# LECTURE 13: EPIGENETICS – IMPRINTING

Reading: Ch. 18, p. 660-664
Problems: Ch. 18, problems 21-24

**Announcements:**

** MIDTERM is Thursday, November 6th, from 7-9 pm. It will cover material through Monday, November 3rd. Room assignments will be finalized the week of the exam and posted on the website. No calculators may be used on the exam. Additional information about the midterm and office hours will be posted on the website.

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In the 1980's, scientists attempted to create gynogenetic or androgenetic diploids in mammals by putting either two female pronuclei or two male pronuclei into mouse eggs and then transferring the eggs into a foster mother. Control embryos derived from fusion of a maternally-derived pronucleus and a paternally-derived pronucleus developed normally, but embryos from the fusion of two maternally-derived pronuclei or two maternally-derived pronuclei did not develop normally. The only possible genetic difference between males and females in this experiment was the sex chromosomes, but even XX animals failed to thrive!

We know now that one of the reasons that mammalian gynogenetic and androgenetic diploids cannot be made is because of an epigenetic phenomenon called **genomic imprinting**. The expression of an imprinted gene depends upon the parent (maternal or paternal) that transmits it. **Epigenetic** means "outside the genes". **Epigenetic inheritance** describes a variant condition that does not involve a change in DNA sequence, yet is transmitted from one somatic cell generation to the next during development and growth of an organism. **Maternal imprinting** means that the allele of a particular gene inherited from the mother is transcriptionally silent and the paternally-inherited allele is active. **Paternal imprinting** is the opposite; the paternally-inherited allele is silenced and the maternally-inherited allele is active.

## Maternal imprinting of *Igf2* in mice

The insulin-like growth factor 2 (*Igf2*) gene in mice is imprinted. Imprinting was discovered at this locus from studies of the transmission of a deletion in chromosome 7 that removed this gene. Mice that inherited the deletion chromosome from the paternal side were small, but mice inheriting the same deletion from the maternal side were normal. Thus, a deletion of *Igf2* only causes a mutant phenotype when transmitted by the father.

A simple model of maternal imprinting seen in a two generation cross:

| | | | |
|---|---|---|---|
| Cross: | del / *Igf2* female | x | *Igf2* / *Igf2* male |
| | ------(  )------ | | ------[ + ]------ |
| | ------[ + ]------ | | ------[ + ]------ |
| Progeny: | ------(  )------$^{m}$ | and | ------[ + ]------$^{m}$ |
| | ------[ + ]------$^{p}$ | | ------[ + ]------$^{p}$ |

Normal sized sons and daughters
Alleles inherited from mom are silent

The sons and daughters of this cross are normal-sized. This is because even though each individual inherits an inactive copy of the *Igf2* gene from their mother (either the deletion or the imprinted allele), they inherit an active allele from their father.

*Note1: There are sons and daughter of both genotypes – this is not a sex-linked trait.*
*Note 2: Superscript m or p indicates whether the allele was inherited from mom (m) or dad (p).*

Some of the sons of the first cross inherit the deletion from their mother and the normal chromosome 7 from their father. If we cross one of these sons (del / *Igf2*) to an *Igf2* / *Igf2* female, we see that about half the progeny are small-sized. This is because they have no active *Igf2* gene; they inherit the deletion from their father and an inactived (imprinted) alelle from their mother.

| | | | |
|---|---|---|---|
| Cross: | del / *Igf2* female<br>------[ + ]------<br>------[ + ]------ | x | *Igf2* / *Igf2* male<br>------(  )------<br>------[ + ]------ |
| Progeny: | ------[ + ]------$^{m}$<br>------[ + ]------$^{p}$ | and | ------[ + ]------$^{m}$<br>------(  )------$^{p}$ |

Half the progeny are small
Alleles inherited from mom are silent

**IMPORTANT**: Do not confuse imprinting with sex linkage. Igf2 is an autosomal gene that is inherited in a silenced state from mom and an active state from dad. It is the sex of the parent that transmits the allele that matters, not the sex of the progeny!

**Old imprints must be erased and new imprints imposed during meiosis.**

Every individual carries a maternal allele and a paternal allele at any one locus. If an allele is maternally imprinted (as in our example above), then when males undergo spermatogenesis, both maternally- and paternally-inheritied alleles are reactivated. When females undergo oogenesis, imprints are imposed on both the maternally- and paternally-inherited genes. Thus when males and females are crossed, the progeny inherit a silenced (imprinted) allele from their mothers and an activated functional (non-imprinted) copy from their fathers.

