

MCDB 4650 Class 30: Last day of class!

Review Session for Final Exam

Tomorrow, Friday 2-3 pm B121

(sorry it's the only time I have available!)

Final Exam (here):

Saturday 1:30 PM

Molecular and Cellular Mechanisms

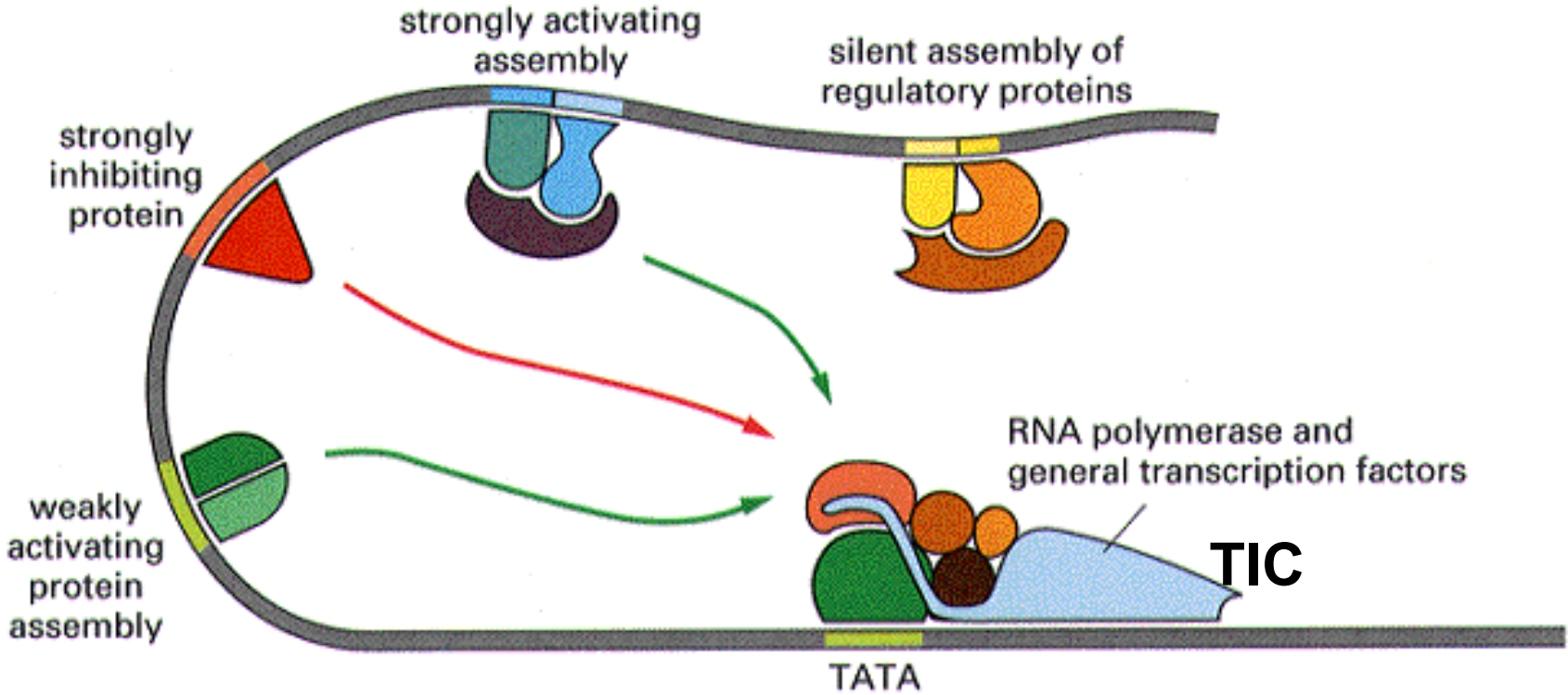
Gene expression in development is controlled at several levels,

e.g. transcription, post-transcriptional processing, post-translational protein modifications.

and it is controlled combinatorially.

e.g. by multiple transcription factors acting positively and negatively with each other and with DNA regulatory elements.

