You can be anything you want to be when you grow up.

STEM CELL PARENTAL ADVICE
Review/discuss eukaryotic gene regulation & mutations

- Methylation
- Restriction enzymes-splicesome
- Non-coding region placement-”junk DNA”
- Micro RNA’s-gene silencers

- Mutations-point level missense chromosomal-inversions
Master Genes

Sequences of DNA found in all animals control formation of proteins necessary for large scale, embryonic development. (Morphogenesis) They code for big scale traits like; head, tail, arms, wings, organs etc.

They are broken down into regions that are called hox genes. “Tool kit” genes-AKA homeobox genes.
Mutations in these genes, or editing and or silencing of these genes can have profound effects on organism development.
Master genes (hox, cont):

These genes are on and off (transcribed/translated) at different times during development. This switching of “on” and ‘off” dictates the differentiation of the cells they regulate.

These influenced cells are called: **totipotent**—(adjective) having the potential for developing into various specialized ways in response to DNA messages carried by the hox genes. (Undifferentiated blastocysts)

Hox genes can influence through; hormones, translation timing and can be influenced by temperature, nutrients…..
Main events impacting the outcome of these genes

- When master genes are “on” and “off”
- Mutations in master genes
- Environmental feedback on master genes (epigenetics)
- Regulation in and around master genes
Embryos develop using **modules**—which are functional structures consisting of genes, the signaling pathways the genes stimulate, and physical structures that result from active genes. These modules are controlled by hox genes.

This means that we can change one module at one time in one group independently of other modules.

This is how mutations with potentially large effects change only one part of the body.
Example: In most salamanders, the webbing between the toes disappears as a hox gene mutation during embryo development causes advanced apoptosis of webbing.

However a separate mutation in those same genes, allows an arboreal species to evolve webbing for better grip.

In other species, mutations can result in polydactylies.
Another example

Duck and chicken embryos both have webbing genes; BMP4, there loci is on the chromosome of limb development. But in the chicken there is a mutation in that gene that instructs cells in the chicken’s foot to undergo apoptosis causing a loss of webbing.

Experimental application of mutated BMP4 into a duck embryo results in a non-webbed foot in a duck.
Figure 20.5 Changes in *gremlin* Expression Correlate with Changes in Hindlimb Structure
Action of genes controlled by genetic switches underlie both the development of an individual organism and the evolution of differences among species.

In the arthropods, morphological changes in species have evolved through mutations in genes that regulate differentiation of segments.
All arthropods have the Hox gene *Ubx*.

Insect *Ubx* has a mutation; Ubx protein from this gene inhibits expression of the *distal-less* gene (*dll*) which is essential for leg formation.

Insect *Ubx* is expressed in the abdomen segments—result: insects have only six legs, none on the abdomen.
Figure 20.7 Mutation in a Hox Gene Changed the Number of Legs in Insects
Similar processes govern development of the vertebral column.

Vertebral column has anterior-to-posterior regions (cervical, thoracic, lumbar, caudal). The regions are controlled by Hox genes.

The characteristic numbers of vertebrae in different species result from genetic changes that expand or contract the expression domain of different Hox genes.
Hox genes are responsible for overall development in all animals:

Manipulation of these genes can have huge impacts on the organism’s phenotypic expression:
Fertilization → Zygote → Cleavage* → Morula → Blastula** → Gastrula → Neurula → Embryo
<table>
<thead>
<tr>
<th></th>
<th>Cleavage</th>
<th>Morula</th>
<th>Blastula</th>
<th>Gastrula</th>
<th>Larva/Embryo</th>
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<td><img src="image9" alt="Frog Gastrula" /></td>
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<tr>
<td>Human</td>
<td><img src="image11" alt="Human Cleavage" /></td>
<td><img src="image12" alt="Human Morula" /></td>
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<td><img src="image14" alt="Human Gastrula" /></td>
<td><img src="image15" alt="Human Larva/Embryo" /></td>
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Evolution of form has not been a result of radically new genes, but has resulted from modifications of existing genes.

