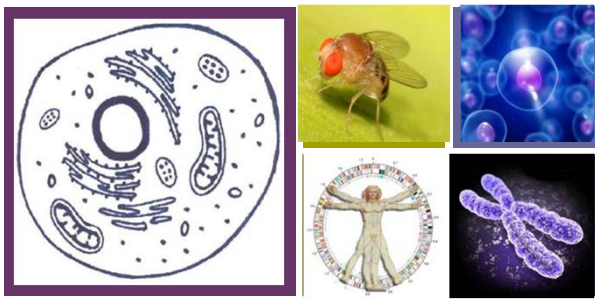
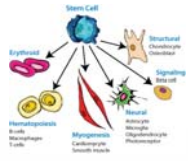


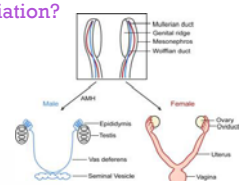
Eukaryotic Development

Presented by: Sean, Daria, Emily, and Maggie

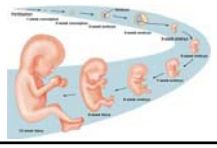
Differentiation vs. Development

What comes to mind when you think of differentiation?



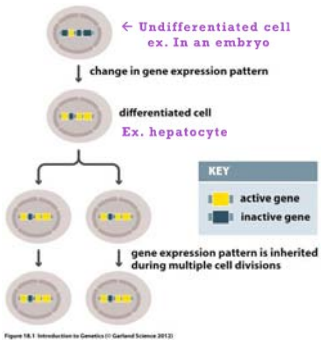


What about development?



Differentiation

- Individual cell acquires specialized function
- Change in pattern of gene expression in a cell
- Change remains and is passed on to future generations
- Example: human cells



Development

- Egg to adult
- Complex series of events
- Order, location, and timing are important
- Events include differentiation
- More on this later…

- Example: Human Development

10^{13} cells differentiated into 400 different types !

Different genes active in different cell types

Remember, humans have 400 cell types !!

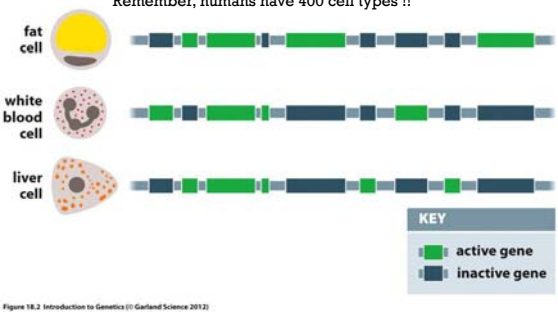


Figure 18.2 Introduction to Genetics (© Garland Science 2012)

4 ways to permanently change gene activity

1. Physical rearrangement of the genome
2. Genetic feedback loops
3. Chromatin modification
4. DNA methylation

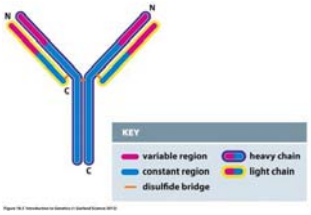
1 & 2 = Sean
3 & 4 = Maggie

Brief Review: Immunology

- Mammalian immune system has many components
  - Lymphocytes differentiate into components
  - B lymphocytes → Immunoglobulins
  - T lymphocytes → T-cell receptors
  - Both of those proteins atach to outer surface of cells. Some immunoglobulins are also released into bloodstream
  - These proteins help to prevent body against invasion of antigens (bacteria, viruses, or any other unwanted substances)