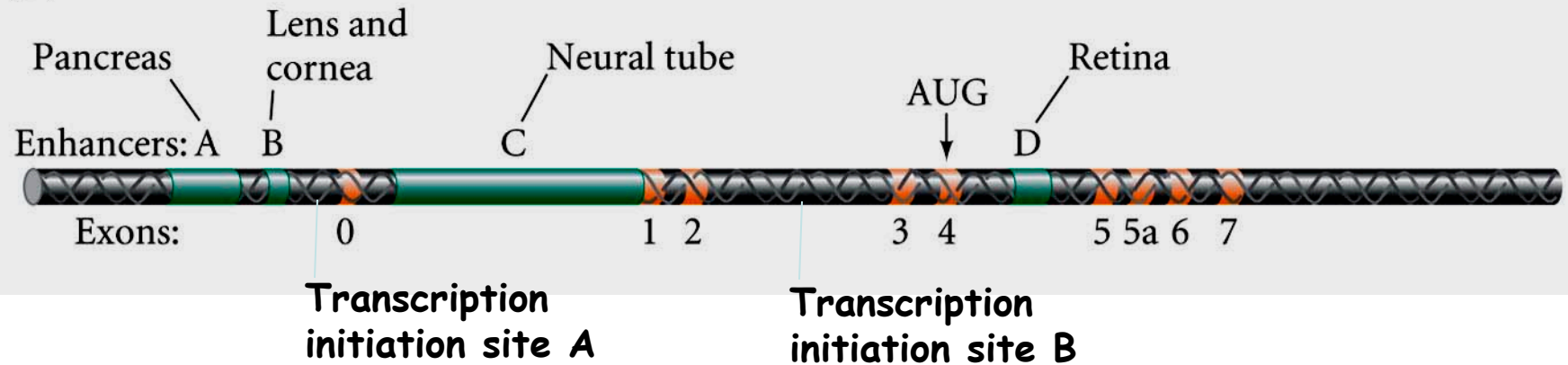
Transcription factors control rate of transcription initiation by the TIC



Below is the *pax-6* gene, which encodes a transcription factor expressed in many different places in the embryo

Orange: exons Green: enhancers (REs)

(B)

