Introduction to Genomic Imprinting

Epigenetics is a higher-order genetic process that works in addition to the direct translation of certain genes (‘epigenetics’ literally means ‘on top of genetics’). Genomic imprinting is a type of epigenetic system whereby some genes are ‘stamped’ or marked in the spermatogonia of the father and other genes are ‘stamped’ or marked in the oocytes of the mother, before they are passed on to the child. Some of these molecularly ‘stamped’ or imprinted genes are expressed only when they come from the mother, while others are expressed only when they come from the father. Only about 1 to 2 percent of human genes are imprinted, but these genes are critical for normal developmental processes. In addition, imprinted pedigrees produce interesting inheritance patterns that are quite different from the inheritance patterns seen in classical Mendelian inheritance.

This unit on Genomic Imprinting is designed for a second year, high school advanced biology elective in genetics for students in grades 11 and 12. These advanced science students will already have previous knowledge of classical Mendelian inheritance patterns. By learning about Genomic Imprinting (an example of non-Mendelian inheritance), the students will appreciate that there’s much more to gene expression than what they learned about in first year biology.

Students’ Misconception to be Addressed in the Unit

“Phenotypes are only changed by mutations in DNA sequences, which result in mutated proteins.”

Indiana State Standards Addressed in the Unit

B.1.23
B.1.26
The supporting theme of scientific thinking

Major Science Concepts Addressed

How are genes turned on and off?

Objectives of the Unit – Students will learn -

1. how defects in genomic imprinting can cause phenotypes that are as severe as those caused by mutations in DNA sequences.
2. how deletions can identify where imprinted genes exist by showing monoallelic expression patterns.
3. that DNA methylation is a mechanism of imprinting.
4. how to interpret restriction enzyme digestion/electrophoresis results to diagnose Angelman syndrome and Prader-Willi syndrome.

**Prerequisite Skills that Students Will Bring to this Unit**

1. pedigree analysis
2. restriction enzyme digestion/electrophoresis data interpretation

**Timeline for the Unit - based upon a block schedule with 90 minute periods (This unit was actually implemented in the last week of April, 2009.)**

**Day 1**

1. Students were given the pre-test (see the hand-out entitled Ruhl-Lossie-Pre-Post-Test.doc along with the answer key entitled Ruhl-Lossie-Pre-test Answer Key.doc). The class average on the pre-test was 29%.

2. **ENGAGEMENT and EXPLORATION -**

The students were divided up into working groups of three. Each group was given one copy each of the five pedigrees (see the hand-outs entitled Pedigree 1.doc, Pedigree 2.doc, Pedigree 3.doc, Pedigree 4.doc, and Pedigree 5.doc, along with their respective answer keys). Each group was also given a 2’ x 2’ white board, white board marker, and eraser. These white boards served as tools that the groups could problem solve and sketch on as they HYPOTHESIZED (and then TESTED) possible modes of inheritance for each pedigree. After the groups finished analyzing the pedigrees, Ruhl handed out the answer keys for each pedigree so that the students could check their answers (FORMATIVE ASSESSMENT). Once it was determined that the students were confident in their pedigree analysis skills, Ruhl gave each group copies of the Star-Belly Sneetches pedigree, printed out in color (see the hand-out entitled Puzzling Pedigree.cwk). The groups struggled for several minutes, wrestling with the task of trying to determine the mode of inheritance of star color in these mythical, Dr. Seuss creatures (star color in these creatures is due to genomic imprinting). None of the groups figured this out, although a couple bright students wanted to know if star color had anything to do with which parent the trait came from. This activity certainly created a ‘need to know’ in these high achieving high school juniors and seniors!
Day 2

EXPLANATION –

Dr. Amy Lossie (Purdue University) gave a lecture/discussion on genomic imprinting based upon the students’ questions about the puzzling pedigree - Inheritance of Star Color in Star-Belly Sneetches (see the PowerPoint lecture entitled Lossie Imprinting.pptx). In this lecture/discussion, the concept of monoallelic expression via deletions was introduced along with DNA methylation.