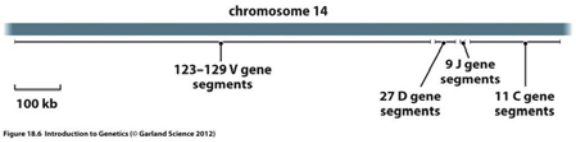
Genome rearrangement

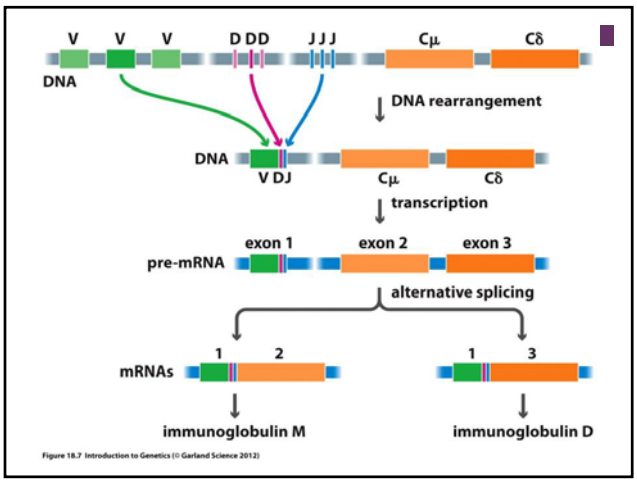
- Example – Lymphocyte Differentation
- Lymphocytes produce immunoglobulins



Genomes for Chains

- That's what's interesting, there isn't a single genome for heavy and/or light chains
- There are instead gene segments on chomosome 14



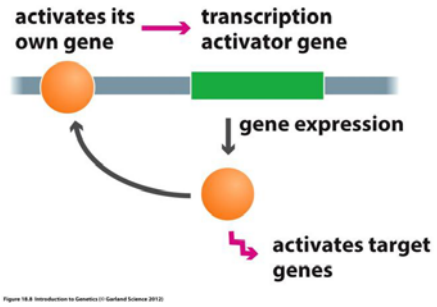


T-cell receptor diversity

- Also based on rearrangement
- V, G, J, and C gene segments are linked in different combinations to produce cell-specific genes
- Each receptor has
  - Two β molecules (similar to immunoglobulin heavy chain)
  - Two α molecules (similar to immunoglobulin κ light chain)

Like immunoglobulins, T-cell receptors embed in cell membrane and enable each lymphocyte to identify and act against its own extracellular antigen.

Genetic Feedback Loop



MyoD protein of vertebrates

- Protein involved in muscle development
- A cell becomes committed to becoming a muscle cell when it has MyoD expression
- *myoD* codes for a transcription activator that targets other genes in muscle cell differentiation
- MyoD protein binds upstream ensuring that its own gene is always expressed.
- Heritable because MyoD transmitted to daughter cells

Another example: Deformed (Dfd)

- Dfd plays a role in *Drosophila* development
  - Without it fly's head has improper dvelopment

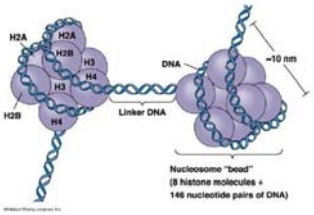
Dfd then needs to be continually expressed in cells that at some point will give rise to insect's head.

Dfd binds to an enhancer upstream of *Dfd* gene

3. Chromatin modification

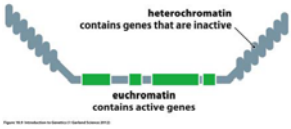
Note: chromatin= DNA + proteins in nucleus

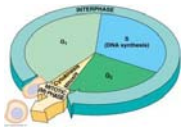
- Induced by nucleosome modification
- Leads to heritable changes in chromatin structure



Happens during interphase

- Heterochromatin = most compact.





* RNA Pol can't access genes in heterochromatin.

- Packaging of regions into heterochromatin influences which genes are expressed
- Primary determinant of cellular differentiation

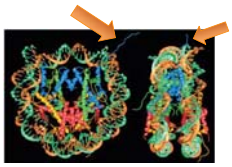
Chemical modification of histones

- Main influence on degree of packaging in different regions

4 types:
- acylation
-methylation
-phosphorylation
-addition of ubiquitin

- Acylation is best studied

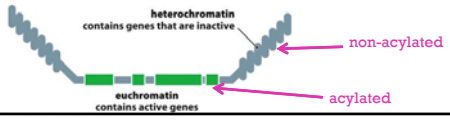
Acylation



- Occurs on lysine (K) residues
- N-terminal regions of histones

Acylation

- Decreases histone affinity for DNA
- Decreases interaction between nucleosomes
- Histones in heterochromatin= NO acylation
- Inactive genes
- Acylated areas are ACTIVE
- Balance of histone acyltransferases (HATs) and histone deacylaces (HDACs).