Developmental genes constrain evolution in two ways:

- Nearly all evolutionary innovations are modifications of existing structures.
- Genes that control development are highly conserved.
Highly conserved developmental genes make it likely that similar traits will evolve repeatedly—parallel phenotypic evolution.

Example: Flight
Environments role?

- Many master genes (hox genes) are epigenetic and therefore are sensitive to external stimuli.
How can humans take advantage of master genes?

- Recombinant DNA organisms (rDNA) are those organisms who have been intentionally genetically modified
- Can also be called “transgenic”
gene therapy
I’m worried that health care has become too impersonal, Doc.

Nonsense... just relax and lie back on the bar code scanner.

Rob Rogers / Pittsburgh Post-Gazette
Frankenfish

HOW THEY COMPARE

GM salmon
Length: 24ins
Weight: 6.6lb

Farm salmon
Length: 13ins
Weight: 2.8lb

*Both fish are 18 months
http://video.pbs.org/video/1701025927/

(41.25)

https://www.youtube.com/watch?v=kgXtktFaADM
4 basic things must be determined to use DNA based technologies

- 1- what is the gene’s sequence?
- 2-where (what locus) is the gene?
- 3-quantity, do we have enough gene material to work with?
- 4-are there mutations involved in the gene?

So, there are many technologies that assist with these needs.
SNPs (Snips)

- Single nucleotide polymorphisms, frequently called SNPs (pronounced “snips”), are the most common type of genetic variation.
- SNPs represent differences in a single DNA building block, called a nucleotide. (A,T,G or C)
- For example, a SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T) in a certain stretch of DNA.
- These can be used as genomic markers or disease indicators.
- They are the foundation of many DNA technologies.
Genetic Engineering?

Key words: Enzyme, gene, desired
Micro-arrays

- Technology multiple genes can be tested at one time to see if mutations are present in a known gene sequence.

- Using a PC walk through this virtual lab:

  http://learn.genetics.utah.edu/content/labs/microarray/
Types of Genetic technology

DNA fingerprinting: Using the order or sequence of nitrogenous base pairs to identify a person.

1. Cut the DNA with restriction enzymes at SNP targets.
2. Place all samples into a gel bed at the negative side, because DNA has an overall negative charge.
3. Apply electricity and wait for each sample to move. (Smaller fragments move faster and farther than larger ones do)
4. Apply a dye to the gel and photograph it, compare all samples. (Rf value)
5. Look for exact matches in the fragments (DNA fingerprints).
6. Uses targeted SNPs DNA because those regions can be unique to each person.
Try these! (Please don’t shout out!)

<table>
<thead>
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<th>Culprit</th>
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Polymerase chain reaction (PCR)- Amplification of any piece of DNA

1. Use heat to break apart DNA that you have. (This is called denaturing)

2. Add DNA polymerase, must come from heat tolerant bacteria. *Thermus aquaticus*

3. DNA polymerase will copy in both missing strands to double the amount of DNA you had at the start.
Eukaryotic gene cloning

1. Isolate the gene you want to clone.
2. Find a bacterial plasmid (bonus DNA).
3. Cut the plasmid to the size of your gene you want to clone. (Use restriction enzymes)
4. Put the cut plasmid and the single gene together with DNA ligase. (sticky ends-5’ and 3’ and corresponding base pairs)
5. Let the bacteria multiply, all bacteria from here will have new gene and produce that protein for you. (competence)
Whole organism Cloning

1. Obtain nucleus from organism you intend to clone.
2. Obtain egg from female.
3. Remove the nucleus from that egg and place in nucleus from organism you want to clone.
4. Apply electric shock.
5. Place dividing egg back into surrogate female for gestation period.
Gene Therapy

Deliver healthy versus of genes to affected individual via vector (virus)
Allow those cells to multiple
Eventually more healthy cells will be present
Ex: cystic fibrosis
**Stem Cells**: Unspecified, totipotent, blank cells that do not have specific function. Uses the blastocyst.
3D Printing (Stem cells) - cloning organs

**HOW IT WORKS**

1. Tissue is taken from animal's muscle
2. Stem cells are extracted from the tissue
3. Muscle cells are grown under tension, to bulk them up
4. The new muscle fibres are minced and turned into burgers
Bio ethics.....