

Week 9 — Tuesday, 26 October

## 19. Drosophila Hox

- Review beSocratic
- Complete dorsal axis specification
- Consider homeotic – Hox genes



Put in your pods  
Start recording  
Share screen!

How is using groupMe going (helpful).

- Using TEAMs devochat

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Genetic studies discovered a role for "microRNAs" in regulating expression gene in *C. elegans*. The figure (→) shows the effect of the lin-4 and lin-7 microRNAs on the levels of lin-14 and lin-41 proteins over developmental time.

In the graph indicate the level of lin14 RNA (as opposed to protein) and describe a possible mutation that would inhibit the effect of lin-4 on lin-14 protein level; how does it act (1)?

how does your model work?

**Behavior depends upon mechanism**

Expression levels

lin-14 protein

lin-41 protein

lin-4 RNA

lin-7 RNA

L1 L2 L3 L4 Adult

1 2 3 4 5 6 7 8 9 10

Consider the following situation. Instead of a cell-less syncytium, assume that a complete cell division occurs followed each nuclear division in the early Drosophila embryo.

How would that alter the gradients of bicoid and nanos proteins in the early embryo? (1)

what would be different?

bicoid RNA

nanos RNA

egg

nucleus

bicoid protein

nanos protein

The patterning of the larval cuticle in *Drosophila* is highly stereotyped. Indicate the anterior-posterior and dorsal-ventral axes in the drawn (→).

Predict the phenotype of an embryo from mother homozygous for mutant for both bicaudal & dorsal.

previously isolated a "fascinating maternal mutation, bicaudal, causes the formation of larvae with two rear ends in mirror-image symmetry".

- \* a new maternal mutant, dorsal, was discovered with very specific loss of ventral pattern elements such that the entire cuticle appears dorsalized"

Make a sketch below (→) and explain what the embryo would look like and why (↓).

explain here (please)

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Consider the expression of HOX genes in a wild type embryo. Predict the pattern of HOX gene expression in a embryo from a homozygous bicaudal mother (→) Explain your predication (↓)

explain your predication

the same as in bicaudal      ← Predict the expression of HOX genes in an embryo from a homozygous dorsal mothers: explain the logic behind your choice (↓).

no effect on HOX expression

impossible to say

no idea

why?

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You are carrying out a screen. Draw the curve (→) that describes the relationship between the number of unique loci (genes) identified and the number of chromosomes scored.

Why does your curve have the shape does? what limits the number of genes that can be mutated to produce a "useful" phenotype (↓)?

explain

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We identify two different mutant alleles,  $m_1$  and  $m_2$ , that map to the same gene. Both produce (as homozygotes) the very similar phenotypes.

Produce a model in which  $m_1m_2$  individuals would be wild type. You can draw (optional →) and describe your model below. Describe (1).

how might it work?

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*C. elegans* Apoptotic signals

A dominant "gain of function" mutant allele in the EGL-1 gene leads to the death of HSN neurons and the inhibition of normal egg laying.

Describe an experiment that would suggest that 1) the EGL-1 gene product is involved in HSN neuron death and 2) that the death of these neurons is responsible for the mutant egg laying phenotype (1).

You are planning a genetic screen using *C. elegans* hermaphrodites homozygous for the EGL-1 mutant.

What types of mutations (in what types of genes) would "rescue" the EGL-1 mutant phenotype? Would expect the mutations you identify to be recessive or dominant and why?

How would you answer these questions?

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As you isolate mutations in genes that rescue the Egl-1 phenotype, describe how you would determine the degree to which your screen was saturated (1). What does saturated mean in this context?

What methods and criteria would you use?

- embryos, rather than eggs
- unable to carry out normal apoptosis, able to compete effectively with wild type embryos (in the wild)
- sorry, their behavior would be impossible to predict since normal apoptosis is essential to embryonic development in *C. elegans*
- no idea

A key point in using the dominant egg laying defective Egl-1 mutant is that they eventually do "lay eggs".

Would you expect these "delayed eggs" to be:

You are designing a construct to carry out homologous recombination to generate a null mutation in your favorite gene (YFG).

Indicate the features of the cassette  
(-) necessary for its targeted insertion and positive selection in embryonic stem cells (used to make transgenic mouse).