For a maternally-imprinted gene:

Egg: ------[ + ]------ (imprinted) x Sperm: ------[ + ]------

Daughter: ------[ + ]------$^{m}$ (not expr)
------[ + ]------ $^{p}$ (expr)

Son ------[ + ]------$^{m}$ (not expr)
------[ + ]------ $^{p}$ (expr)

*Gametogenesis: During gamete formation, it is thought that all imprints are erased early.*

During oogenesis, imprints are then imposed upon both Igf2 copies, regardless of whether they were inherited from mom or dad. This ensures that the daughter passes on only inactive copies.

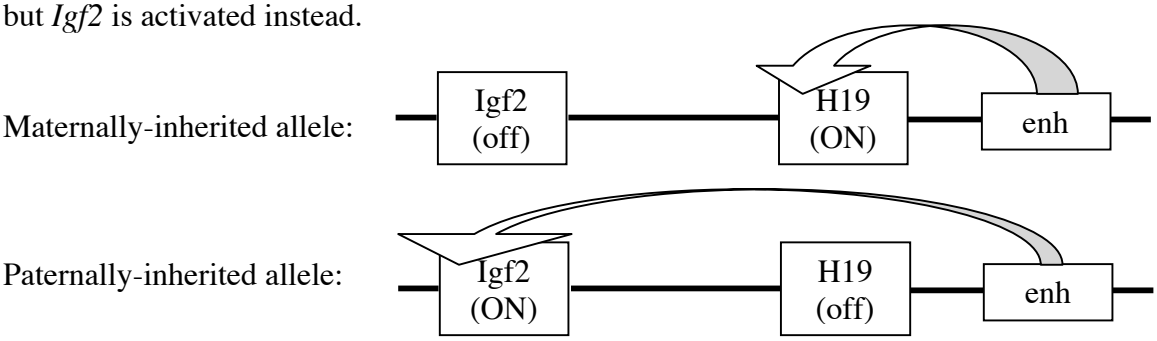
During spermatogenesis, imprints are erased upon both Igf2 copies, regardless of whether they were inherited from mom or dad. This ensures that the son passes on active copies.

Gametes: ------[ + ]------ (imprinted)

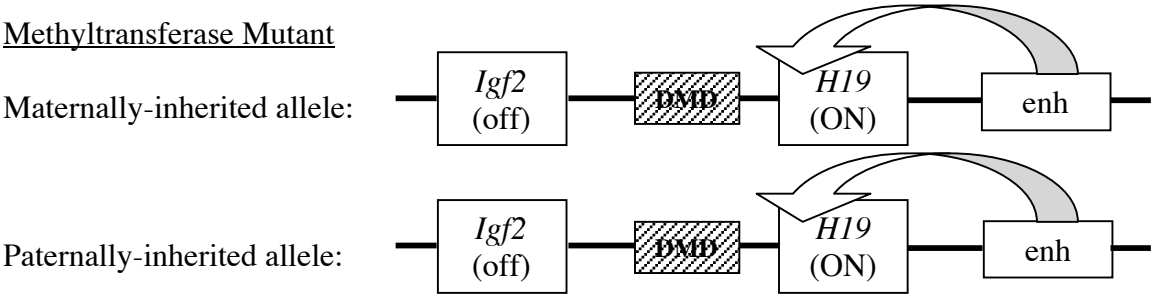
Gametes: ------[ + ]------

**A model for imprinting at the *Igf2* locus.**

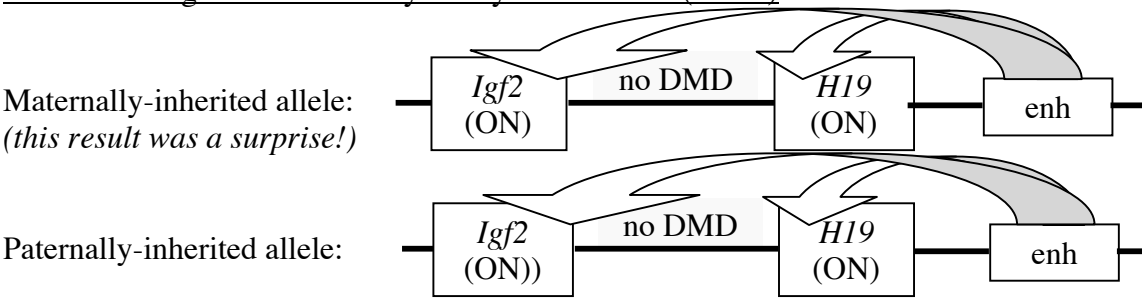
Insight into how imprinting at *Igf2* might function came from the discovery that a linked gene, called *H19*, was imprinted in the opposite fashion (paternally, instead of maternally). One model (an "enhancer competition" model) is that the two linked genes, *Igf2* and *H19* share an enhancer. The enhancer prefers to turn on the *H19* gene. Since there is no imprinting at the maternally-inherited *H19* locus, the adjacent enhancer turns on *H19* and not *Igf2*. Because there is imprinting at the paternally-inherited *H19* locus, *H19* is not expressed from this chromosome, but *Igf2* is activated instead.




