated regions (DMRs)
- Imprinting centers or imprinting control elements (ICs/ICEs)

How are imprinted genes silenced?



Dnmt-/- mice-

Many imprinted genes (e.g. H19) reactivated

..but, Igf2 and Igf2r are silenced

Mechanism- Methylation interferes with transcription factor binding

Problems with model-

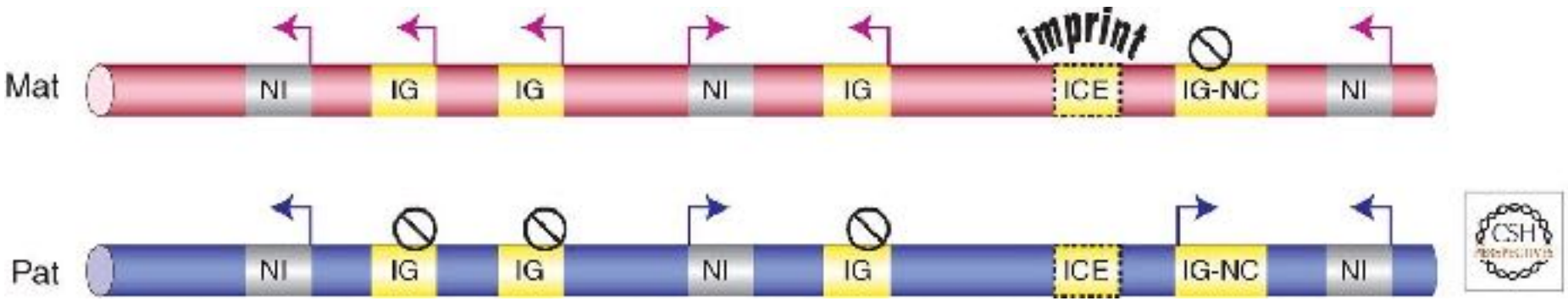
1. Promoters of silent Igf2 and Igf2r alleles are unmethylated
2. One gene, Mash2, is unaffected by loss of methylation

S. Tilghman, Cell 96:185

Imprint Control Element(ICE)

- Gametic Differentially Methylated regions (gDMR)
- Control expression of one or more imprinted genes in a cluster
- Often associated with long non-coding RNA promoters
- Definative proof that something is an ICE: KO→→Loss of Imprinting (LOI) of one or more distant genes

Imprinted Genes are Often Clustered



- Multiple protein coding imprinted mRNAs
- At least one ncRNA
- Can also contain NON-imprinted genes

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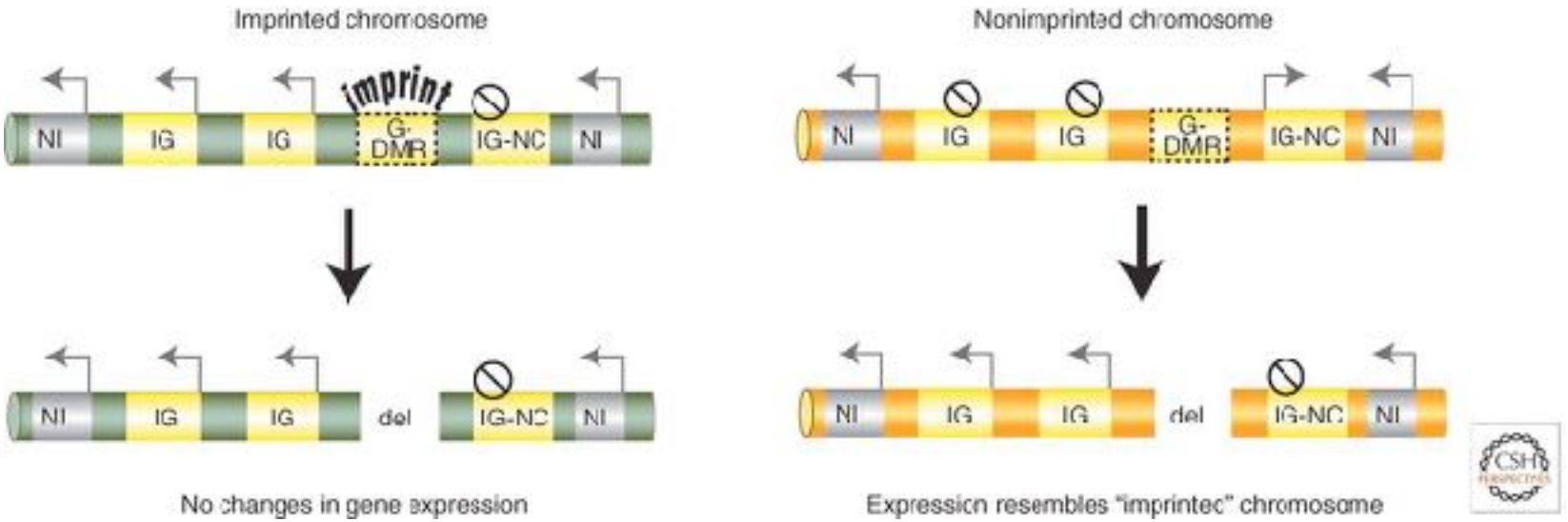
Imprinted expression is regulated by gDMR (ICE)



If you delete the DMR on the chromosome with the imprint→→ no change
If you delete the DMR on the chromosome without the imprint →→expression resembles imprinted chromosome

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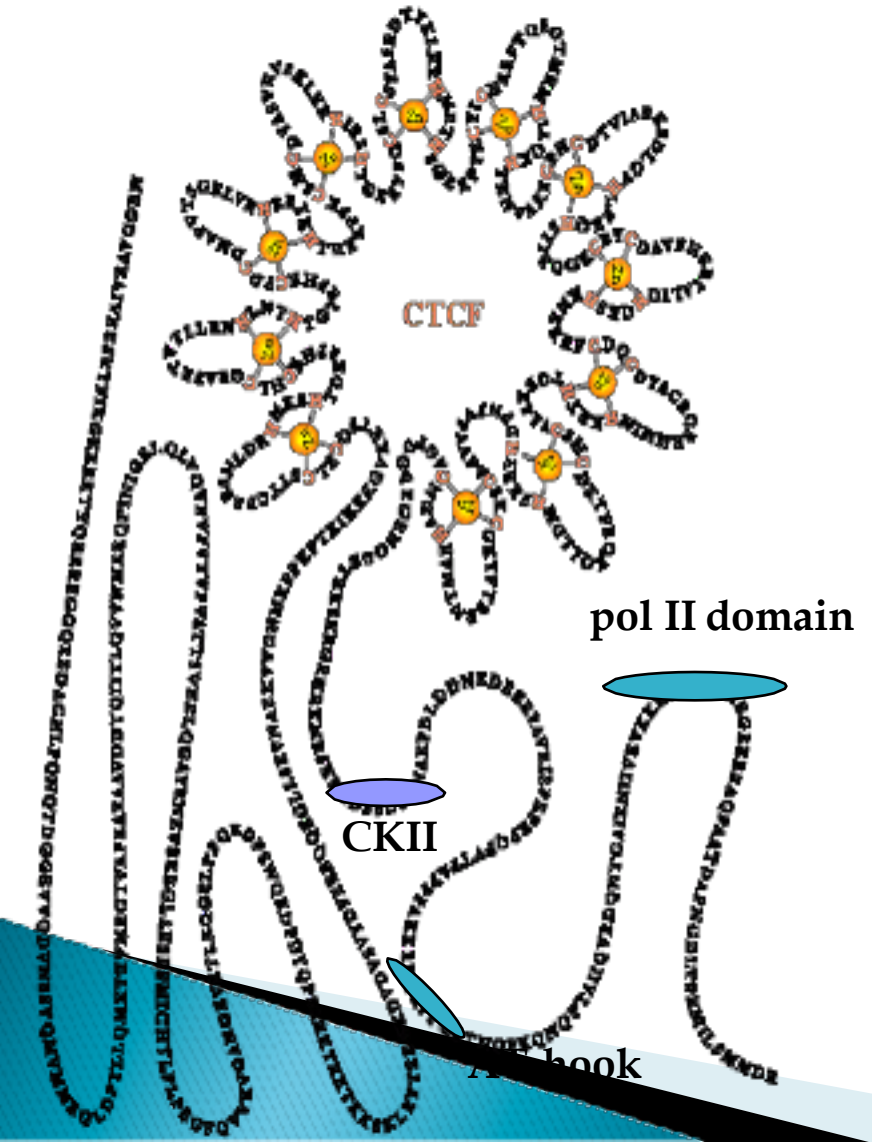
How do ICEs regulate imprinted genes?