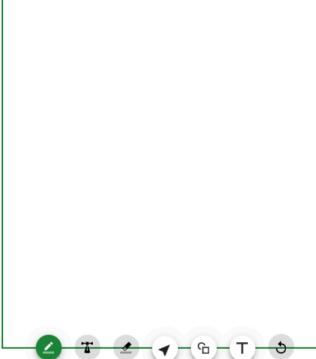
What is the point (usefulness) of negative selection in this process? (1).



What methods and criteria would you use?

Can you imagine a set of conditions under which it would be possible to make a homozygous mutant animal using the CRE-LOX system? Describe your thinking using a drawing (-) and words (1).

What would have to be true.



How would you describe the effects of a CRISPR-Cas9 mediated mutagenesis event? (we did not cover this explicitly in class, but we are curious as to what you already know)

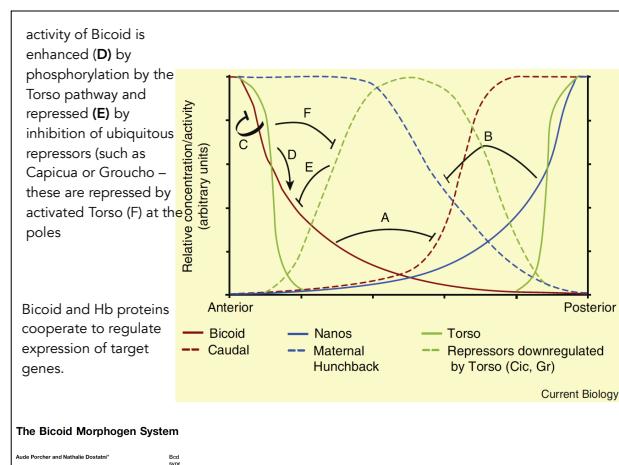
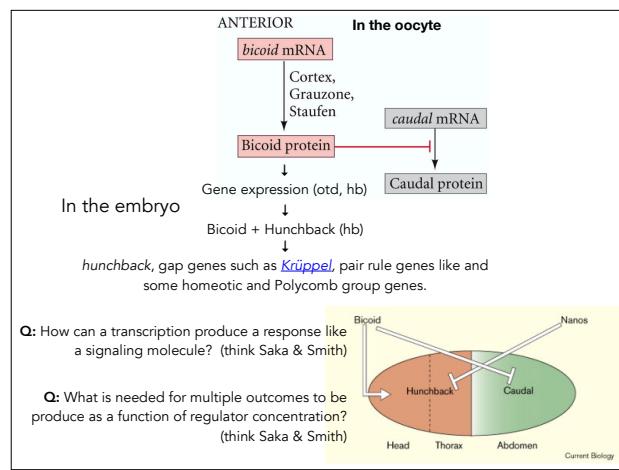
- small deletion
- a SNP
- a frameshift
- a promoter defect
- no idea

Start by letting us know what already know about CRISPR-Cas9 mediated mutagenesis?

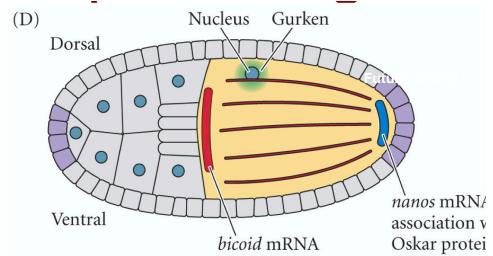
← What types of mutations would **not** expect to arise from a CRISPR-Cas9 mutagenesis event?

here

your reasoning →



### Dorsal–ventral patterning in the oocyte



**Bicoid RNA**

wt

*grk*

Gurken acts earlier in oogenesis – Bicoid RNA is localized to posterior end of oocyte in *Gurken*<sup>-/-</sup> females

Q: What processes may be involved in Gurken RNA localization?

example: Squid – protein binds Gurken RNA (in the nucleus)  
Involved in Gurken RNA localization and translational repression  
Then search for Squid-associated proteins (and so on)