Other chemical modifications

- Methylation → lysine (K) and arginine (R)
- Phosphorylation → serine (S)
- Ubiquitin → binds lysines in C-terminal region

◆ **Different sites interact**
- determines degree of chromatin packaging taken up by a stretch of DNA

Methylation examples

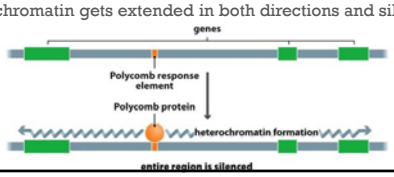
- Lysine 9 of histone H3→
  - binding site for HP1 protein
  - induces chromatin packaging
  -silences gene expression
- Blocked by double-methylation of lysine 4
- Therefore, methylation of lysine 4
  = open chromatin structure
  = associated with active genes

Histone Code??

- Growing awareness of variety of modifications
- Possible Histone Code:
- **The pattern of chemical modifications species which regions of the genome are expressed at a particular time.**
- Examples exist in which histone modification is directly linked to cellular differentiation
- *Drosophila*
- Still don't know how histone modification patterns are inherited during cell division

Polycomb and trithorax in *Drosophila*

- *Polycomb*: multigene family
  30 genes code proteins
  Bind Polycomb response elements (10kb of DNA)
  induce formation of heterochromatin
  trimethylation of Lys 9 & 27 of histone H3
  induces chromatin packaging
- Heterochromatin nucleation of Polycomb
- Heterochromatin gets extended in both directions and silences genes

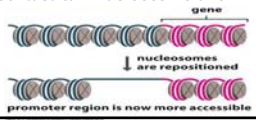


Polycomb and trithorax in *Drosophila*

- Silenced genes control development of head, legs, etc.
  **location, timing.
- Polycomb proteins do NOT determine which genes are silenced
  -genes already repressed
  -Polycomb *maintains* this
- Heterochromatin induced by Polycomb is heritable !
  (therefore permanent)

Polycomb and trithorax in *Drosophila*

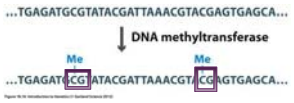
- Trithorax proteins opposite Polycomb
- Open chromatin state & active genes
- Targets same genes that are silenced by Polycomb
- Perform **nucleosome remodeling**
  - repositioning of nucleosomes within target region
- Not chemical
  -energy-dependent process
  -weakens contact b/w nucleosome and DNA



← RNA Pol can bind and transcribe

4. DNA Methylation

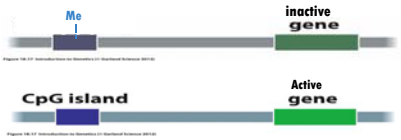
- Chemical modification of DNA rather than nucleosome
- Silences regions of genome (entire chromosomes?)
- Methylation of Cytosines by **DNA methyltransferases.**
- Not random
  5'-CG-3'
  5'-CNG-3' (in plants)
  N=any of the four nucleotides



How do we know there's a link between methylation and gene expression ?

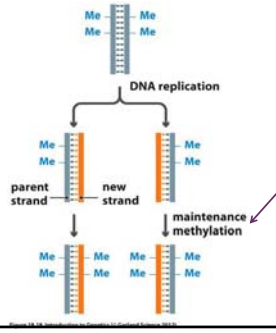
The Link: CpG islands

- In humans, 40-50% of genes located close to CpG islands
- CpG island= 1kb sequence with rich CG content
- CpG islands unmethylated in housekeeping geens
- CpG islands associated with tissue-specific genes:
  - **methylated** in tissues where the gene is **not expressed**
  - **unmethylated** in tissues where the gene is **expressed**



How is the pattern inherited?

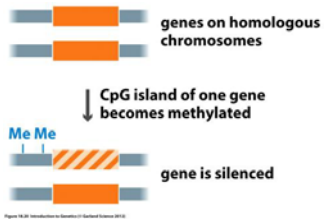
- Maintenance methylation by methyltransferase (Dnmt1)



Dnmt1 scans along and adds methyl groups to CpG islands corresponding to parental ones

Genomic Imprinting and methylation

One of a pair of genes is silenced



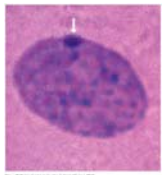
- When chromosomes are passed on to offspring, the silenced set of genes pay be inherited.
- Imprinted copy of chromosome can be maternal or paternal

Genomic Imprinting and methylation

- Imprint control elements (close to imprinted genes) mediate methylation of imprinted regions
- Still don't know why imprinting happens.
- Mice with 2 copies of maternal genome fail to develop properly

