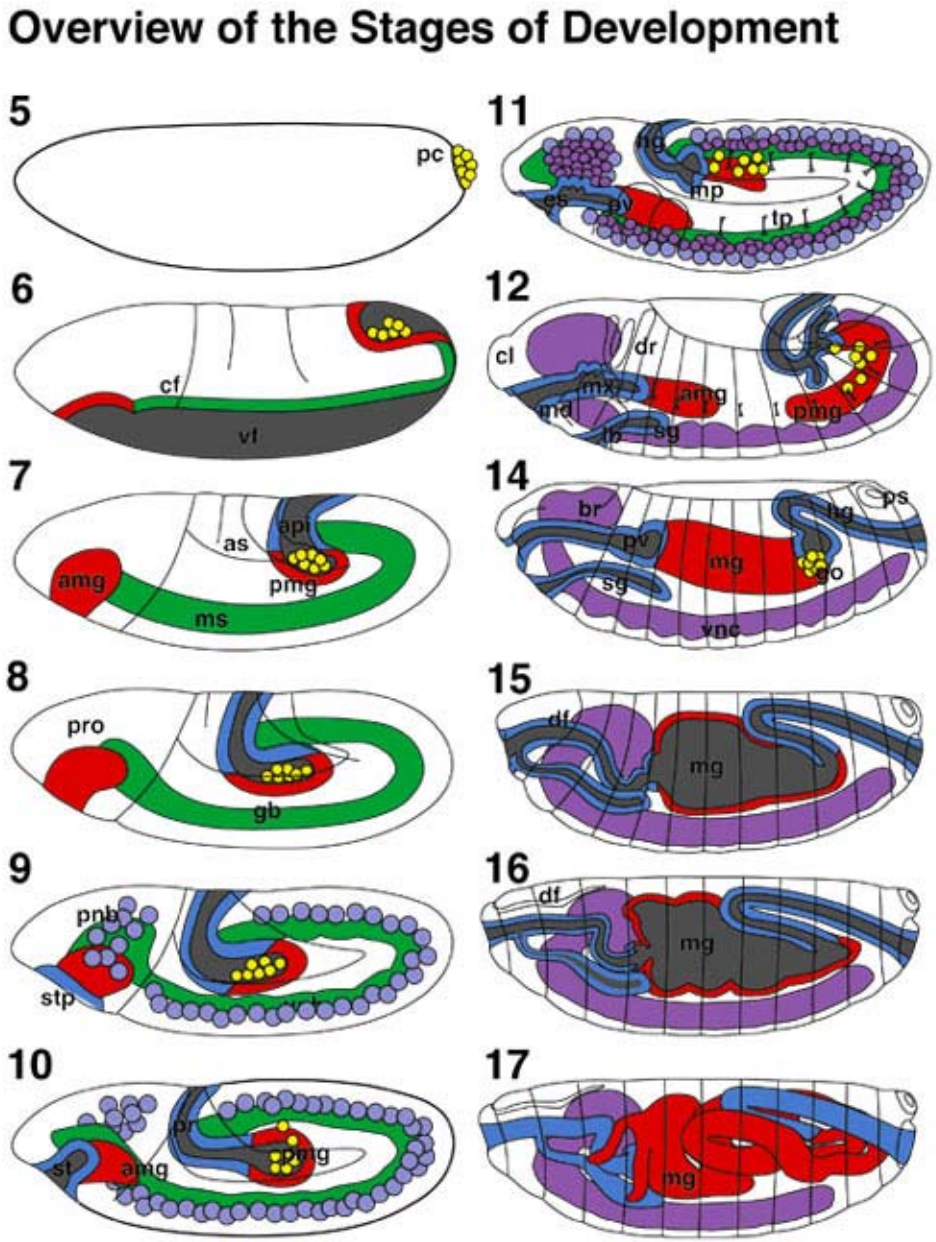
# Axis determination in flies

Sem 9.3.B.5

Animal Science




All embryos are in lateral view (anterior to the left). Endoderm, midgut; mesoderm; central nervous system; foregut, hindgut and pole cells in yellow.