Insulator (enhancer blocking) model

Non-coding RNA model

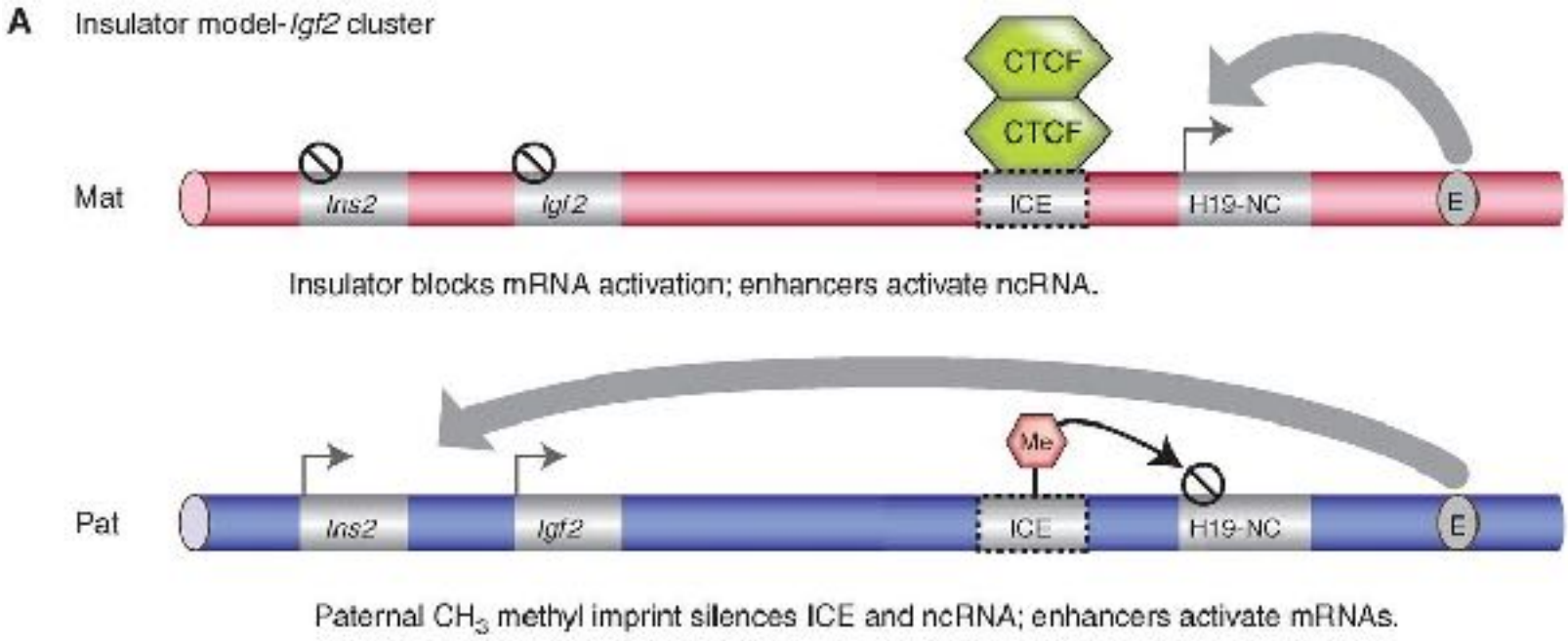
CTCF: CCCTC Binding Factor



- Highly conserved 11ZF nuclear phosphoprotein
- DNA targets recognized by combinatorial usage of 11 Zn fingers
- Protein interaction domains
- transcriptional activation, repression, silencing and chromatin insulation

Ohlsson, Renkawitz, Lobanenkov (2001) TIGs

Chromatin Boundary/ Insulator Model: Igf2 Cluster



The *H19* lncRNA is methylated, most likely because of spreading from the 2-kb distant methylated ICE, and silenced

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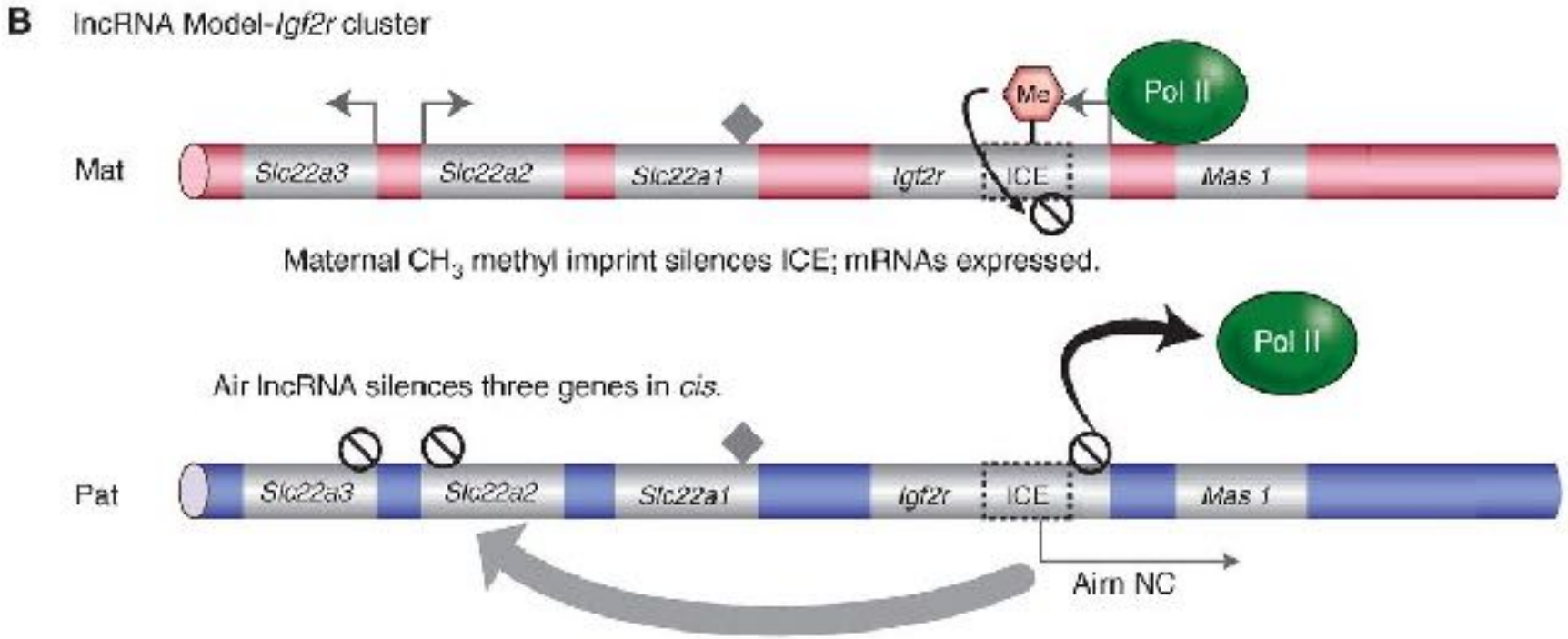
Lnc RNA Model
Long-non-coding RNA