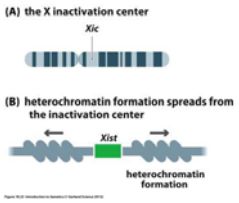
X-inactivation

- A special form of imprinting
- Inactivation of 80% of genes on one of the X chromosomes in a female mammalian cell (Fs have two X chromosomes)
- Prevents females from synthesizing X-coded proteins at twice the rate of males
- Inactive X stays in nucleus as a **Barr Body** (all heterochromatin)



X-inactivation

- Early embryonic development
- Controlled by X-inactivation center (Xic)
  - present on both X's
- One Xic initiates formation of heterochromatin
  -spreads over whole chromosome in a few days



- Xist protein transcribed into non-coding RNA
  - copies coat the chromosome as hc is formed
- Histones modification and DNA methylation occur too
- Heritable

Coordination of Gene Activity During Development

Model Organisms

Positional information

Development Gene Activity

Genes in Plant Development

Model Organisms

- Complex developmental processes in higher eukaryotes
- Parallels between between developmental processes in different organisms
- **Model Organism**: an organism that is relatively easy to study so can be used to obtain relevant information to the biology of a second organism that is more difficult to study.

-examples: fruit fly (*D. melanogaster*), nemotode (*C. elegans*)

Caenorhabditis elegans

- 1960's- Sydney Brenner
- Advantages:

-easy to grow in laboratory

-has short generation time

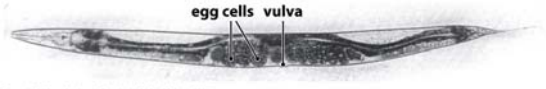
-transparent at all stages of life cycle

-every cell division in pathway previously identified

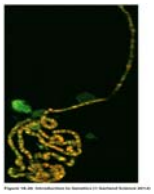
-complete connectivity of cells in the nervous system has been mapped

-small in size

-entire sequence is known



Drosophila melanogaster



- 1910- Thomas Morgan
- Advantages:

-small in size

-minimal nutritional requirements

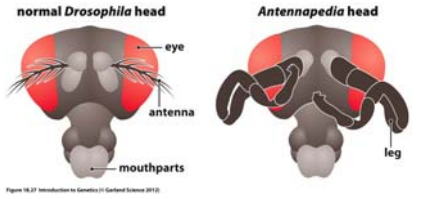
-presence in natural populations of occasional variants with easily recognized genetic characteristics

-small genome

-gene isolation is aided by the presence in the salivary glands of "giant" chromosomes

Antennapedia

- Discovered in 1915
- Mutant with legs where eyes should be
- First indication that mutation can affect not just phenotype but also the underlying body plan of an organism

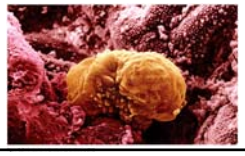


Cell-to-cell Signaling and Positional Information

- **Positional information**: information that enables a cell to follow the differentiation pathway that is appropriate for its particular location in a developing organism.
- *C. elegans*:

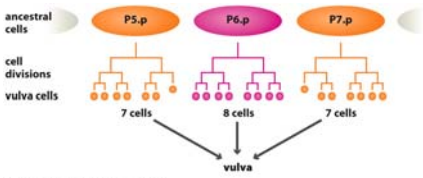
  -cell-to-cell signaling is critical for the establishment of positional information within a developing embryo

  -hermaphrodites and vulva derived from three **vulva progenitor cells**

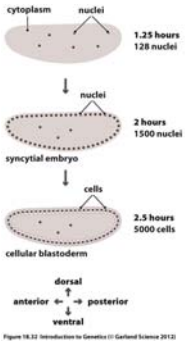


Vulva Progenitor Cells

- P4.p, P6.p & P7.p located in a row under-surface of the developing worm
- Each committed to differentiation pathway and production of vulva cells
- P6.p- primary vulva cell fate
- P5.p & P7.p- secondary vulva cell fate
- 22 resulting cells reorganize their positions to construct the vulva
- Vulva development must occur in the correct position relative to the gonad
- Role of anchor cell



Positional Information in the Fruit Fly Embryo with Maternal Genes

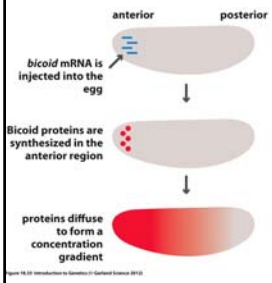


- **Syncytium**: a cell-like structure with a mass of cytoplasm and many nuclei.
- **Blastoderm**: the structure that forms when individual cells start to appear around the outside of the syncytium of a developing fruit fly embryo.