Day 3

EXTENSION/ELABORATION –

Amy Lossie and Joe Ruhl led the students on a field trip to the Indiana University Medical Center in Indianapolis (organized by Lossie), where they met with two genetic counselors (Mandy Miller and Abby Stevens) for two hours. The genetic counselors were excellent, enthusiastic communicators and the students thoroughly enjoyed this opportunity to interact with professionals in the real world of human genetics. Amy Lossie packed lunches for the students who were most appreciative!

Day 4

EXPLANATION/FORMATIVE ASSESSMENT –

Ruhl led a follow-up discussion/clarification of Prader-Willi syndrome, Angelman syndrome, deletions, imprinting, and methylation (See PowerPoint lecture entitled Intro to ImprintingRuhl.ppt). At the end of the lecture students were again directed to the Sneetches pedigree. Each student was given a copy of chromosome pair #15 – A (see the hand-out entitled Normalsneetch.jpg), chromosome pair #15 – B (see the hand-out entitled ASsneetch.jpg), and chromosome pair #15 – C (see the hand-out entitled PWSsneetch.jpg). As the Sneetches pedigree was shown on the slide screen, Ruhl pointed to a Sneetch in the pedigree. The students were instructed to hold up the correct picture of the chromosome 15 pair (A, B, or C) that the Sneetch pointed to would actually have. Ruhl would then advance to the next slide, which revealed the correct chromosome pair superimposed on the Sneetch previously pointed to (a blue star on a Sneetch is analogous to Prader-Willi syndrome, a red star is analogous to...
Angelman syndrome, and no star on the belly is analogous to normal in humans). While clicking through slides 55 through 65, as each student held up his/her paper for each Sneetch pointed to, Ruhl was able to quickly glance around the room to check for the students’ understanding and to provide immediate feedback. At times, when Ruhl perceived that more feedback was needed, he would spend a few minutes reviewing with the students which parent a particular Sneetch got each of his/her chromosomes from. This also provided an opportunity to review the fact that a parent will ‘erase’ the imprints on his/her chromosomes, ‘stamp’ them with his/her own imprint, and THEN pass them on to the offspring.

**Day 5**

**EVALUATION (SUMMATIVE)** –

Students were given the post-test (this test is identical to the pre-test that was given on day number 1, and is used to measure growth throughout the unit). The class average on the post-test was 89% (compared to the class average of 29% on the pre-test).
Pre-Test  Introduction to Epigenetics

1. A normal individual has _______ alleles of each gene.

2. A normal individual inherits _______ allele(s) of each gene from his or her mother, and _____ allele(s) of each gene from his or her father.

3. Genes that follow Mendelian inheritance ‘rules’ are expressed from:
   a. The maternal allele(s) only
   b. The paternal allele(s) only
   c. Both the maternal and paternal alleles
   d. Either the maternal or paternal allele, but not both

4. Genes that are imprinted are expressed from:
   a. The maternal allele only
   b. The paternal allele only
   c. Both the maternal and paternal allele
   d. Either the maternal or paternal allele, but not both

5. What percentage of genes in the genome is imprinted?

6. Name one imprinted gene.

7. Give an example of a molecular ‘stamp’ that the cell uses to determine parental origin of a particular imprinted gene.

8. a. Describe the characteristics of Prader-Willi syndrome.

   b. Which chromosome is affected in persons with Prader-Willi syndrome?

   c. What is the most common kind of chromosomal defect observed in patients with Prader-Willi syndrome?
9. a. Describe the characteristics of Angelman syndrome.

b. Which chromosome is affected in persons with Angelman syndrome?

c. What is the most common kind of chromosomal defect observed in patients with Angelman syndrome?

**Diagnosis of Angelman and Prader-Willi Syndromes**

The following figure is a map of the promoter of the gene used to diagnose Angelman and Prader-Willi syndromes.

10. Which gene does this cartoon represent?
Background information for question 11.

X depicts locations where the restriction enzyme, Xba I cuts genomic DNA. There are two Xba I sites in this region. N depicts locations where Not I cuts genomic DNA. There are two Not I sites in this region.

Xba I cuts all genomic DNA. Not I only cuts unmethylated DNA. Therefore, if the Not I sites are methylated, Not I will not be able to digest the DNA.