lncRNA: Non-protein coding transcripts longer than 200 nucleotides

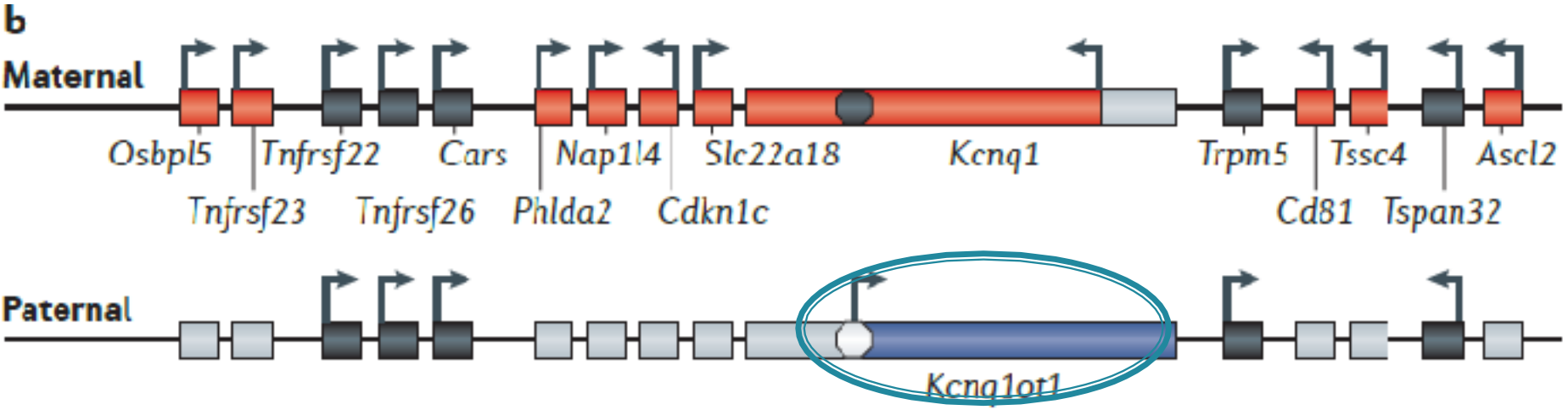
- Xist in X chromosome inactivation
- Airn at the Igf2r locus
- Kcnq1ot1 at the KvDMR1 locus

lncRNA model: Igf2r cluster



The Airn lncRNA promoter lying in the unmethylated ICE **is expressed** and **silences** *Igf2r* (in part by kicking off RNA polymerase II), *Slc22a2*, and *Slc22a3* in *cis*.

Kvdmr1 Cluster



- Kcnq1ot1 lncRNA expression from paternal allele silences genes in cis
- Methylation on maternal allele represses Kcnq1ot1 which allows expression of genes in cis

How do lncRNAs silence genes in cis?

- ncRNA may "recruit" repressive chromatin modifier complexes that suppress transcription (e.g. polycomb group [PcG] proteins)

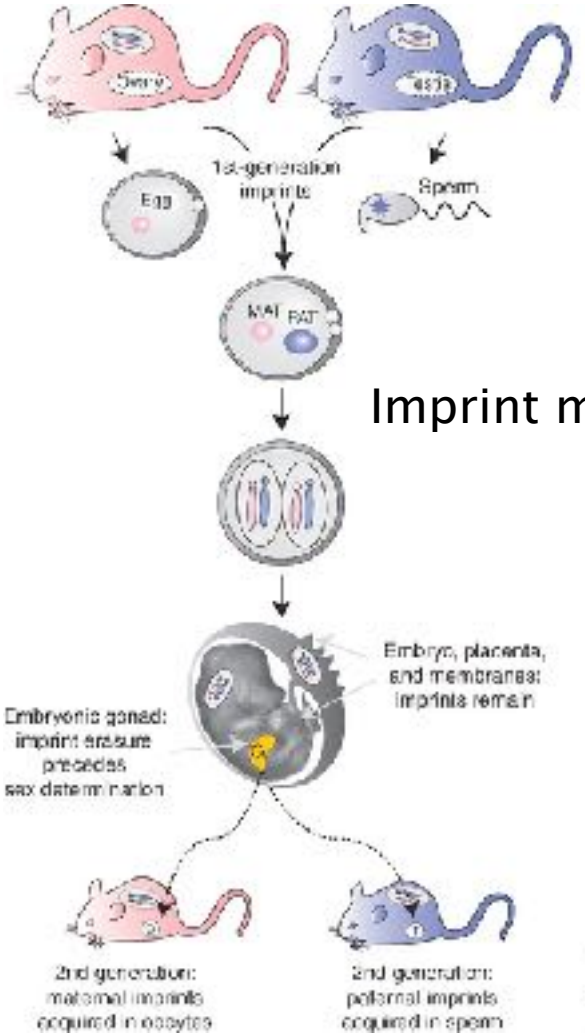
- ncRNA may "target" the chromosome domain to a nuclear compartment that is devoid of RNA polII and enriched in PcG proteins or may help set up the compartment and bring along the imprinted domain.

- the act of transcription (of the ncRNA) through the domain may affect the binding of a regulatory protein complex
  - Opens it up and repressors bind?
  - Knocks off activating proteins?

Lifecycle of an Imprint

Imprint acquisition and erasure in mammalian development.

Imprints are present in gametes



Imprint maintained after fertilization

Imprintes ERASED in gonad before sex determination

Imprints are acquired by gametes

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How can methylation machinery distiniguish maternal vs. paternal allele?

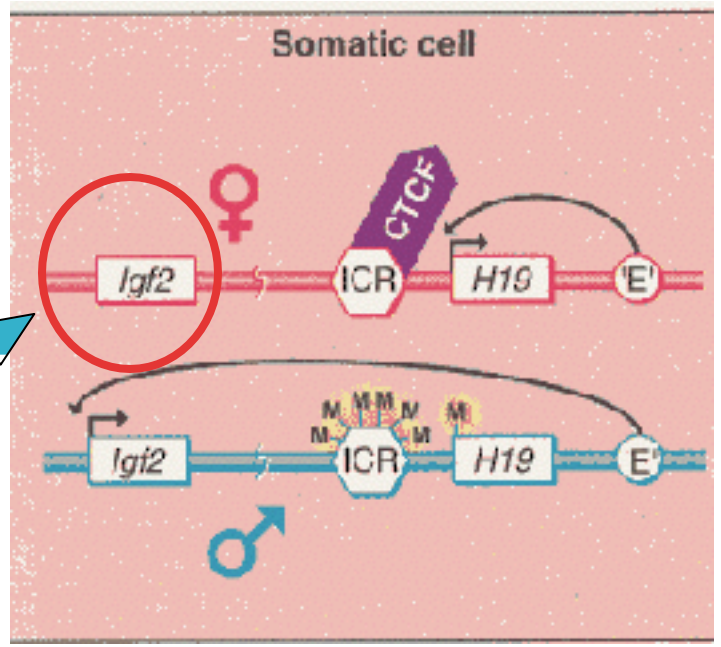
- The parental alleles are differentially marked by DNA methylation during gametogenesis when the genomes are in separate compartments.
  - Abramowitz & Bartolomei 2013:
    - "transcription, histone modifications and higher order chromatin are employed either individually or in combination to set up parental imprints"
  - Prontera & Donti :Front Genet. 2014; 5: 294. –
    - « minimal variations of temperature can induce different allele-specific epigenetic modifications, and that the gonads are particularly sensitive to this process »

Genomic Imprinting and Cancer

Imprinting in the news

- Colon — Potential blood test to screen for colon cancer

Loss of Imprinting (LOI) of the Igf2 gene correlates with colon cancer



Result= Maternal Igf2 gene is turned on

- With family history- 5X more likely to show LOI
- Polyps detected- 3X more likely to show LOI
- Personal history- 22X more likely to show LOI

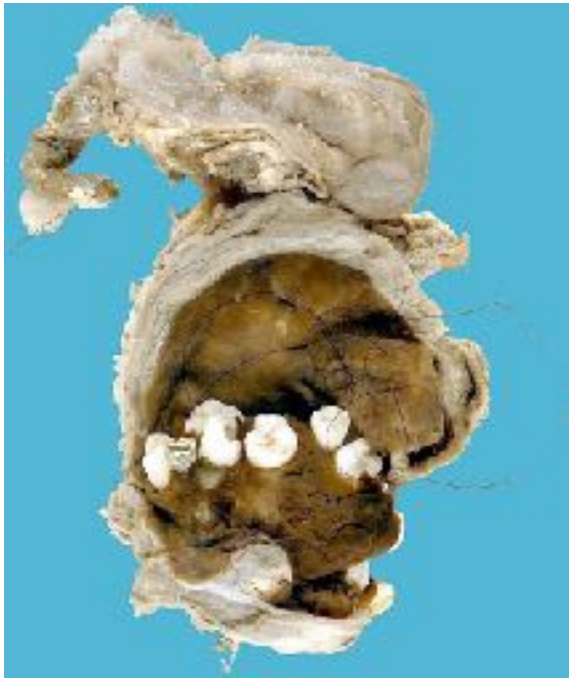
Source- AP News, March 14, 2

Genomic Imprinting Involvement in Cancer

- Hydatidiform moles (Molar Pregnancy): caused by sperm combining with an egg that has lost its DNA (sperm reduplicates →→46 chromosomes all of PATernal origin)