  -Before the blastoderm stage, positional information has begun to be established

- Positional information is the ability to distinguish anterior from posterior and dorsal from ventral

Maternal Genes

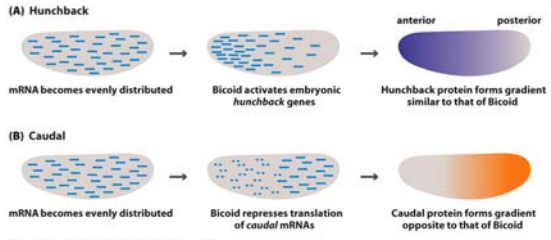


- Most of these proteins are translated from mRNAs & injected into the embryo by the mother
- **Maternal Gene**: a fruit fly gene that is expressed in the parent & whose mRNA is subsequently injected into the egg, after which it influences development of the embryo.
- Example: *bicoid*
  - -transcribed in the maternal nurse cells
  - -*bicoid* proteins are translated from the mRNA and diffuse through the syncytium, setting up a concentration gradient

Maternal Genes

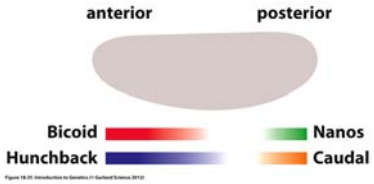
- Other maternal effect gene products involved in setting up the A-P gradient:

  -hunchback, nanos & caudal



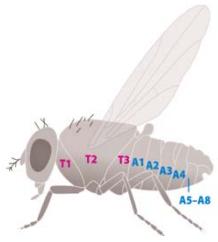
Maternal Genes

- Nanos mRNA is transported to the posterior part of the egg and attached to the cytoskeleton and its protein represses hunchback mRNA translation.



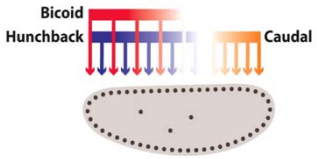
Positional information to segmentation pattern

- Body segments: 3 head, 3 thorax (1 pair of legs), and 8 abdomen
- Each point in syncytium has unique protein signature



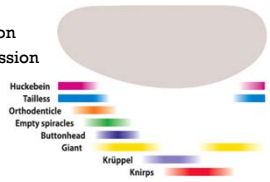
Gap Genes

- Segmentation increases with the expression of gap genes
- Bicoid, Hunchback, and Caudal are also transcription activators (targeting gap genes in nuclei)
- Identity of gap genes expressed depends on the concentration of gradient proteins along the A/P axis (positional info important)

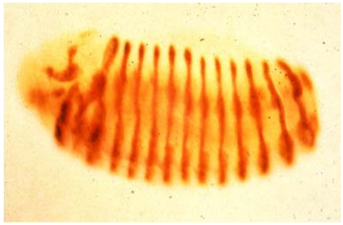


Gap Genes

- Activation of gap genes:activated directly by Bicoid, Hunchback, and Caudal
  eg. *buttonhead*, *empty spiracles*, *orthodenticle* (Bicoid control)
- Indirect activation by transcription factors
  eg. *huckebein* and *tailless*
- Repressive effects
  eg. Bicoid represses *knirps* expression
- Gap genes regulate their own expression

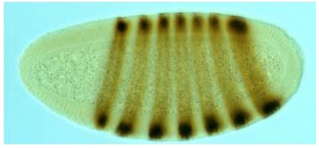


Pair-rule Genes



- Establish the basic segmentation pattern
- Transcription of these genes is dependent on the concentrations of gap genes products the segmentation at this point in development is a "striped" pattern
- Set of cells expressing particular combination of pair-rule genes