In order to diagnose Angelman and Prader-Willi syndrome, DNA from patients is digested with Xba I and Not I. The DNA is then size fractionated by gel electrophoresis, and transferred to a nylon membrane. The DNA from this region is visualized by hybridization with a probe, depicted above.

When the DNA is digested with Xba I alone, the probe hybridizes to a 4.3 kb fragment, which is the size of the region between the two Xba I sites.

When the DNA is digested with Not I alone, the probe will detect a 4.3 kb fragment if the DNA is methylated (because Not I will not be able to digest the methylated DNA), and a 0.9 kb fragment if the DNA is unmethylated.

In this region, the paternal allele is unmethylated in normal individuals, while the maternal allele is methylated. Therefore, Not I can be used to differentiate between the unmethylated (Paternal) allele and the methylated (Maternal) allele, and is an easy method to diagnose Angelman and Prader-Willi syndrome patients.

Normal (NI) individuals have both a methylated and an unmethlated allele.

Angelman syndrome (AS) patients only have an unmethlated allele.

Prader-Willi syndrome (PWS) patients only have a methylated allele.

11. In the figure on the previous page, mark which lanes correspond to:

   a. Normal Individuals (NI)—explain your decision.
   
   b. Angelman Syndrome Patients (AS)—explain your decision.
   
   c. Prader-Willi Syndrome Patients (PWS)—explain your decision.
Pre-Test Introduction to Epigenetics

1. A normal individual has __2__ alleles of each gene.

2. A normal individual inherits __1__ allele(s) of each gene from his or her mother, and __1_ allele(s) of each gene from his or her father.

3. Genes that follow Mendelian inheritance ‘rules’ are expressed from:
   a. The maternal allele(s) only
   b. The paternal allele(s) only
   c. Both the maternal and paternal alleles
   d. Either the maternal or paternal allele, but not both

4. Genes that are imprinted are expressed from:
   a. The maternal allele only
   b. The paternal allele only
   c. Both the maternal and paternal allele
   d. Either the maternal or paternal allele, but not both

5. What percentage (or approximate number) of genes in the genome is imprinted? 1-2 percent (or about 200 genes)

6. Name one imprinted gene. Snrpn, Ube3a, H19, Igf2, Dlk1, Gtl2, Kcnq1, Kcnq1ot1, etc. I will get you a list.

7. Give an example of a molecular ‘stamp’ that the cell uses to determine parental origin of a particular imprinted gene.
   DNA methylation, histone modifications (acetylation, methylation, ubiquitination, phosphorylation, sumoylation)

8. a. Describe the characteristics of Prader-Willi syndrome.
   Hypogonadism, poor muscle tone, failure to thrive, floppy baby syndrome, obesity, obsessive/compulsive, behavioral abnormalities, short stature, hyperphagic (always hungry/eating), moderately mentally impaired

b. Which chromosome is affected in persons with Prader-Willi syndrome? Chromosome 15

c. What is the most common kind of chromosomal defect observed in patients with Prader-Willi syndrome?
   Deletion of Paternal Chromosome 15q11-q13

9. a. Describe the characteristics of Angelman syndrome.
Movement disorder (general lack of body coordination), failure to speak, happy affect, severely mentally impaired, hypopigmented (fair hair and skin),

b. Which chromosome is affected in persons with Angelman syndrome? Chromosome 15

c. What is the most common kind of chromosomal defect observed in patients with Angelman syndrome? Deletion of Maternal Chromosome 15q11-q13

Diagnosis of Angelman and Prader-Willi Syndromes

The following figure is a map of the promoter of the gene used to diagnose Angelman and Prader-Willi syndromes.

10. Which gene does this cartoon represent? 

**SNRPN**

Background information for question 11-12.
Xba I cuts all genomic DNA. *Not* I only cuts unmethylated DNA. Therefore, if the *Not* I sites are methylated, *Not* I will not be able to digest the DNA.

In order to diagnose Angelman and Prader-Willi syndrome, DNA from patients is digested with *Xba* I and *Not* I. The DNA is then size fractionated by gel electrophoresis, and transferred to a nylon membrane. The DNA from this region is visualized by hybridization with a probe, depicted above.

When the DNA is digested with *Xba* I alone, the probe hybridizes to a 4.3 kb fragment, which is the size of the region between the two *Xba* I sites.