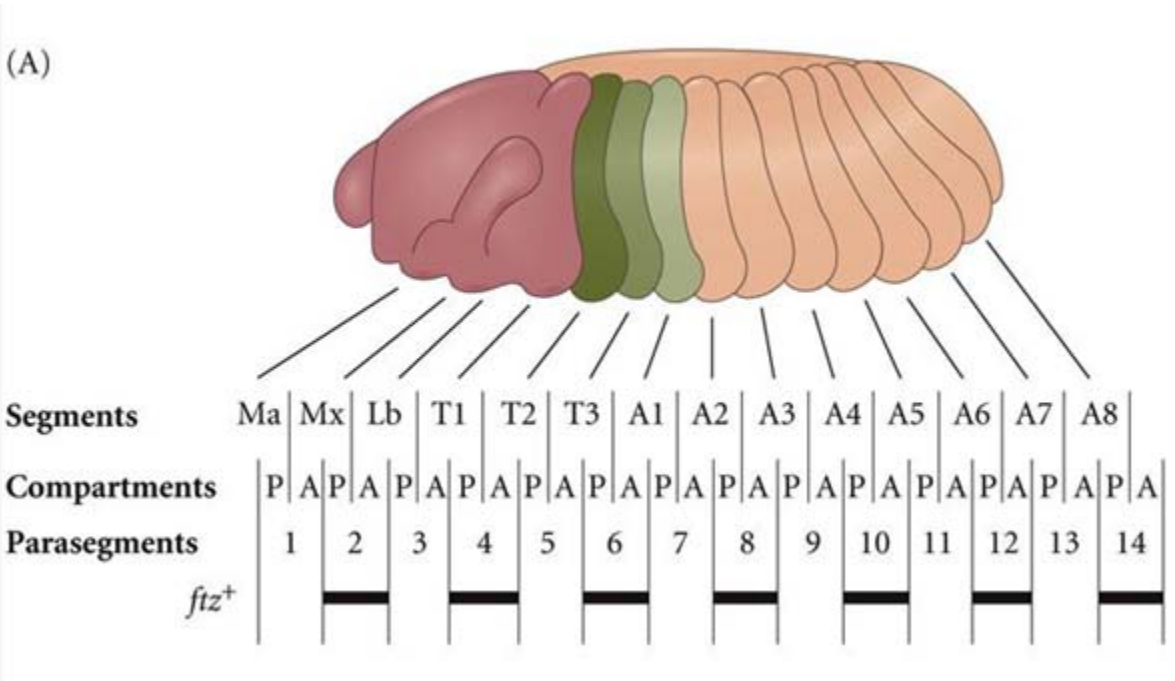
(*amg*) (Anterior midgut rudiment; (*br*) brain; (*cf*) cephalic furrow; (*cl*) clypeolabrum; (*df*) dorsal fold; (*dr*) dorsal ridge; (*es*) esophagus; (*gb*) germ band; (*go*) gonads; (*hg*) hindgut; (*lb*) labial bud; (*md)* mandibular bud; (*mg*) midgut; (*mg*) Malpighian tubules; (mx) maxillary bud; (*pc*) pole cells; (*pmg*) posterior midgut rudiment; (*pnb*) procephalic neuroblasts; (*pro*) procephalon; (*ps*) posterior spiracle; (*po*) proventriculus; (*sg*) salivary gland; (*stp*) stomodeal plate; (*st*) stomodeum; (*tp*) tracheal pits; (*vf*) ventral furrow; (*vnb*) ventral neuroblasts; (*vnc*) ventral nerve