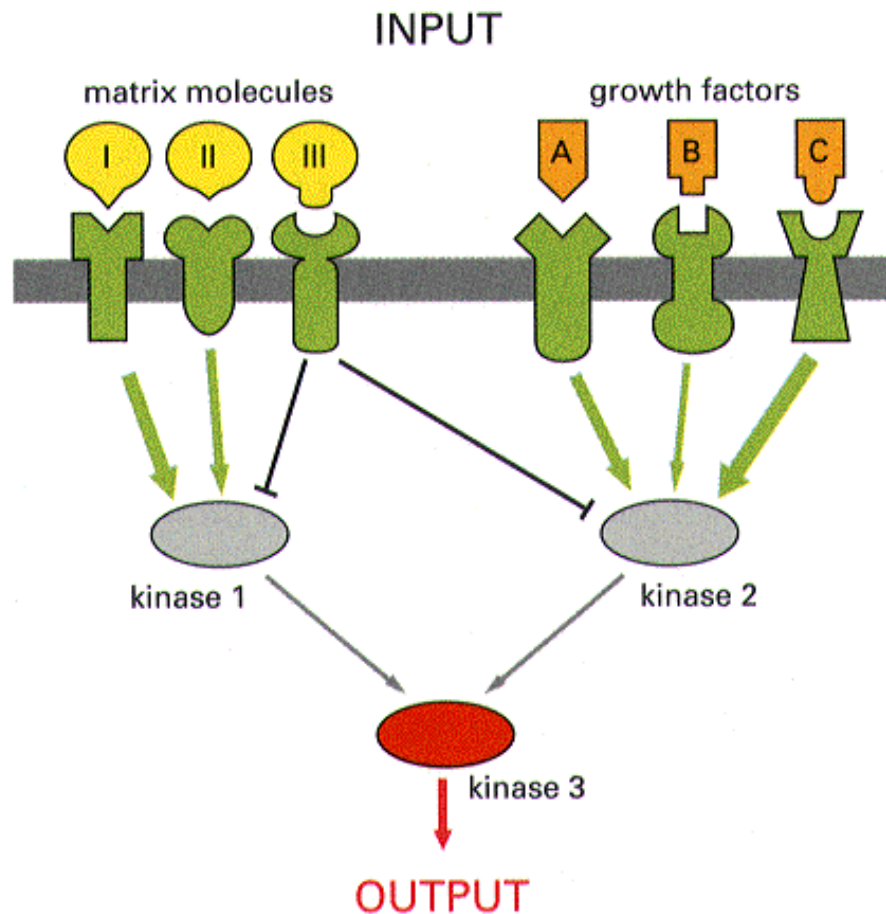


Given this information, what do you expect is true of the RNAs and proteins that will be made from the *pax-6* gene?

- Two different length RNAs and two different length proteins can be made
- Two different length RNAs are made; the protein is the same length assuming no alternative splice sites
- Only transcription site A will be used; one size protein will result
- Only transcription site B will be used, one size protein will result.

Molecular and Cellular Mechanisms

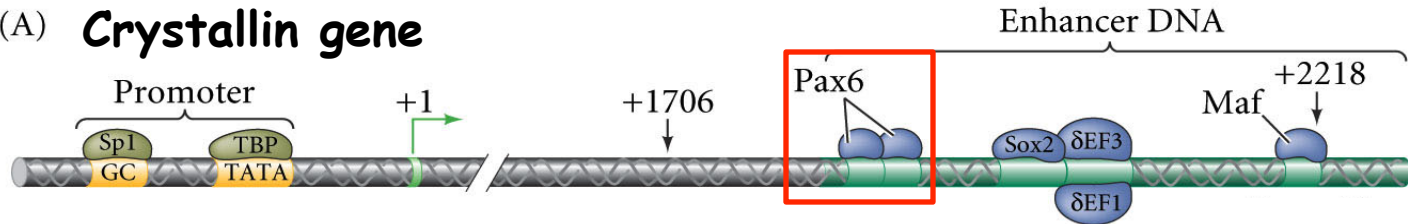
Signaling pathways (a small number) allow cells during development to communicate with each other, and they act combinatorially to control gene expression, cell behavior



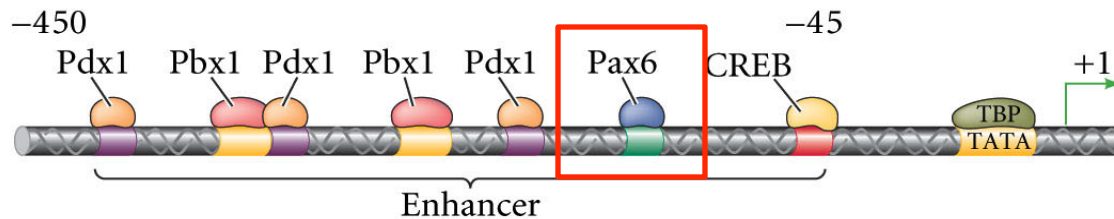
Remember that combinatorial controls often turn off genes where they are not supposed to be expressed, as well as turning on genes where they are supposed to be expressed.

Both of the genes shown below have large enhancer regions to which the transcription factor Pax-6 can bind.

(A) **Crystallin gene**



(B) **Somatostatin gene**



What is the best explanation for why crystallin protein is found only in the eye, while somatostatin protein is found only in the pancreas?

- Pax6 binding is only required for transcription of one, not both, of these genes.
- Pax6 can act as either an enhancer or silencer.
- Different additional transcription factors are required in each cell type.
- The enhancer regions to which Pax6 binds are different in cells of the pancreas cells vs. cells of the eye.