What is the "imprint"? Imprinting has been correlated with DNA methylation. When DNA is heavily methylated, it tends to be transcriptionally inactive. When mice mutant for a methyltransferase (a primary enzyme required for DNA methylation) are made, *H19*, and not *Igf2*, is transcribed from both maternally- and paternally-inherited chromosomes.

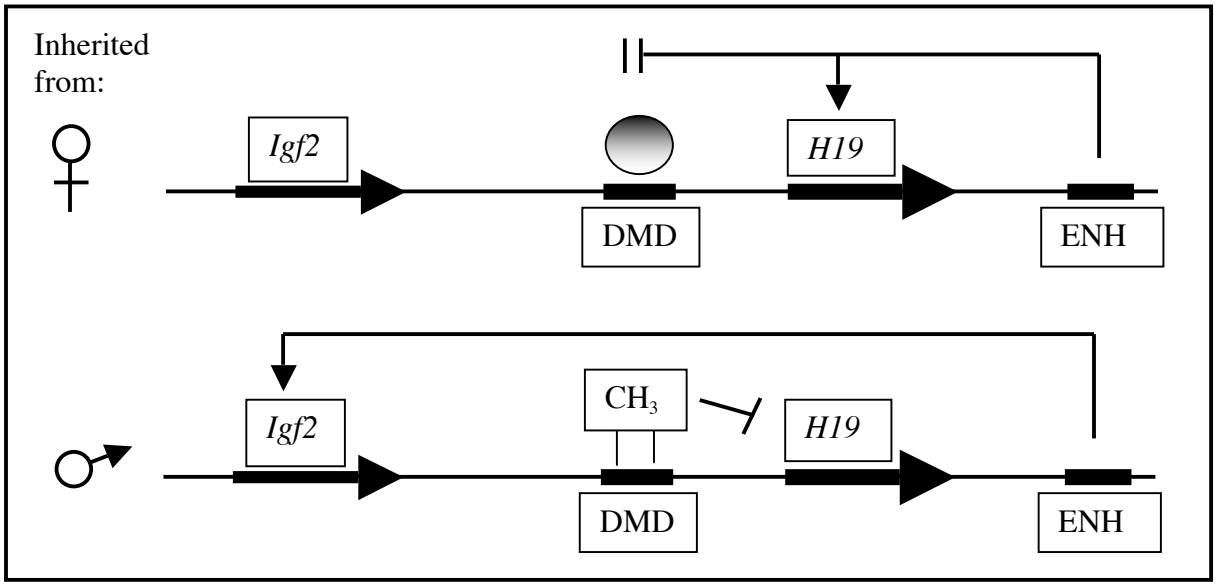



But a mouse mutant for a methyltransferase could affect the expression of many genes, so this experiment is not as good as a mutation that affects only the Igf2 locus. There is a differentially methylated domain (DMD) at the *H19* / *Igf2* locus that is usually heavily methylated on the paternally-derived chromosome. When investigators made a mouse in which the DMD is deleted, one sees an increase in *H19* expression and a decrease in *Igf2* expression from the paternally-inherited chromosome, just as one might expect. However, both H19 and Igf2 are expressed from the maternally-inherited chromosome!




*Class, I had the data right in my notes, but wrong on my diagram (corrected here). As we deduced in class, Igf2 is biallelically expressed (levels are slightly different in the 2 situations, but that's not critical for you to consider!)*

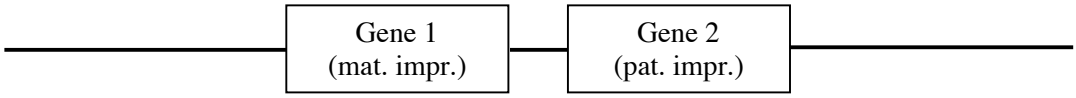
This shows that the region identified as the DMD in fact serves two purposes: (1) the region becomes methylated (thus silencing H19 expression and allowing Igf2 expression) on the paternally-inherited chromosome and (2) the region acts as an <u>insulator</u> on the maternally-inherited allele and prevents the enhancer from activating Igf2 gene. In fact, there is a protein CTCF that binds the unmethylated DMD (on the maternally-inherited chromosome) -- this protein blocks the enhancer from turning on the IGF2 gene. Thus, the mutation in which the DMD is deleted will remove the binding site for this protein and allow the enhancer to activate the maternally-inherited *Igf2* gene.