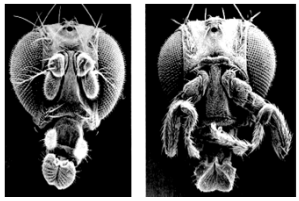
Segment Polarity Genes



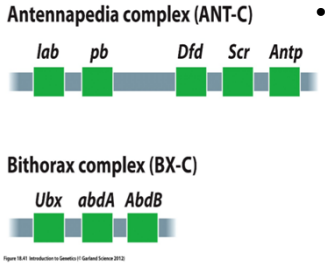
- Provide greater definition to stripes set size and precise location to future segments
- Positional info from maternal effect gradient has led to a sharply defined segmentation pattern

Segment identity: Homeotic selector genes

- Pair-rule and segment polarity genes establish a pattern of segmentation
- Identity of individual segments the responsibility of homeotic selector gene
- Homeotic selector genes first discovered in *Antennapedia* (mutant fruit fly with a pair of legs in place of antennae)



- Genetic mapping: clustered in two groups on chromosome 3
  - ANT-C (head and thorax determination) and BX-C (abdomen determination)
  - Correct activation dependent on positional information (gap and pair-rule proteins)

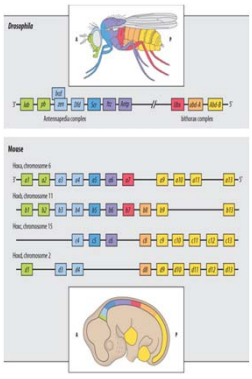


- Selector gene products:
  - transcription activators that initiate differentiation
  - repressive effects ensure maintenance of differentiation
    - Polycomb = inactive heterochromatin over unexpressed selector genes

Homeotic selector genes involved in vertebrate development

- **Homeobox genes:** group of transcription factors containing 60 amino acid homeodomain (which is encoded by 180 bp homeobox sequence)
- The homeodomain allows proteins to bind to DNA= transcription activators
- Not all selector genes
  eg. *even-skipped* and *fushi tarazu* (pair-rule genes), *engrailed* (segment polarity)

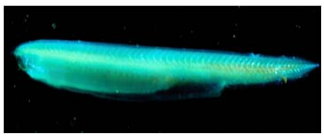
Homeoboxes in other organisms



- Present in wide variety of animals, from nematodes to humans
- Homeo selector genes, specify body plan like ANT-C or BX-C
  eg. *HoxC8* in mice = extra pair of ribs
- HOM-C: the homeotic gene complex that consists of ANT-C and BX-C clusters
- Vertebrates: HoxA, HoxB, HoxC, and HoxD
- Additional clusters related to added complexity of vertebrate body plan
- Expressed co-linearly

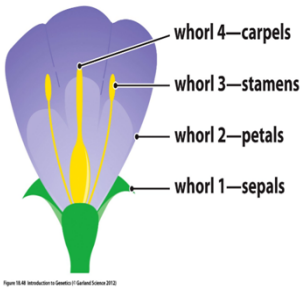
Evolutionary Relationships: HOM and Hox

- HOM cluster and four Hox clusters have genes at equivalent positions
- Multiple rounds of duplication in ancestral species
- Amphioxus has two Hox clusters (protovertebrate)
- Ray-finned fish have seven
  - Vast range of body plan variations



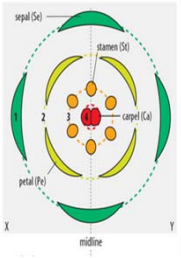
Homeotic genes underlie plant development

- Structure of flower determined by homeotic genes
- Model organism: *Arabidopsis thaliana* (genome = 123 Mb)
- All flowers constructed from four concentric whorls
  - Whorl 1: sepals
  - Whorl 2: petals
  - Whorl 3: stamens
  - Whorl 4: carpels

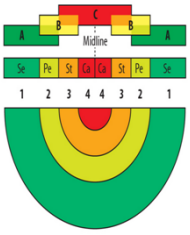


ABC Model

- No homeodomain proteins, but mutations in certain genes lead to homeotic changes in floral architecture
- Three types of homeotic genes: A, B, and C



Whorl 1: A-type genes (*apetala1* and *apetala2*)
Whorl 2: A-type acting with B-type genes (*apetala3*)
Whorl 3: B-type acting with C-type gene (*agamous*)
Whorl 4: C-type gene acting alone



The floral identity genes encode homeotic proteins

- A, B, and C homeotic gene products = transcription activators
- DNA-binding domain in proteins = MADS box
  - Also present in other plants, fungi, and animals
- *curly leaf*: gene whose product acts like Polycomb
  - maintains differentiated state of cell by repressing inactive homeotic genes