Using genetics to understand development

Forward genetics with certain organisms is a powerful approach to finding the genes that control developmental processes.

e.g. screening for mutants, mapping, gene identification, protein identification

Reverse genetics is a powerful approach to defining functions of cloned genes, in addition to experiments using mutants.

e.g. Targeted knockouts, RNAi, reporter constructs, etc

The components of the signaling pathway that determine the genes required for vulval fate determination in *C. elegans* were determined by (Choose the most useful approach)

- a) biochemical analysis of gene product activities.
- b) epistasis testing.
- c) forward genetics.
- d) reverse genetics.
- e) mosaic analysis.

The order of interactions among gene products involved in vulval fate determination was determined by -- ? (Choose the most useful approach):

- a) biochemical analysis of gene product activities.
- b) epistasis testing.
- c) forward genetics.
- d) reverse genetics.
- e) mosaic analysis.

You know the following information about mutants in the pathway of genes that help to specify the vulva in *C. elegans*:

<u>Mutant gene</u>	<u>Phenotype</u>
(If mutations)	
let-60	Vul; all Pnp cells make epidermis
lin-1	Muv; all Pnp cells contribute to vulva
let-60; lin-1	Muv; all Pnp cells contribute to vulva

Of the pathways shown below, which best describes the above data? (Pathways show a gene's normal function.)

- a) $lin-1 \dashv\vdash let-60 \longrightarrow vulval\ fate$
- b) $lin-1 \longrightarrow let-60 \dashv\vdash vulval\ fate$
- c) $let-60 \dashv\vdash lin-1 \dashv\vdash vulval\ fate$
- d) $let-60 \longrightarrow lin-1 \dashv\vdash vulval\ fate$

Understanding embryonic development

Development requires establishment of asymmetries

first in the early embryo, later in other cells and tissues

"Give me one asymmetry, and I can explain development."
Sydney Brenner, 1960s

Cells in an embryo become "determined" for different developmental fates by two major mechanisms:

asymmetric segregation of cytoplasmic factors during cell division,

and in response to signals from other cells.

But now that we understand the mechanisms, both are just gene expression and signaling pathways all over again.

Maternal-effect lethal mutants

P ₀	+/+	mutagenize		
↓				
F ₁	<i>m</i> /+	“m” represents a loss of function in a maternal gene required for survival		
↓		Mate F ₁ animals together (or allow to self fertilize)		
F ₂ embryos:		+/+	<i>m</i> /+	<i>m</i> / <i>m</i>
embryo will		live	live	?

Question: If *m* is a strict maternal effect lethal mutation, *m*/*m* embryo will

- a) live.
- b) die.

Mesoderm arises near the equator (in the marginal zone) of the *Xenopus* blastula because

a) animal hemisphere cells in this region are autonomously determined to become mesodermal precursors.

b) vegetal hemisphere cells in this region are autonomously determined to become mesodermal precursors.

c) animal hemisphere cells in this region receive signals from underlying vegetal hemisphere cells and become mesodermal precursors.

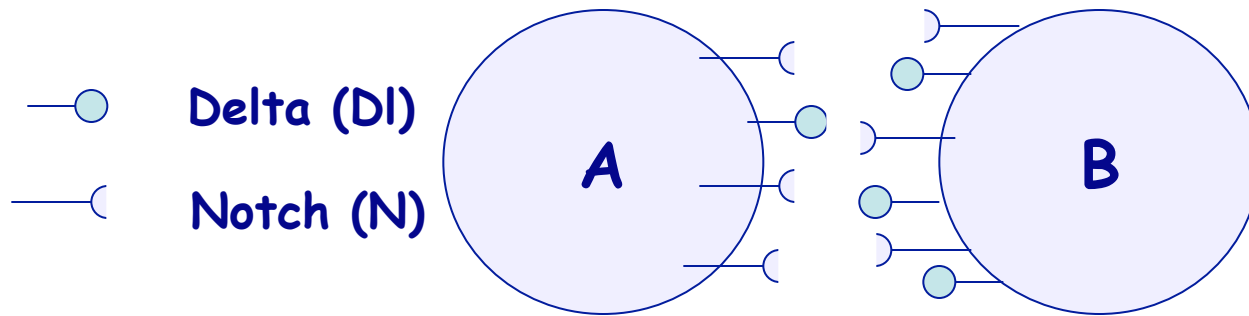
d) vegetal hemisphere cells in this region receive signals from overlying animal hemisphere cells and become mesodermal precursors.