# The segmentation genes

- So far I have shown you that you can change the fate of a cell by the protein gradients like bicoid, nanos, torso etc
- This specification of cell fate is flexible and can still be altered in response to signals from other cells.
- Ultimately, the cells undergo a transition from this loose type of commitment to an irreversible determination
- The transition from specification to determination in *Drosophila* is mediated by the **segmentation genes**

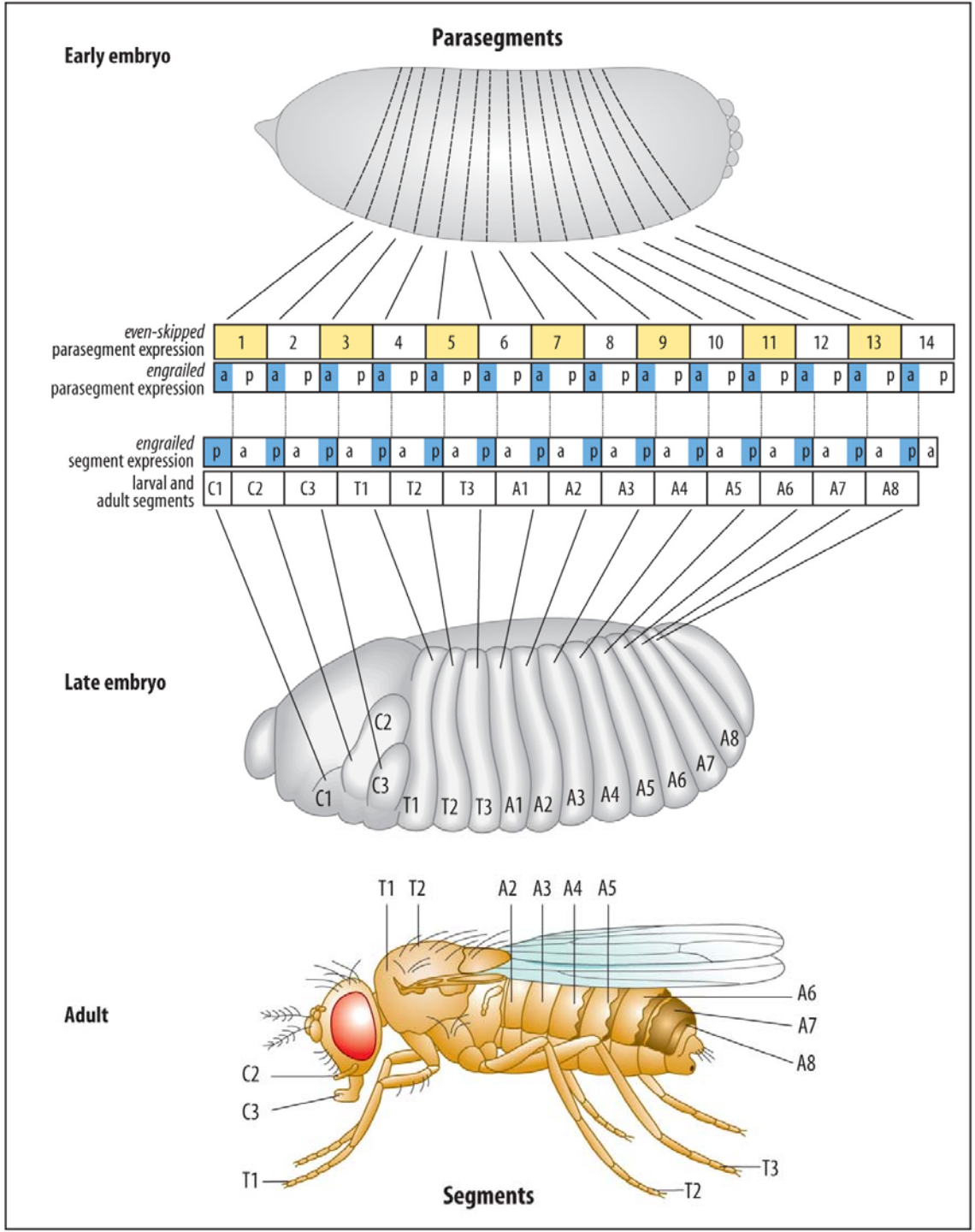
# Segments and parasegments

- True segmentation, or metamerism, is usually considered to be the repetition along the anterior–posterior axis of a structural unit that comprises a suite of characters involving the entire body
- Parasegments are regions of the embryo that are separated by mesodermal thickenings and ectodermal grooves. The parasegments of the embryo do not become the segments of the larva or adult; rather, they include the posterior part of an anterior segment and the anterior portion of the segment behind it.



# Actually

While the segments are the major anatomical divisions of the larval and adult body plan, they are built according to rules that use the parasegment as the basic unit of construction.



# Segmentation genes

- Three classes of segmentation genes and they expressed sequentially
  - The Gap genes: these are repressed or activated by maternal affect genes and they define broad units of embryo
  - The Pair rule genes: the proteins of neighbouring Gap genes interact with each other to activate the transcription of these genes. These affect parasegments
    - Genes upto this point regulate syncitial embryo
  - The segment polarity genes: these are responsible for maintaining certain repeated structures within each segment. These are also transcription factors that with the help of main gradient factors defines each segment into repetitive parasegments
- After the parasegmental boundaries are set, the pair rule and gap genes interact to regulate the homeotic selector genes, which determine the identity of each segment.