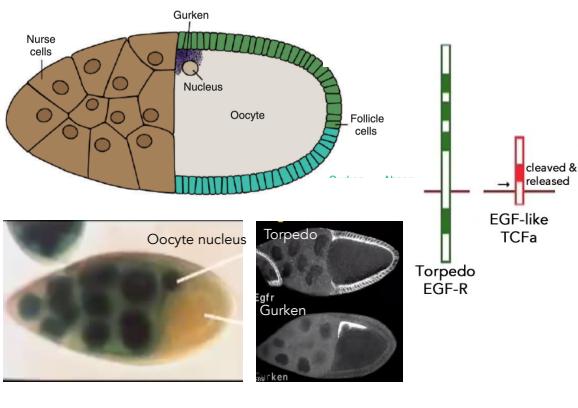
Screen for mutations that lead to dorsalized embryos  
↓  
Look for oocytes with mislocalized Gurken RNA

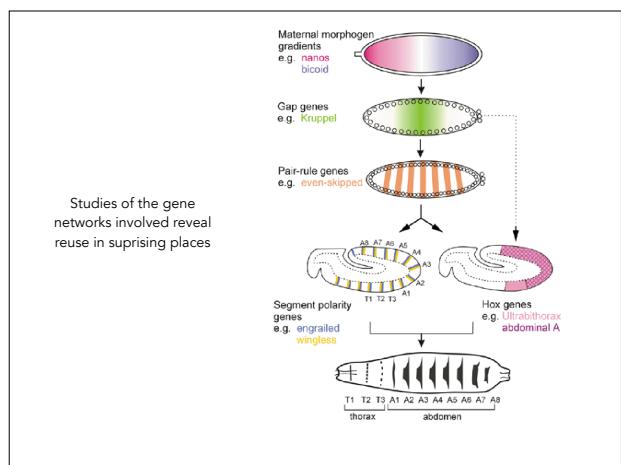
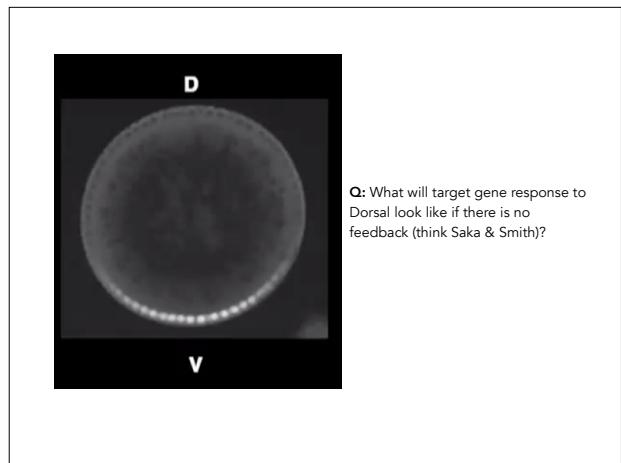
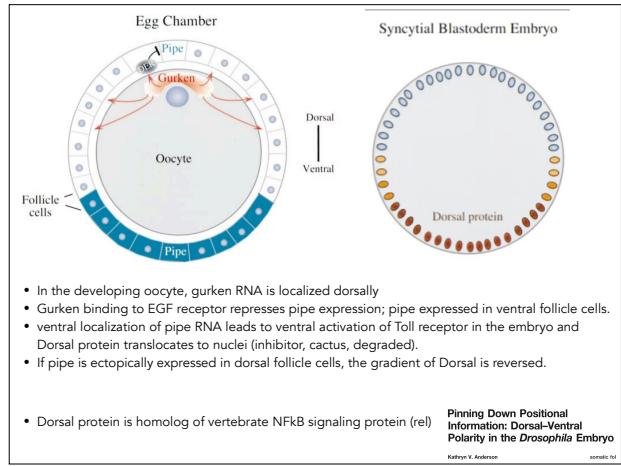
wild type