The dorsalizing factors that induce formation of the organizer in *Xenopus* embryos

- a) arise at the point of sperm entry and move across the animal pole toward the organizer region.
- b) arise at the vegetal pole and move dorsally toward the organizer region.
- c) arise at the animal pole and move dorsally toward the organizer region.
- d) are present in ventral vegetal cells before the organizer forms.
- e) are present in dorsal animal cap cells before the organizer forms.

Which of these statements is true in the *Xenopus* embryo?

- a) The organizer induces formation of neural ectoderm by locally inhibiting an inhibitor of neural cell fates in the animal cap.
- b) The organizer induces formation of neural ectoderm by locally inducing animal cap cells that would otherwise adopt epidermal fates.
- c) The organizer cells use activated b-catenin as a master transcriptional regulator.
- d) The organizer secretes factors that activate formation of the Nieuwkoop center.



The neuroblast fate is prevented when a cell has bound Notch receptors. In a normal embryo, which of the two cells above will become a neuroblast?

- A
- B
- Neither
- Both

If an embryo were mutant for Delta (no Delta protein present on either cell above), which cell will become a neuroblast?

- A
- B
- Neither
- Both

Understanding embryonic development

In general, cells become more restricted in their developmental potential as embryonic development proceeds.

This progressive determination involves first combinatorial control by transcription factors, but later gene silencing by epigenetic changes in DNA and chromatin.

Understanding embryonic development

Sheets of cells (epithelia) can rearrange to change shape and move

e.g. convergent extension

Cells in an epithelium can leave it and become mesenchymal. Mesenchymal cells can join an epithelium.

e.g. during gastrulation.

Which of the following is NOT true of the organizer?

- a. Induces gastrulation
- b. Inhibits ventral ectoderm
- c. Converts ventral mesoderm to intermediate mesoderm
- d. Involutes through the dorsal lip of the blastopore
- e. Becomes intermediate mesoderm

Understanding embryonic development

Single cells can migrate through tissues,

and receive instructions on where to go and what genes to express from chemical signals along the way.

e.g. neuronal pathfinding, neural crest cells, hematopoietic stem cells. (Note: just signaling pathways again.)

Patterns of cells with different fates and behaviors can arise in response to gradients of diffusible signaling factors (morphogens),

and then be maintained by local cell interactions.

Morphogens can be intracellular (e.g. transcription factors in the *Drosophila* syncytial blastoderm embryo), but are usually extracellular. Again, signaling pathways, for both pattern determination and maintenance.

Understanding embryonic development

Overall patterning of animal body plan involves segmentation (or the equivalent establishment of repeating metamereric units of pattern)

and then control of segment identities by Hox genes and chromatin remodeling.

Hox proteins are master transcription factors

What establishes the anterior boundary of each Hox gene in *Drosophila*?