# In an embryo

Maternal

*bicoid*

Establish Polarity

Gap

*hunchback*

Divide embryo Into regions

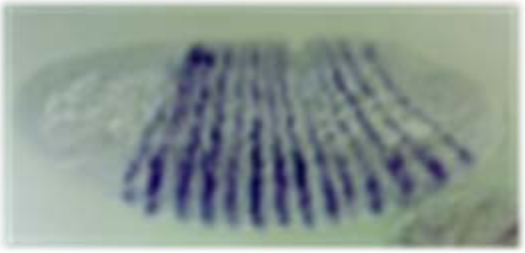
Pair rule

*even-skipped*
*fushi-tarazu*

Establish segmental plan

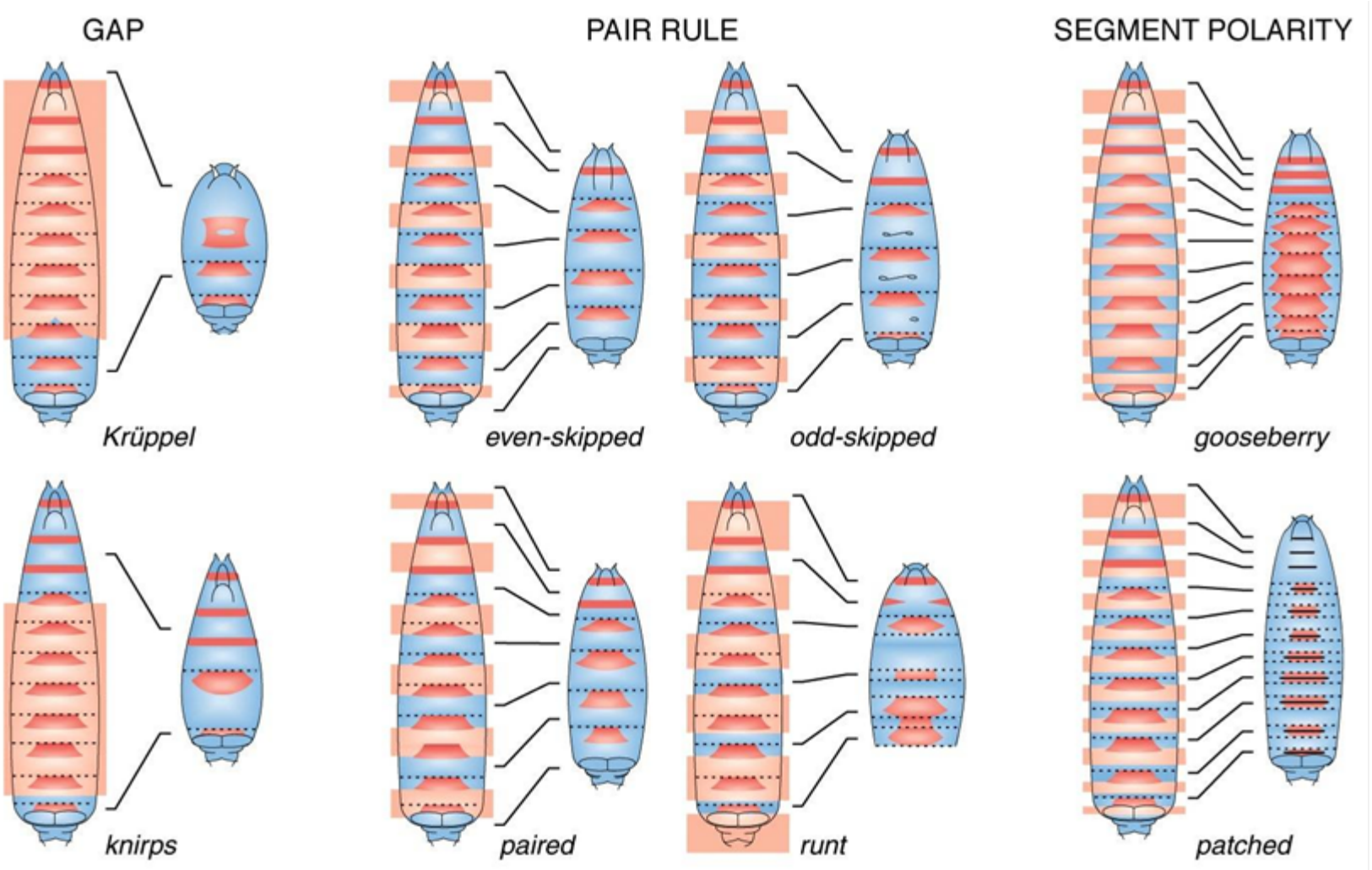