- Ovarian teratomas: germ cell tumors that arise from parthenogenetic (46 chromosomes all of maternal origin)

- These tumors indicate that an imbalance between maternal and paternal genomes causes neoplasia



Genomic Imprinting in Cancer

- Mice engineered to be “imprint-free” are tumor prone
- Normal imprinting of some genes is disrupted in tumors
- Beckwith-Wiedemann Syndrome(BWS): a cancer predisposition syndrome results from abnormal expression of imprinted genes on chromosome 11

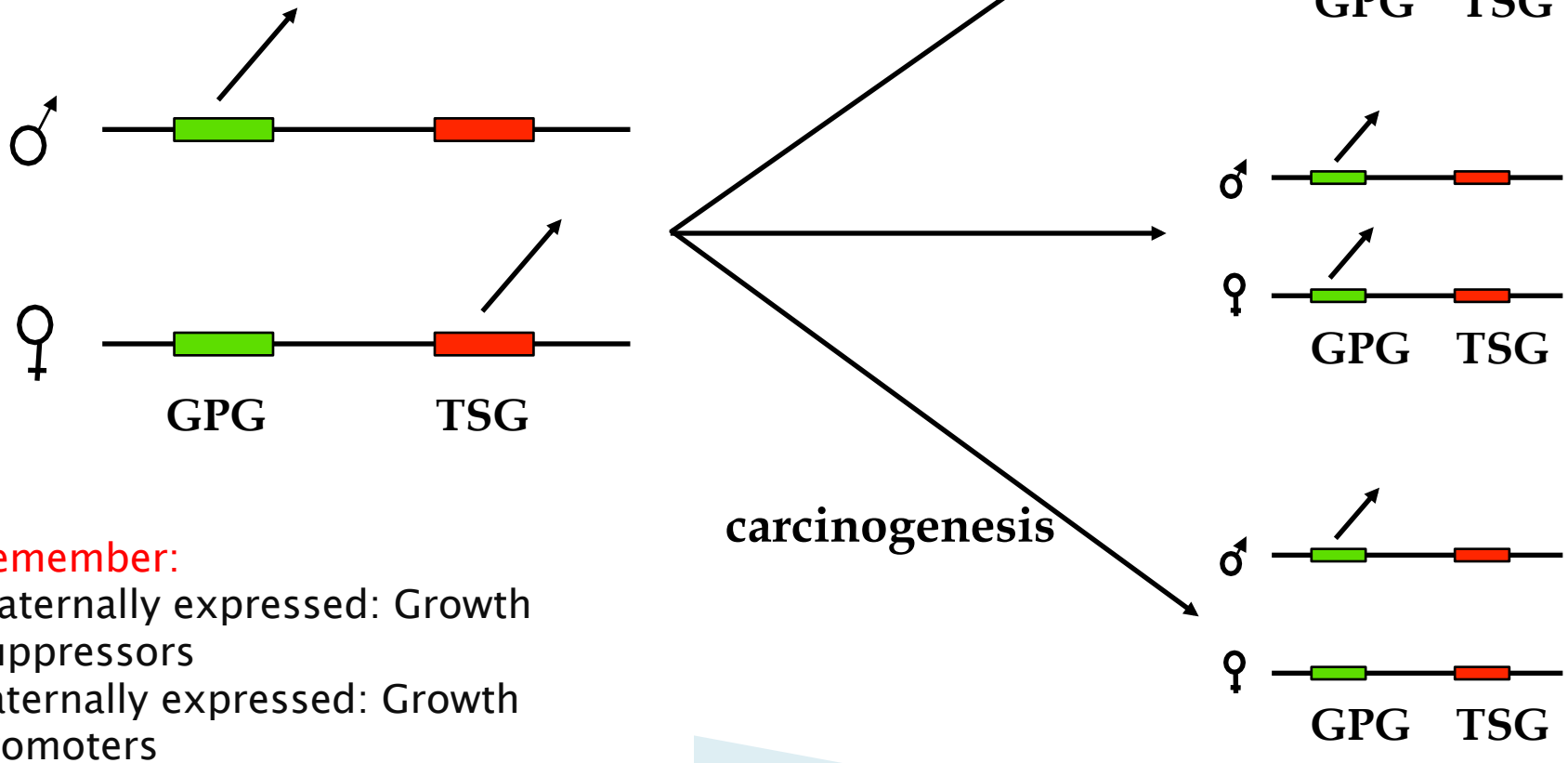
Loss of Imprinting (LOI) in Tumors

Loss of imprinting of Tumor Suppressor and Growth Promoting genes

Tumor Suppressor Gene

TSG

GPG Growth Promoting Gene



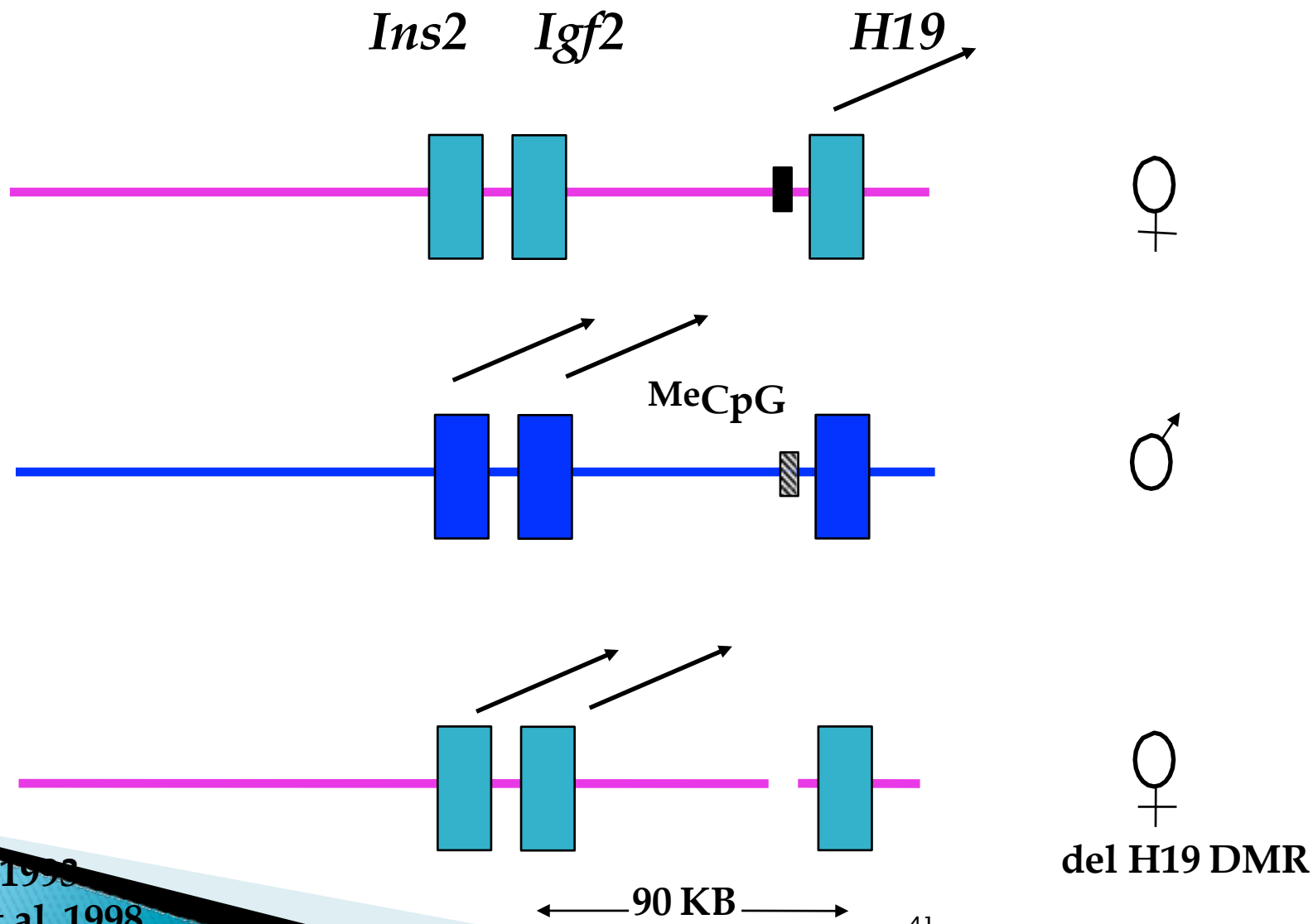
Remember:
Maternally expressed: Growth suppressors
Paternally expressed: Growth Promoters