- a. gap gene combinations
- b. pair rule gene combinations
- c. A and B
- d. segment polarity genes
- e. the boundary of expression of other Hox genes

Many genes are evolutionarily conserved

Deep homology: genes have similar sequence, expression pattern, and function

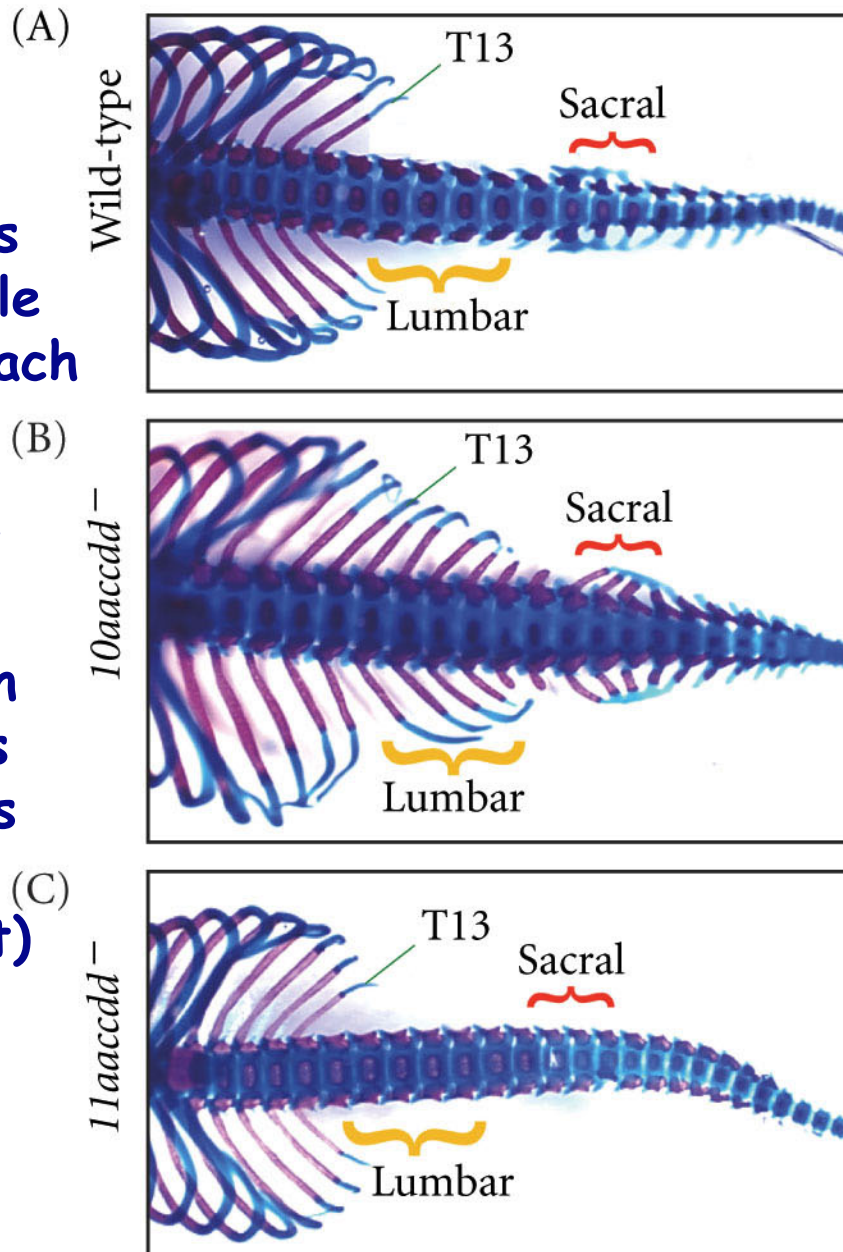
Random mutations can lead to changes in gene expression pattern or function

Genes can also be duplicated

If a mutation helps the organism survive, it is likely to be maintained

Vertebrates have multiple copies of each Hox gene

Hox loss of function mutations in vertebrates (all paralogs must be knocked out)



The phenotype of the loss of function of the Hox10 cluster can be described as

a. posterior to anterior transformation

b. anterior to posterior transformation

Gene replacement by homologous recombination

When a linear DNA construct is introduced into a mammalian cell, insertion at random nicks will always be more frequent than HR events, which must therefore be selected or screened for.

Remember that HR by definition requires two identical (or nearly identical) DNA sequences that recognize each other, pair, and then exchange a DNA segment.