**<u>Imprinting in humans</u>**

Imprinting has been implicated in a variety of human disorders, including Prader-Willi syndrome (OMIM 176270) and Angelman syndrome (OMIM 105830). Prader-Willi syndrome children have small hands and feet, underdeveloped gonads and genitalia, short stature, mental retardation, and obesity. Children with Angelman syndrome have red cheeks, large jaws, large mouth and tongue, mental and motor retardation, and a happy disposition. Both syndromes are associated with deletions in a region of chromosome 15. (In fact, it is not uncommon for imprinted genes to be clustered in the genome.) This region contains at least two imprinted genes, one maternally imprinted and one paternally imprinted. If a child receives a chromosome in which a large deletion removes function of these genes from their father and an (inactive) copy of the maternally imprinted gene from their mother, they will have Prader-Willi Syndrome. If a child receives the deleted chromosome from their mother and an (inactive) copy of the paternally imprinted gene from their father, they will have Angelman syndrome.

Let's say a spontaneous deletion occurs on chromosome 15 that deletes at least 2 genes:




If deletion is inherited from mom, no gene 2 product is made → Child has Angelman syndrome
If deletion is inherited from dad, no gene 1 product is made → Child has P-W syndrome
*Phenotype of offspring depends upon which parent transmits the deleted chromosome!*

Take a look at the pedigrees in your book (Fig. 18-15e) and convince yourselves that you could recognize a paternal and maternal imprinting in a human pedigree. General guidelines are (1) in paternal imprinting, half the progeny of affected females will be affected and (2) in maternal imprinting, half the progeny of affected males will be affected.

**Why does imprinting exist?**

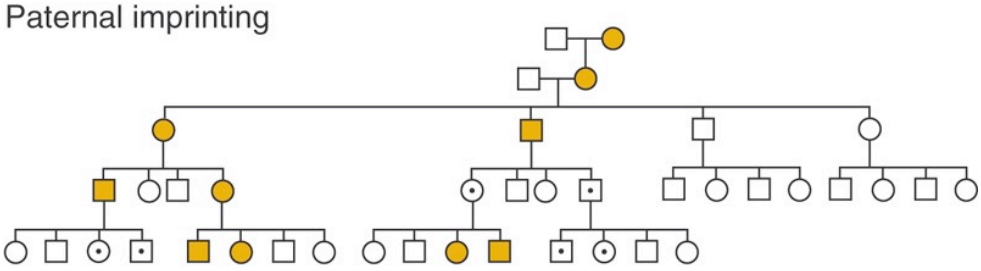
Imprinting seems to put individuals at a disadvantage, giving them only one chance for an active copy of the gene. One potential explanation, the Haig hypothesis, is that imprinting reflects the "battle of the sexes". Males want to transmit genes that normally retard embryonic growth in a "silenced" form; this ensures that his progeny would grow larger and more rapidly than other embryos in the uterus that might have been fathered by different males. This would increase the chances of his genes being successfully passed on. Severe overgrowth of embryos can be a disadvantage to the mother, so she counteracts by transmitting "silenced" copies of genes normally responsible for enhancing growth. All of her embryos would have an equal chance of survival and would do so without draining all of her energy. This hypothesis could explain why imprinting has only been observed in mammals, the only animals where embryos can compete in utero for maternal resources. It is also interesting to note that many, but not all, of the imprinted genes known to date are involved in embryonic growth control.

Imprinting Pedigrees (from Figure 18.15 in your book)



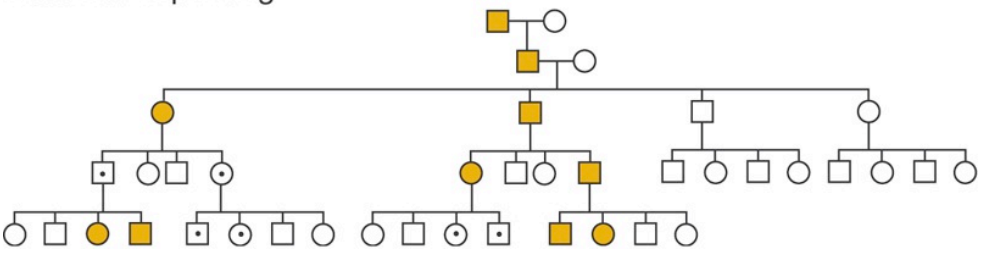
(e) Genomic imprinting and human disease

Paternal imprinting



In paternal imprinting, the paternally-inherited allele is inherited in a silent state. Half the progeny of affected females will be affected, regardless of their gender.

Maternal imprinting



In maternal imprinting, the maternally-inherited allele is inherited in a silent state. Half the progeny of affected males will be affected, regardless of their gender.