IGF2

Potent fetal mitogen(growth stimulator) and survival (apoptosis antagonist) factor.
LOI leads to double dose of Igf2

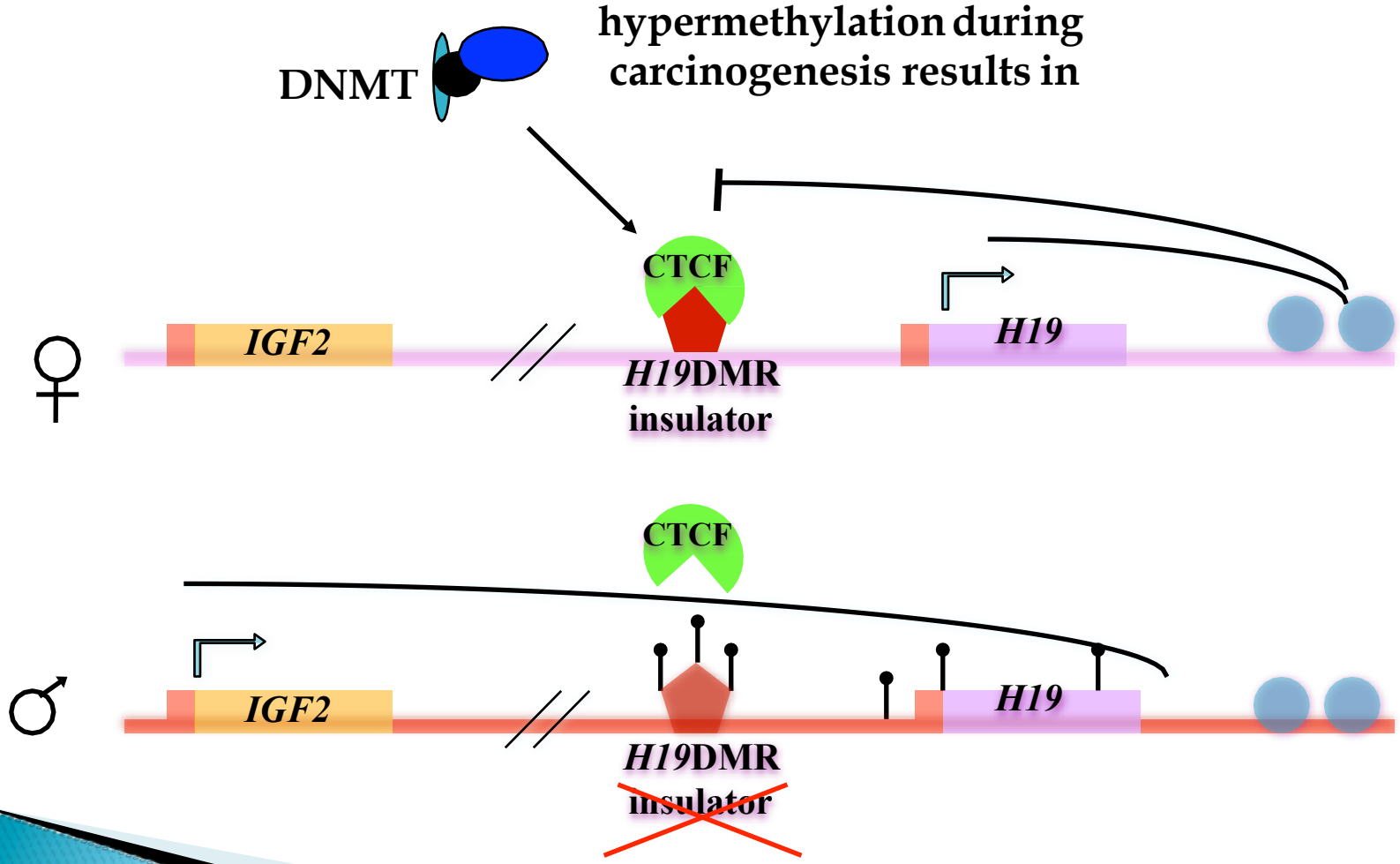
How is Igf2 imprinting deregulated?

LOI of H19/Igf2 via deletion of ICE leads to double dose of Igf2, a potent growth factor



Leighton et al. 1998
Thorvaldsen et al. 1998

LOI of the *Igf2/H19* genes by abnormal methylation of maternal *H19* ICR



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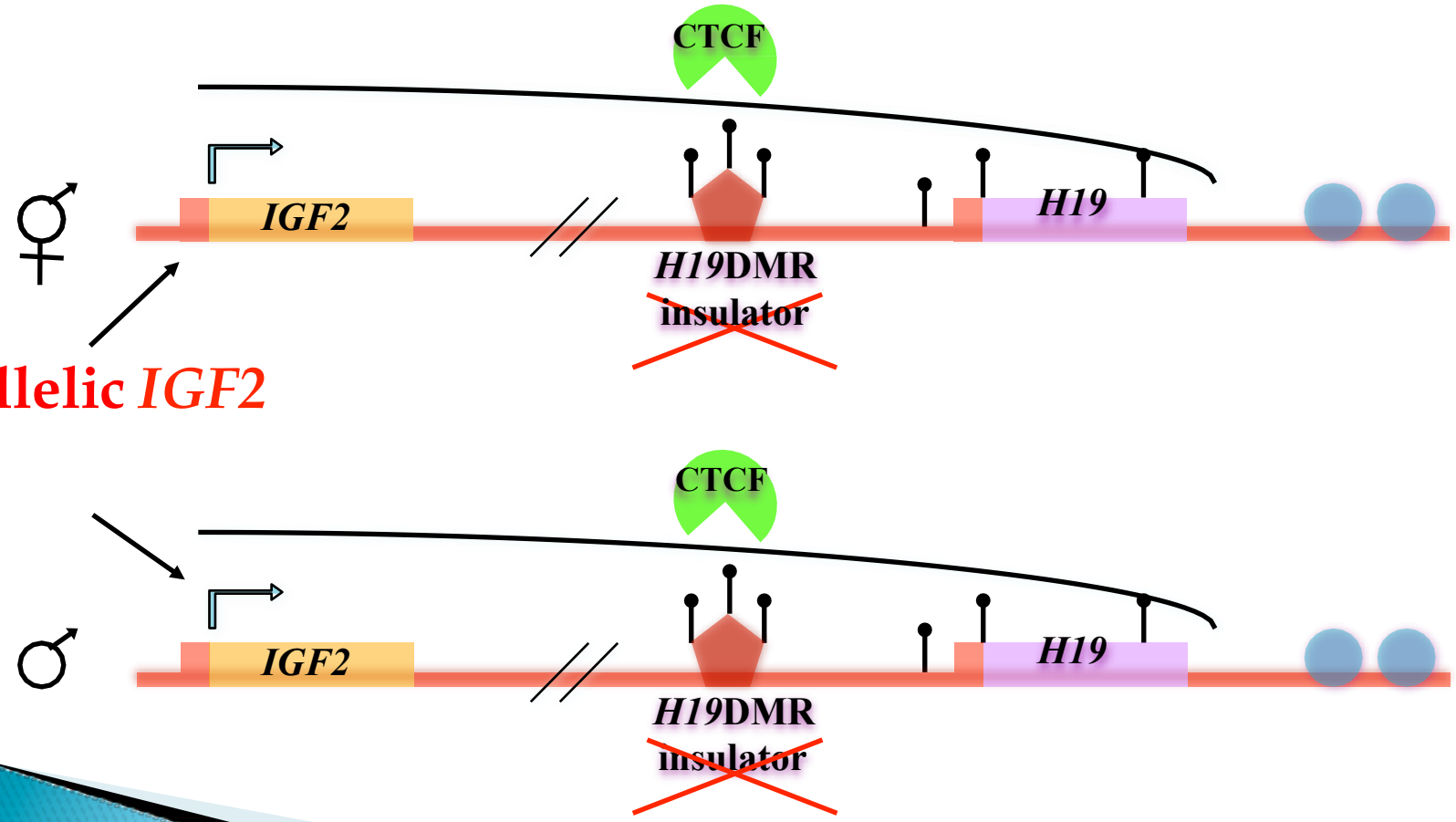


Now we have 2 copies of this growth promoter (oncogene)!!!