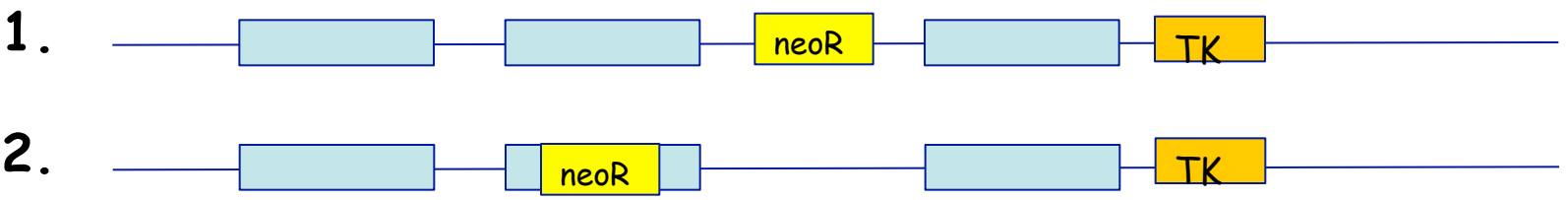
If HR is successful with the constructs below (the ES cell incorporates the construct shown),

- a. Cells with construct 1 will survive in the presence of neomycin
- b. Cells with construct 2 will survive in the presence of neomycin
- c. Cells with either of these constructs will survive in the presence of neomycin

Normal gene: 3 exons



Engineered constructs:

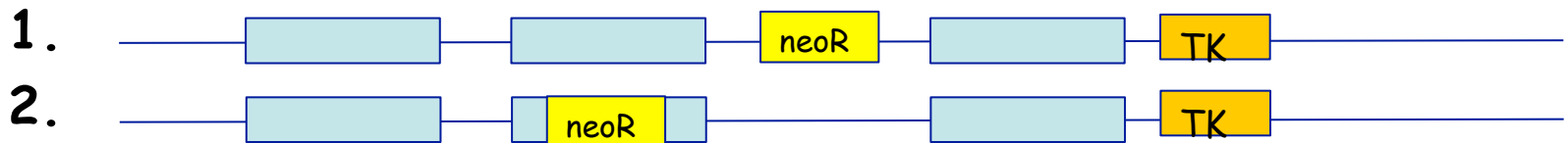


If you manage to make transgenic mice (using homologous recombination) with one of the engineered constructs below:

- Mice who are homozygous for construct 1 will have a knockout phenotype for this gene
- Mice who are homozygous for construct 2 will have a knockout phenotype for this gene
- Mice homozygous for either of these constructs will have a knockout phenotype for this gene
- Neither lines of mice homozygous for these constructs would have a knockout phenotype



Engineered constructs:



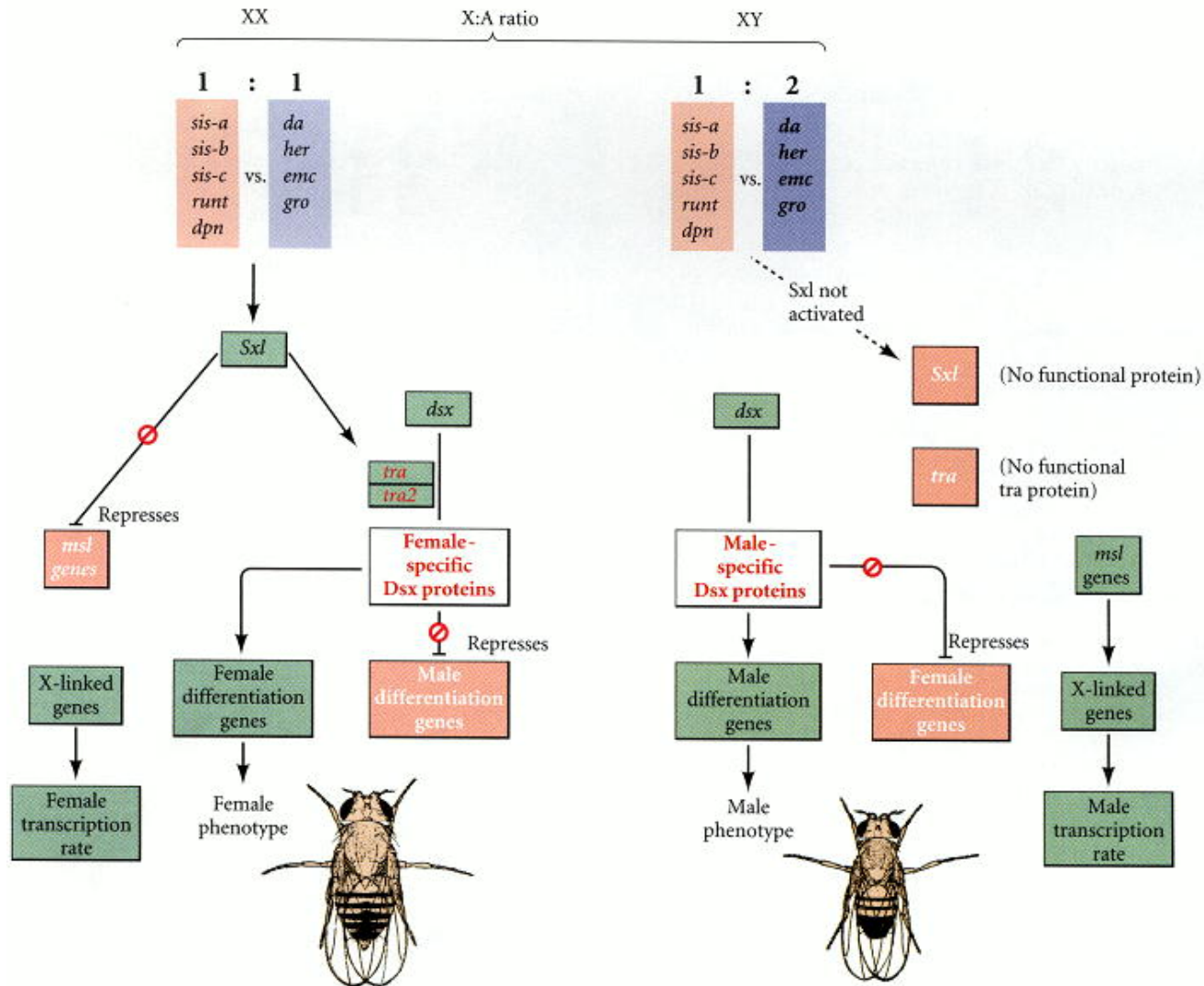
Understanding embryonic development

Animals have evolved a variety of chromosomal and gene control mechanisms for determining sex and maintaining an optimal sex ratio,

and for dosage compensation of sex-chromosome-linked genes in heterogametic species with chromosomally determined sex.

In which chromosomal combination would a gain of function mutation in the *Sxl* (sex lethal) gene be lethal?

- a. XX
- b. XY
- c. Neither
- d. Both



Imprinting

There's nothing special molecularly about imprinting; it's just gene silencing (methylation and histone modification) that occurs in a few genes during gametogenesis, and so affects the next generation.

In humans, more than 70 genes are imprinted (silenced) during gametogenesis.

This means that for each of these genes, you have only one functional copy; the other one was silenced in either the sperm or the egg you came from.

When a heterozygous $Igf2\Delta/+$ male is mated to a normal female ($Igf2+$) mouse, the resulting $Igf2\Delta/+$ embryos also die as above. However, if a heterozygous $Igf2\Delta/+$ female mouse mates with an $Igf2+$ male, the resulting $Igf2\Delta/+$ embryos develop normally. These results suggest that:

- a) The $Igf2$ gene is imprinted in males.
- b) The $Igf2$ gene is imprinted in females.