Segment polarity

*engrailed*



Set boundaries of segments

# Mutants

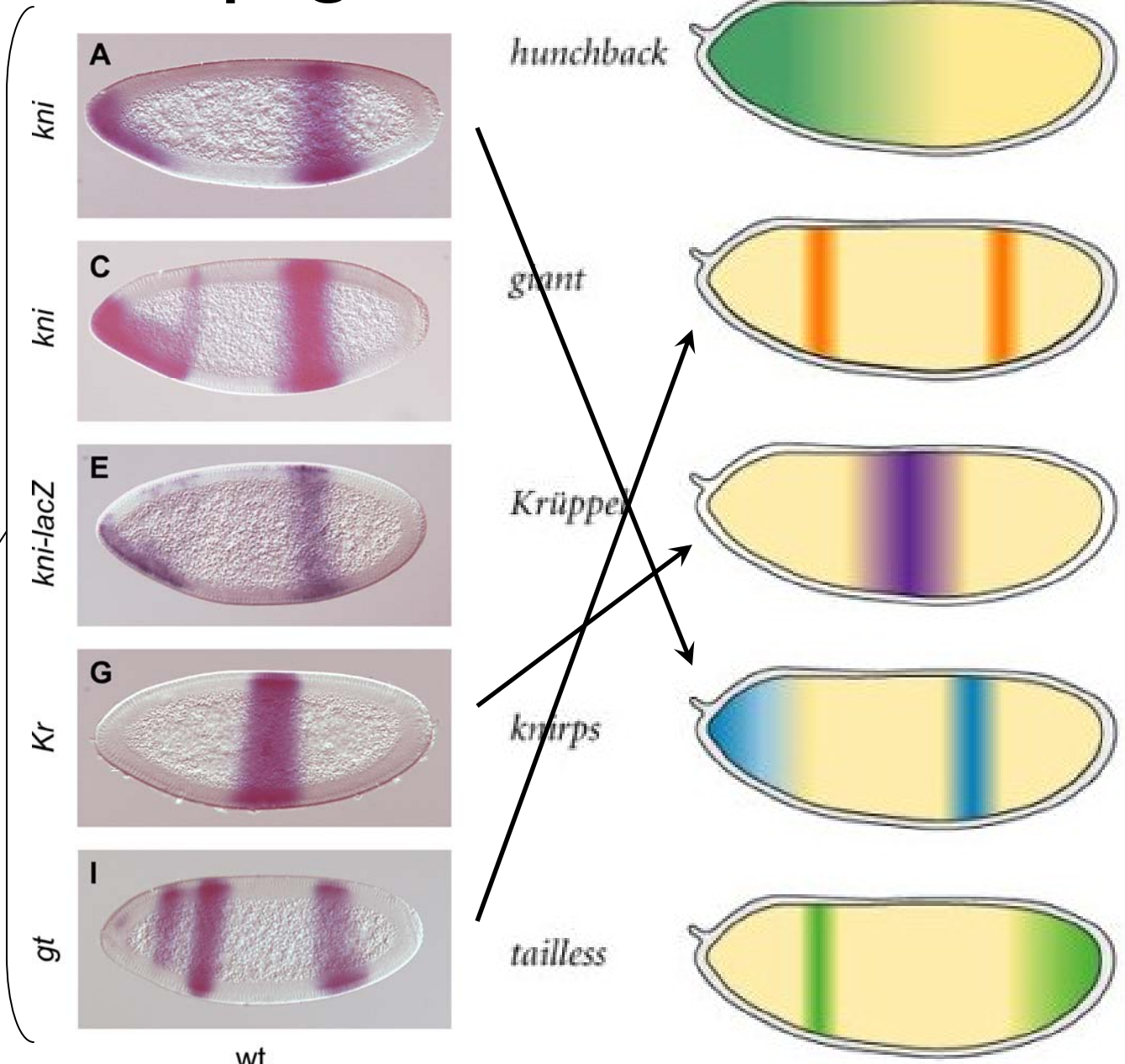

GAP
PAIR RULE
SEGMENT POLARITY
Krüppel
even-skipped
odd-skipped
gooseberry
knirps
paired
runt
patched