When the DNA is digested with *Not* I alone, the probe will detect a 4.3 kb fragment if the DNA is methylated (because *Not* I will not be able to digest the methylated DNA), and a 0.9 kb fragment if the DNA is unmethylated.

In this region, the paternal allele is unmethylated in normal individuals, while the maternal allele is methylated. Therefore, *Not* I can be used to differentiate between the unmethylated (Paternal) allele and the methylated (Maternal) allele, and is an easy method to diagnose Angelman and Prader-Willi syndrome patients.

Normal (NI) individuals have both a methylated and an unmethylated allele.

Angelman syndrome (AS) patients only have an unmethylated allele.

Prader-Willi syndrome (PWS) patients only have a methylated allele.

11. In the figure on the previous page, mark which lanes correspond to:

   a. Normal Individuals (NI)—explain your decision.
   Since normal individuals have both a maternal and a paternal allele, *Not* I will only cut the unmethylated, paternal allele. The maternal allele will not cut. The probe will therefore, hybridize to a 4.3 kb maternal allele and a 0.9 kb paternal allele.

   b. Angelman Syndrome Patients (AS)—explain your decision.
   Since AS patients only have the paternal allele, which is unmethylated, *Not* I will cut, and the probe will hybridize to a 0.9 kb fragment.

   c. Prader-Willi Syndrome Patients (PWS)—explain your decision.
   Since PWS patients only have the maternal allele, which is methylated, *Not* I will not cut, and the probe will hybridize to a 4.3 kb fragment.

12. Which allele of *SNRPN* is expressed?
   Maternal (unmethylated)
Pedigree 1

In the pedigree below, shaded individuals are affected by a genetic condition. Could the condition in this pedigree be:

Autosomal dominant?  
Autosomal recessive?  
X-Linked dominant?  
X-Linked recessive?  
Y-Linked?
Pedigree 1

In the pedigree below, shaded individuals are affected by a genetic condition. Could the condition in this pedigree be:

- Autosomal dominant? Yes
- Autosomal recessive? No
- X-Linked dominant? No
- X-Linked recessive? No
- Y-Linked? No
Pedigree 2

In the pedigree below, shaded individuals are affected by a genetic condition. Could the condition in this pedigree be:

Autosomal dominant? __________
Autosomal recessive? __________
X-Linked dominant? __________
X-Linked recessive? __________
Y-Linked? __________
Pedigree 2

In the pedigree below, shaded individuals are affected by a genetic condition. Could the condition in this pedigree be:

- Autosomal dominant? Yes
- Autosomal recessive? Yes
- X-Linked dominant? No
- X-Linked recessive? Yes
- Y-Linked? No
Pedigree 3

In the pedigree below, shaded individuals are affected by a genetic condition. Could the condition in this pedigree be:

- Autosomal dominant? __________
- Autosomal recessive? __________
- X-Linked dominant? __________
- X-Linked recessive? __________
- Y-Linked? __________
Pedigree 3

In the pedigree below, shaded individuals are affected by a genetic condition. Could the condition in this pedigree be:

- Autosomal dominant? Yes
- Autosomal recessive? No
- X-Linked dominant? No
- X-Linked recessive? No
- Y-Linked? No
Pedigree 4

In the pedigree below, shaded individuals are affected by a genetic condition. Could the condition in this pedigree be:

Autosomal dominant? _________
Autosomal recessive? _________
X-Linked dominant? _________
X-Linked recessive? _________
Y-Linked? _________
Pedigree 4

In the pedigree below, shaded individuals are affected by a genetic condition. Could the condition in this pedigree be:

- Autosomal dominant? ___ Yes ___
- Autosomal recessive? ___ Yes ___
- X-Linked dominant? ___ Yes ___
- X-Linked recessive? ___ No ___
- Y-Linked? ___ No ___
Pedigree 5

In the pedigree below, shaded individuals are affected by a genetic condition. Could the condition in this pedigree be:

Autosomal dominant? __________
Autosomal recessive? __________
X-Linked dominant? __________
X-Linked recessive? __________
Y-Linked? __________
Pedigree 5

In the pedigree below, shaded individuals are affected by a genetic condition. Could the condition in this pedigree be:

Autosomal dominant? __Yes____
Autosomal recessive? __Yes____
X-Linked dominant? __No____
X-Linked recessive? __Yes____
Y-Linked? __Yes____
What is the mode of inheritance of the red and blue stars (having no star on the belly is normal)?
Genomic Imprinting: How did the Sneetches Get Their Stars?