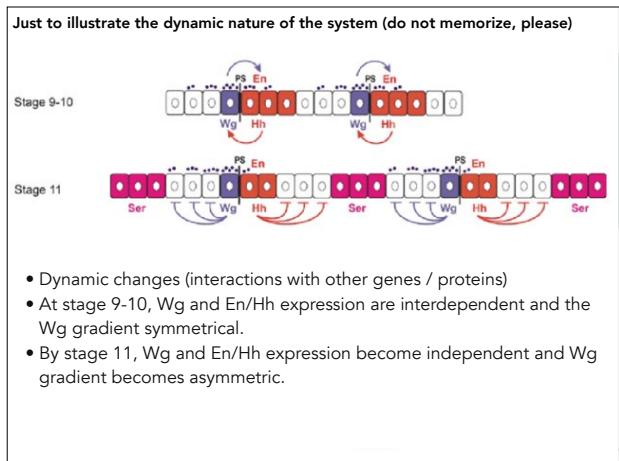
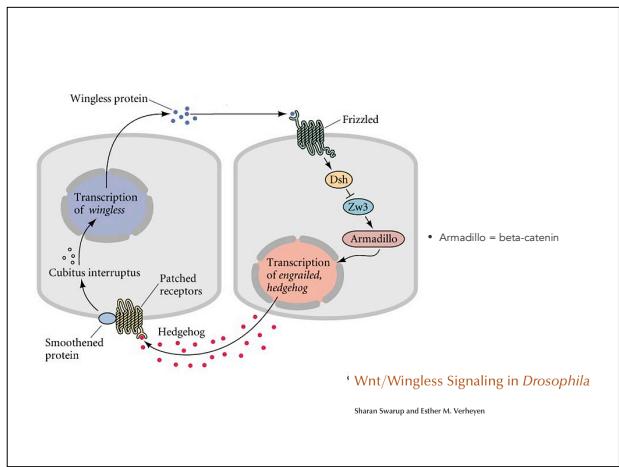
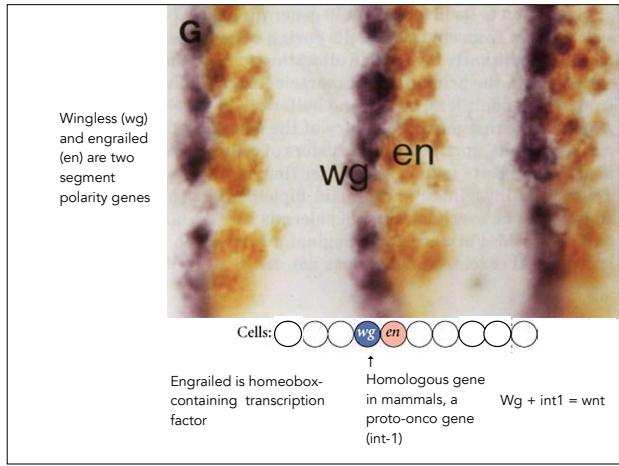
*gurken*

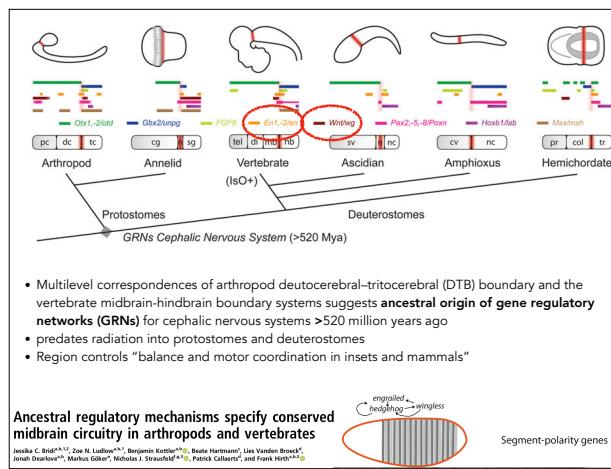
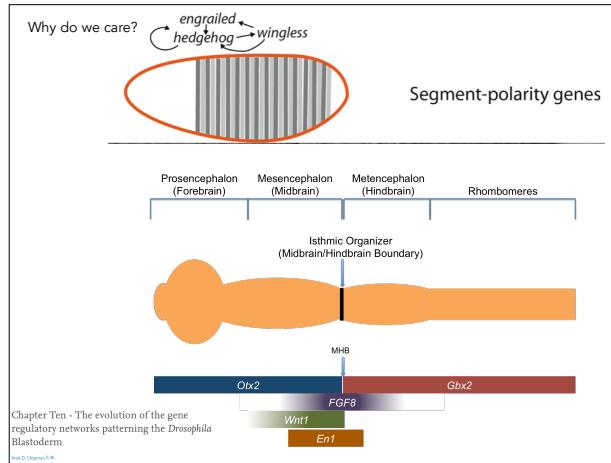
mutant

### Dorsal–ventral patterning









Next we move to mammals!