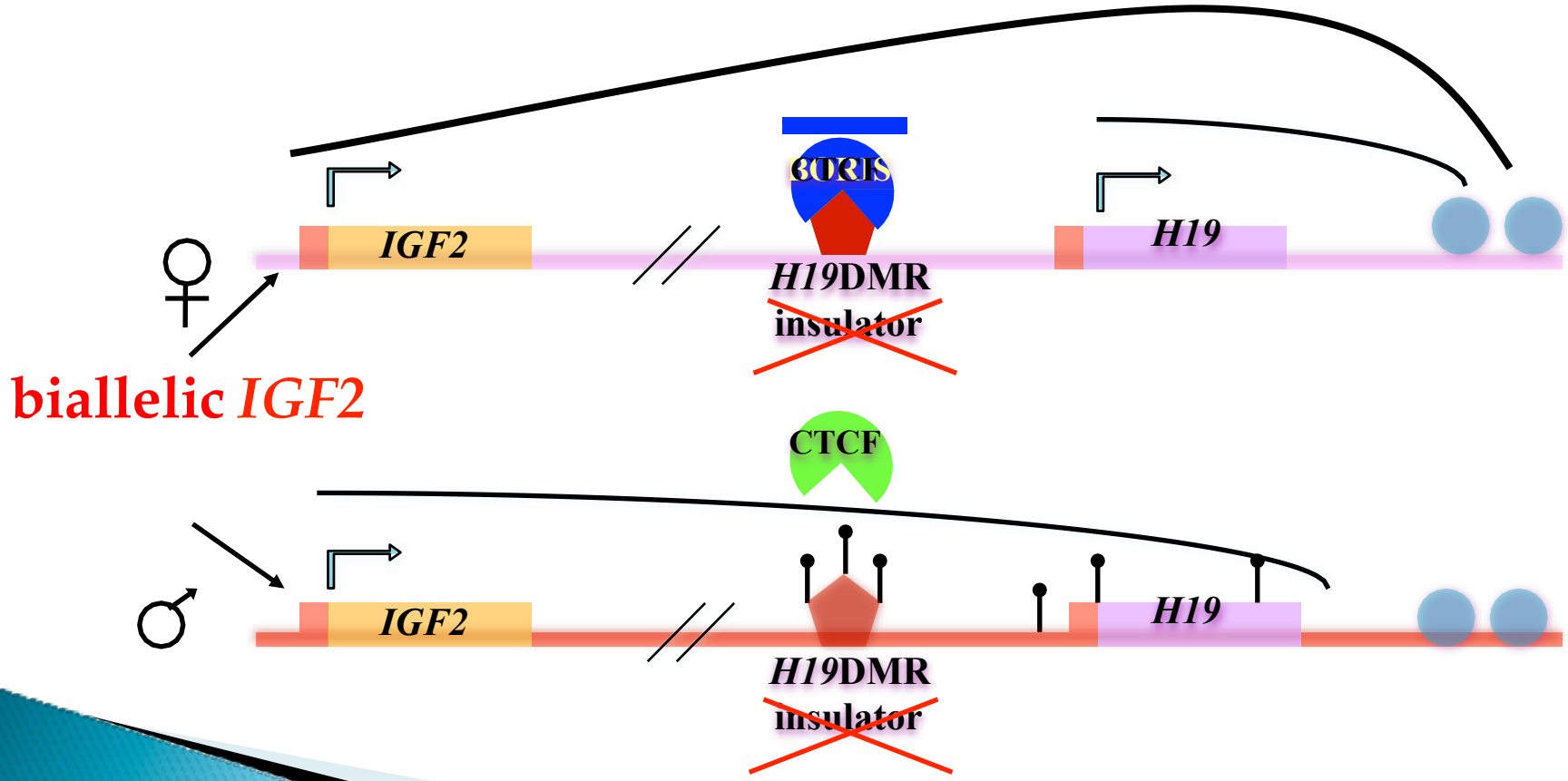
BORIS (CTCFL, CTCF-like) (Brother Of Regulator of Imprinted Sites)

- Has very similar 11-zinc finger domains to CTCF
  - can bind potentially bind to CTCF binding sites

- Remainder of protein is completely different than CTCF
- Expression is restricted to spermatogonia and pre-meiotic spematocytes when imprints are being set up.
- BORIS is expressed in many cancer cell lines along with CTCF
- Ectopic expression of BORIS in cancer cells may disrupt CTCF function

Loss of imprinting of the *Igf2/H19* genes by ectopic expression of BORIS

Displacement of CTCF by BORIS?



Loss of imprinting (LOI) of *IGF2* in tumors

- Wilms tumor
- Adrenocortical carcinoma
- Rhabdomysarcoma
- Hepatoblastoma
- Hepatocellular carcinoma
- Breast cancer
- Lung cancer
- Colorectal cancer
- Leiomyosarcoma
- Testicular germ cell cancer
- Renal cell carcinoma
- Kidney clear cell carcinoma
- Choriocarcinoma
- Cervical carcinoma
- Oesophageal cancer
- AML

- Many tumors have LOI of the *IGF2* gene
- This results in two active copies of *IGF2* and possibly the silencing of one or more maternally expressed 11p15 imprinted genes.

Other genes showing LOI in cancer

- PEG1 in lung cancer
- TP73 in gastric cancer
- CDKN1C in pancreatic cancer (loss of expression)

Cdkn1c (Cyclin-Dependent Kinase inhibitor 1c)

Cell cycle inhibitor (growth suppressor/TSG), normally expressed exclusively from the maternal allele

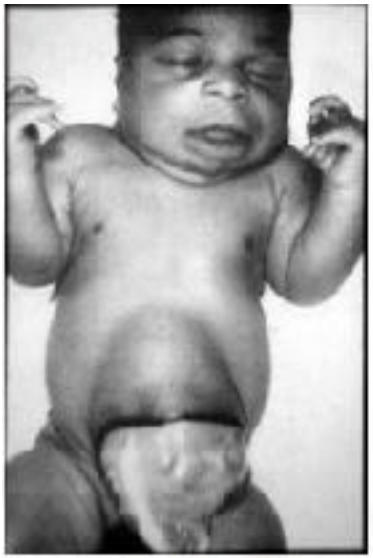
Located in Kcnq1ot1 imprinted cluster

Beckwith-Wiedemann Syndrome (BWS)

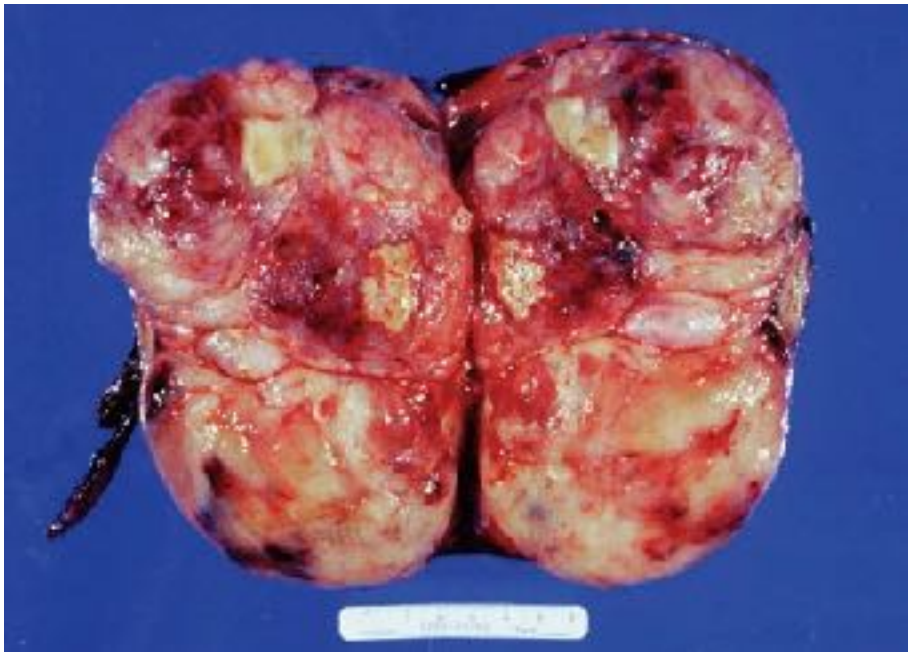
- Overgrowth and cancer predisposition syndrome- 1000-fold increased risk to specific embryonal tumors (i.e. Wilms' tumor)
- Imbalance in the expression of one or more 11p15.5 imprinted genes (contains both the IGF2/H19 cluster and the KvDMR1 cluster)
- Main causes: Overactivity of IGF2 / loss of CDKN1C expression
- >50% of cases due to abnormal DNAme