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Department of Animal Sciences
Purdue University
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Genomic Imprinting demonstrates that the genomic DNA and transcriptional capacity of selected genes are different on the maternal and paternal alleles of these genes.

In contrast to Mendelian genes, which are expressed from both the maternal and paternal alleles, imprinted genes are expressed from either the maternal OR paternal allele.

Why Use the Mouse
• Small animal with short generation time
• Genetic tools
  — Gene targeting, transgenes
  — Excellent genomic resources
• Identify disease genes
  — Study mechanism/progression
  — Understand cellular basis
  — Develop diagnostic/treatment methods

The Start of Mouse Genetics
• 17th Century Asia—mouse fanciers
  — Albino, dwarf and waltzer
• 1900—Abbie Lathrop—Granby, MA
  — >11,000 animals, cashed in on Victorian craze
  — 1902—received order from:
    • WE Castle—Harvard
      — Mouse colony burned down
      — Black, silver, frizzy, behavioral traits
      — His undergraduate student:
• 1906—CC Little
  — 1909—Inbred strains (brother/sister matings)
  — With $ from industry, started:
• 1929—Jackson Laboratory (JAX)
  — Now, 2500 strains housed
• 1940s—Specific Locus Test—Bill Russell
• 1960s—Mouse Embryology
  — Beatrice Minz and Anne McClaren
• 1982—Transgenics—Palmiter and Brinster
• 1987-89—ES cells and knockouts—Capecchi, Evans and Smithies
The male and female germlines are different

Miyoshi et al., 2006

Mammals need both a maternally and a paternally derived genome for normal development: parthenogenesis is not successful

Miyoshi et al., 2006
The male and female germlines are different

Haig’s Hypothesis: A Molecular and Evolutionary Battle of the Sexes

- Imprinting evolved due to a ‘conflict of interest’ between mothers and fathers
- Maternally expressed genes preserve the fitness of all progeny by limiting access to maternal resources
- Paternally expressed genes maximize fetal access to maternal resources at the expense of subsequent progeny

Imprinting in PWS and AS

Prader-Willi Syndrome
- Lack of pat 15q11-13
- Small at birth
- Hyperphagia/obesity
- Mild dev delay
- Behavioral problems

Angelman Syndrome
- Lack of mat 15q11-13
- Profound speech defect
- Poor motor skills
- Severe dev delay
- Happy affect

Imprinted Expression

PWS Gene

AS Gene

P M

P M

Imprinted Expression
Imprinted Expression

Imprinted Genes
- Mono-allelic: Expressed from either the Maternal OR Paternal allele
- Tissue-restricted imprinting (i.e. only the brain is mono-allelic)
- Approximately 200 imprinted genes
- Paternally expressed genes promote growth
- Maternally expressed genes inhibit growth

Egypt

How does the embryo know which alleles come from Mom and which alleles come from Dad?

Epigenetic Gene Regulation
- "on top" of genetics
- Heritable changes that affect expression, but not the primary DNA sequence

The maternal and paternal genomes are ‘marked’ epigenetically during development, and these marks must be erased and reset at each generation
Epigenetic Marks
- Allele-specific (imprinted) & spatio-temporal expression imprints or patterns caused by:
  - Differential DNA methylation imprints
  - Alterations in chromatin structure
  - Expression of non-coding RNAs

DNA Methylation
- Post-replication addition of a CH3 group to Cytosines within a CpG dinucleotide
- Interacts with histone modifications and non-coding RNAs to regulate transcription
  - Tissue-specific
  - Temporal
  - Imprinted

In germ cells of boys, the maternal marks must be erased and re-established as paternal marks.

DNA methylation Marks DNA in adult cells

DNA methylation Marks DNA in adult cells
DNA methylation marks DNA in adult cells.

DNA from mother
DNA from father

DNA Marks are erased and reset in the germline.

DNA from mother
DNA from father

DNA from mother
DNA from father

In girls, the paternal marks must be erased and re-established as maternal marks.

DNA methylation marks DNA in adult cells.

DNA from mother
DNA from father

DNA from mother
DNA from father

DNA from mother
DNA from father

DNA methylation marks DNA in adult cells.

DNA from mother
DNA from father

DNA from mother
DNA from father
DNA Marks are Erased and reset in the germline

DNA from mother

DNA from father

DNA Methylation and Imprinting

PWS Gene

AS Gene

DNA Methylation and Imprinting

PWS Gene

AS Gene

Imprints are Erased and Re-established in Germ Cells

How is PWS Inherited?

PWS is a defect on the paternal allele of Chr 15

Surani, Nature 2002
How is AS Inherited?
PWS is a defect on the paternal allele of Chr 15

How do the stars change color?

Epigenetic Changes Influence Gene Expression at Many Types of Genes.

Diet and Other Environmental Factors can Alter Epigenetic Modifications, Thereby Changing Gene Expression.

For Kai, the Best Dog Ever
Did you have any trouble trying to figure out the mode of inheritance of star color in the Star-Belly Sneetches?

That’s because this trait is controlled by another genetic system that works in addition to the direct translation of certain genes.

Epigenetics - “on top of genetics”

Genomic imprinting
Genomic imprinting - the example of epigenetics at work in the Star-Belly Sneetches

Genomic imprinting - the information in certain genes is active only when it passes to a child through the sperm or egg.

Genomic imprinting - a small number of genes are stamped with a “memory” of which parent they came from.

Let’s see how imprinting works . . .
Biallelic

the way most of our genes work

AA

A

A

protein

protein
Monoallelic

\[ \begin{align*}
A & \rightarrow \text{protein} \\
A & \rightarrow \text{protein}
\end{align*} \]

\[ \begin{align*}
\cancel{A} & \rightarrow \text{protein} \\
A & \rightarrow \text{protein}
\end{align*} \]

\[ \begin{align*}
\cancel{A} & \rightarrow \text{protein} \\
\cancel{A} & \rightarrow \text{protein}
\end{align*} \]

\[ \begin{align*}
\cancel{A} & \rightarrow \text{No protein} \\
A & \rightarrow \text{protein}
\end{align*} \]
Imprinting explains what we saw in the Star-Belly sneetches.

Imprinting but Star-Belly sneetches are make-believe.

Imprinting a real-life human example showing the results of imprinting.

Prader-Willi syndrome

Prader-Willi syndrome
Both Prader-Willi and Angelman syndromes are caused by a deletion of the very same segment of chromosome #15.

How can this same deletion produce two very different syndromes?

About 156 genes are imprinted in mammals.

Approximately 88 of these are active (i.e. expressed) on the maternally inherited chromosome.

Approximately 68 of these are active (i.e. expressed) on the paternally inherited chromosome.
So the product of this sperm and egg (embryo) has about 1% of its gene pairs working in a monoallelic form.

In fact, these particular gene pairs **MUST** be monoallelic in order for normal embryo development to occur.

So just what IS it that makes this allele inactive?

It’s the adding of methyl groups onto the allele.

(Remember: “epi-” means “on top of” - **EPIGENETICS** )

It’s the adding of methyl groups onto the allele.

(Remember: “epi-” means “on top of” - **EPIGENETICS** )
So just what IS it that makes this allele inactive?

It's the adding of methyl groups onto the allele.

Remember: "epi-" means "on top of" - **EPIGENETICS**

Some of the genes that are imprinted in the egg and in the sperm lie in a region of the long arm of chromosome 15.

S and U are two different imprinted genes. The paternal allele of the S gene is active, while the maternal allele of the U gene is active.

If this region of chromosome 15 is deleted, the embryo would have the S allele working and the U allele from Mom working.

The embryo with this pair of chromosomes would have the S allele from Dad working and the U allele from Mom working.
If this region of chromosome 15 is deleted

then this embryo has no functioning S allele, resulting in Prader-Willi syndrome.

If the same region of THIS chromosome 15 is deleted

then this embryo has no functioning U allele, resulting in Angelman syndrome.