BWS Clinical Presentation

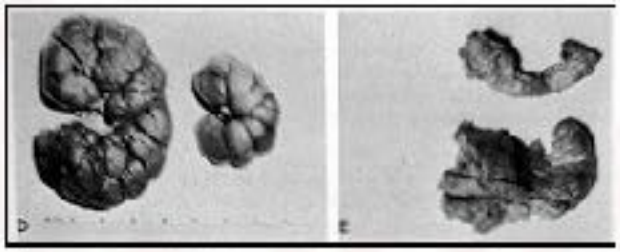
Wilm's tumor



Omphalocele



Macroglossia



BWS and normal kidneys

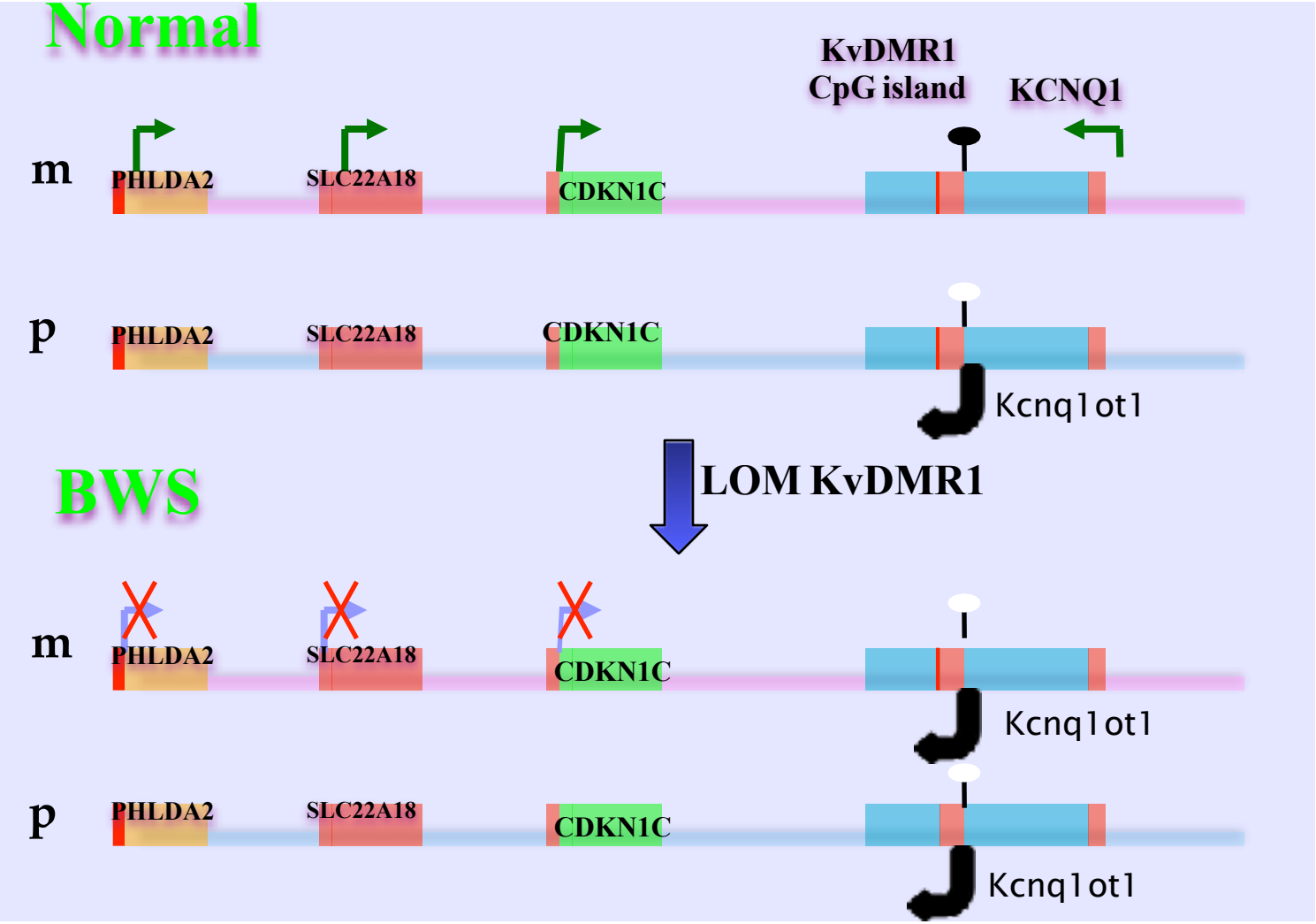
BWS and normal pancreas

Ear Creases

BWS Molecular Subtypes

- 15% heritable (autosomal dominant)
  - Inherited point mutations of Cdkn1c
    - Loss of a growth suppressor →→overgrowth
- 85% sporadic
  - 5% to 10% – point mutation at Cdkn1c
    - Loss of growth suppressor →→overgrowth
  - 10% to 20% – Paternal UPD chromosome 11
    - overexpression of IGF2 and "loss of expression" of CDKN1C and other maternally-expressed genes
  - 2% to 7% – Hypermethylation of H19 ICR
    - Higher risk of cancer
  - Rare – Microdeletion of ICR

LOM (Hypomethylation) at KvDMR1 and BWS



LOM of KvDMR1 →→"activation" of KvDMR1 repressive function which prevents the expression of *CDKN1C* and other maternally-expressed genes

Conclusions

- Imprinted genes are expressed monoallelically, meaning that they are expressed from only one allele. In contrast to a majority of the genome, which is expressed biallelically.
- In genomic imprinting, the way a gene is expressed is dependent on who's chromosome it was inherited from (Mom's or Dad's), the sex of the offspring does not matter
  - Some imprinted genes are expressed from the maternal allele and repressed on the paternal
  - Some imprinted genes are expressed from paternal allele and repressed on maternal allele
- Imprints are erased and reestablished in the germ cells during development, this is important because sperm need to pass on certain imprints and eggs need to pass on different ones to their future offspring.

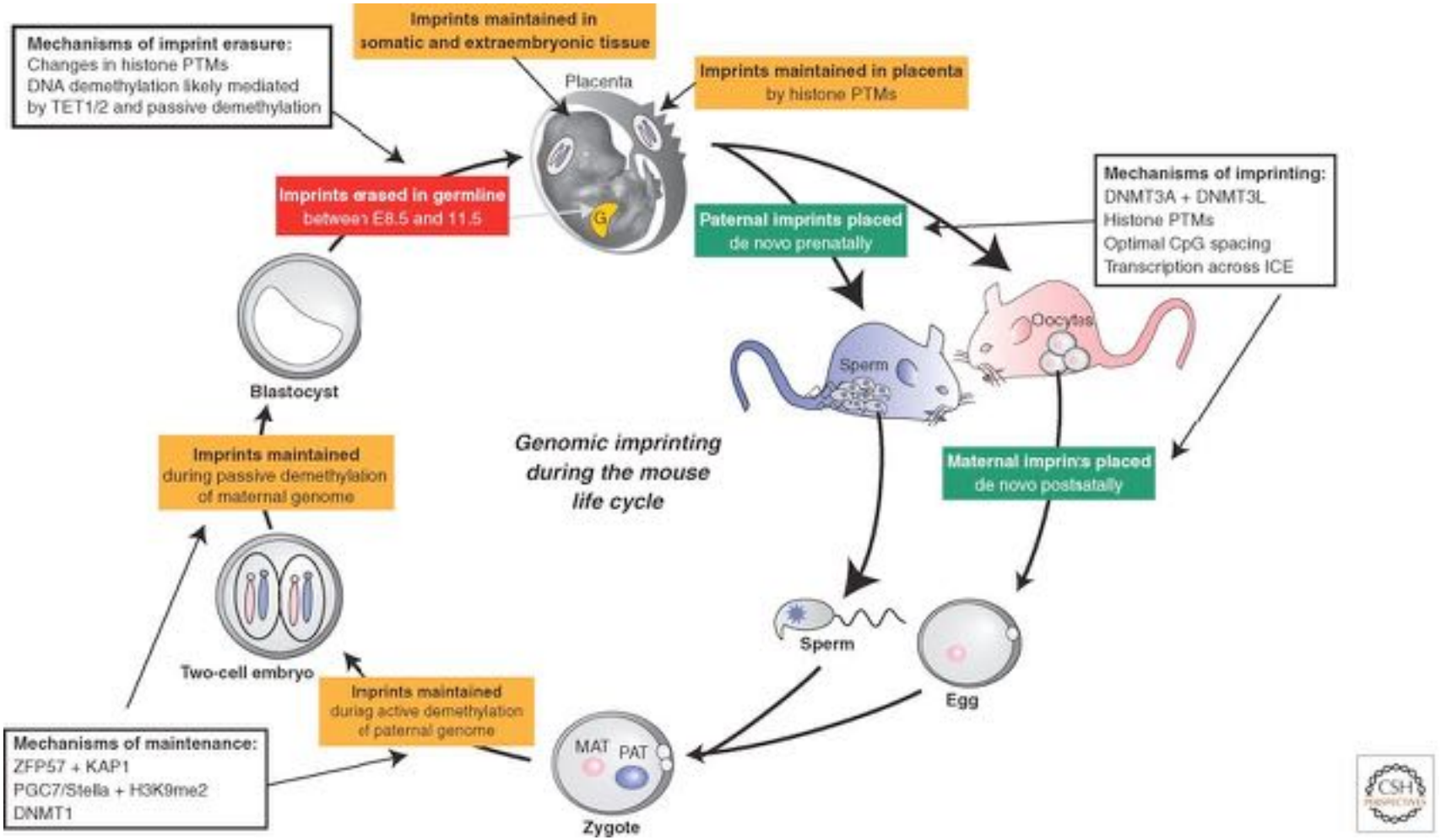
Conclusions

- DNA methylation is the mark found on one of the parental alleles in areas termed differentially methylated regions (DMRs).
- Sequence features, Specific chromatin signature at DMRs and transcription through DMRs may all play an important role in targeting imprinted genes for methylation
- Imprint Control Regions (ICRs) are found at DMRs and you may see the 2 terms used interchangeably in the literature. If a region is truly an ICR, deletion should result in LOI of one or more genes in cis. 2 examples of ICRs: H19 DMR & KvDMR1
- ICRs are often associated with insulators or long ncRNAs
- The H19/Igf2 cluster and the KvDMR1 cluster are both located on human chr 11p15.5 (distal mouse chr7)
- BWS is an imprinting disorder characterized by overgrowth and cancer predisposition. Overexpression of Igf2 and loss of expression of C[illegible] are often responsible for the BWS

Conclusions

- Igf2 is a potent fetal mitogen and survival factor, it is normally expressed exclusively from the paternal allele, but in many cancers and BWS imprinting of this gene is lost and 2 copies are now expressed
  - LOI may result from PAT UPD, hypermethylation of the H19 ICR, BORIS displacment of CTCF, etc.
- Cdkn1c is a cell cycle inhibitor (growth suppressor), it is normally expressed exclusively from the maternal allele, but in certain cancers and BWS, LOI leads to complete loss of expression.
  - LOI may result from point mutations, PAT UPD, LOM at KvDMR, etc.

Establishment, maintenance, and erasure of genomic imprints in mouse development.



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Helpful Reading

Nature Reviews: Genetics: 2011

TIMELINE

Genomic imprinting: the emergence of an epigenetic paradigm

Anne C. Ferguson-Smith

Genomic Imprinting in Mammals
Denise P. Barlow1 and Marisa S. Bartolomei

Barlow D P , and Bartolomei M S Cold Spring Harb Perspect Biol 2014;6:a018382

Practice Questions

- Define Genomic Imprinting
- What is UPD?
- 2 main mechanisms of regulation in imprinted clusters, how they work and an example of a cluster that employs them and an example of how they might be deregulated.
- What is the significance of imprint erasure in the developing embryo?
- What may play an important role in targeting imprinted genes for methylation?
- What is an ICR?
- 2 Main genes involved in etiology of BWS, how is their expression changed from normal? Most common molecular subgroup.
- Mechanism by which Igf2 may lose imprinting.
- What is the relationship between CTCF and BORIS with respect to the Igf2/H19 cluster? (i.e. How would abberrant expression of BORIS affect CTCF and imprinting in the cluster?)

Mice engineered to be "imprint-free" are tumor prone

- made ES cells that could be manipulated to:
  - inactivate Dnmt1 and thus lose all methylation and then turn Dnmt1 back on
  - found that global methylation was restored but methylation at imprinted genes and ICRs was not
  - described as imprint-free (IF)

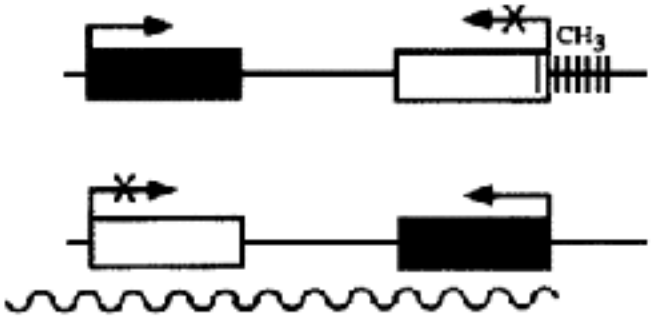
- used these IF ES cells to make chimeric mouse embryos from which they derived IF mouse embryo fibroblasts (MEFs)
  - these IF MEFS were found to become spontaneously immortalized at high frequency; they were easily transformed (using cooperating viral oncogenes)

- 100% of chimeric adult mice made with imprint-free ES cells developed tumors; tumors were derived from IF-ES cells
- concluded that Loss of Imprinting (LOI) alone can predispose cells to tumorigenesis by leading to immortalization which lowers the threshold for transformation

Holm et al. Cancer Cell 8:433 (2005.)

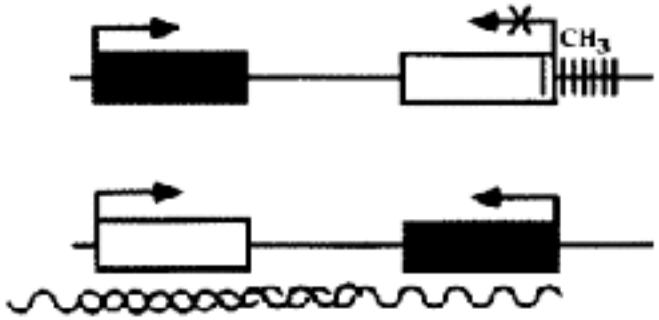
How are imprinted genes silenced?

D. Transcriptional anti-sense interference



Mechanism- Antisense transcription of unmethylated chromosome blocks sense strand transcription

E. Post-transcriptional anti-sense interference



Mechanism- Antisense RNAblocks sense strand transcription

S. Tilghman, Cell 96:185

What makes gDMRs/ICEs targets for the de novo methylation machinery?

- Sequence features
- Specific chromatin signature at DMRs
  - may mediate DNA methyltransferase access to DNA.
- Transcription through the DMR
  - Most known maternally-methylated DMRs are located within transcription units
  - May be required to create or maintain open chromatin so the DNAme machinery has access