# Gap genes

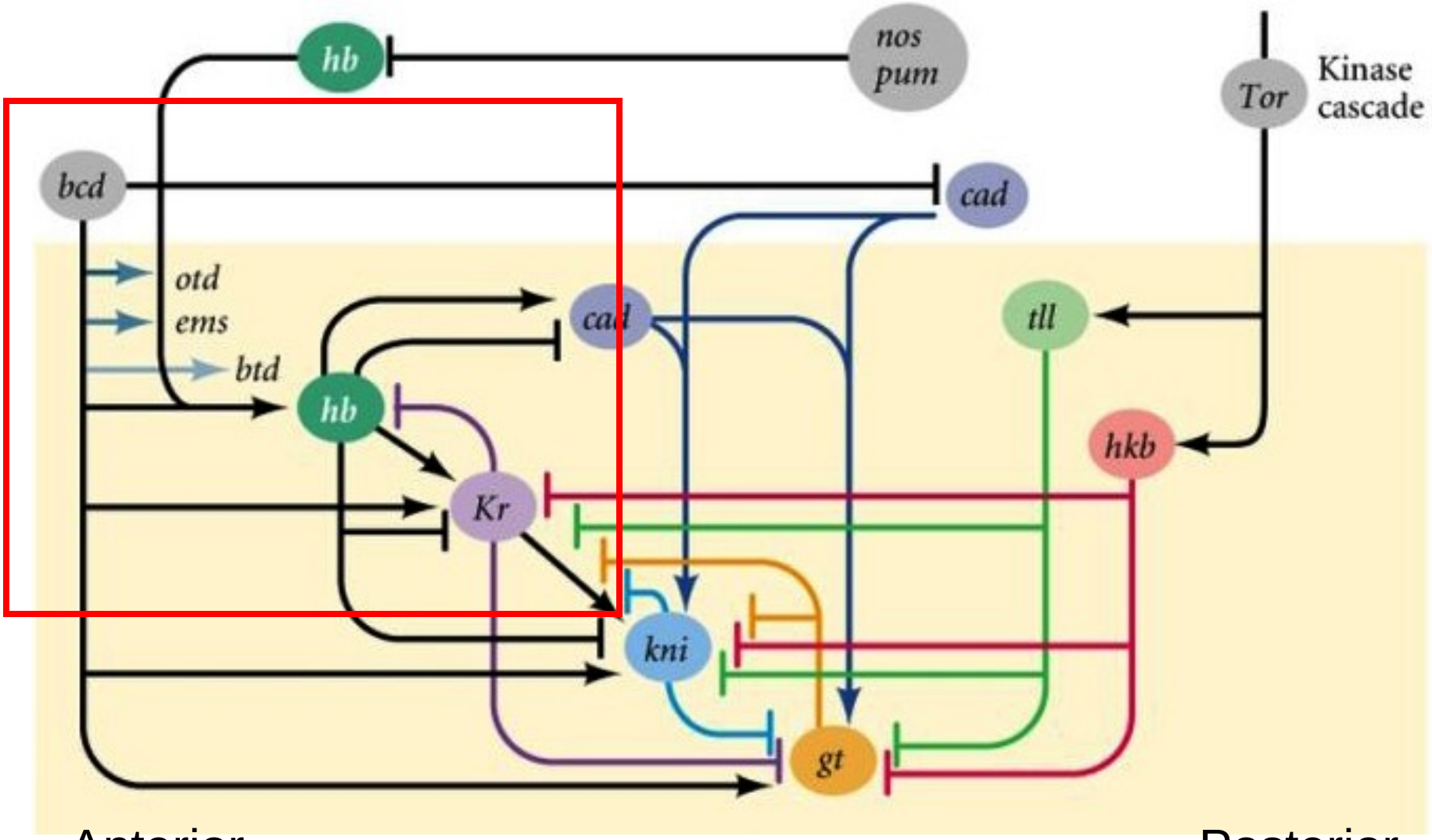
These are the first sets of genes transcribed/translated after maternal affect genes

Eg: *hunchback (hb), giant (gt), Krüppel (Kr), knirps (kni), tailless (tll)*

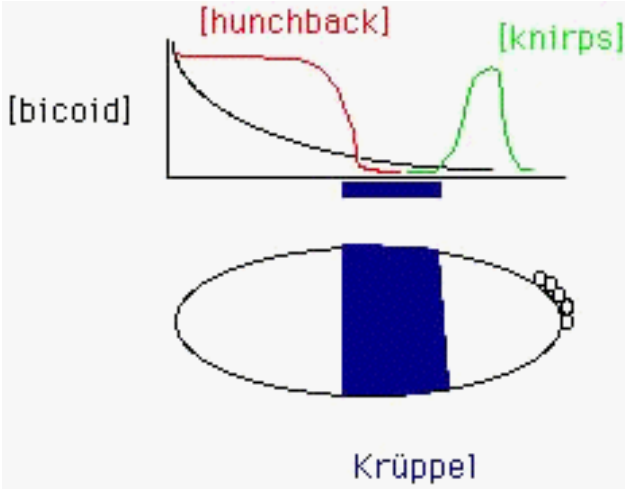
mRNA



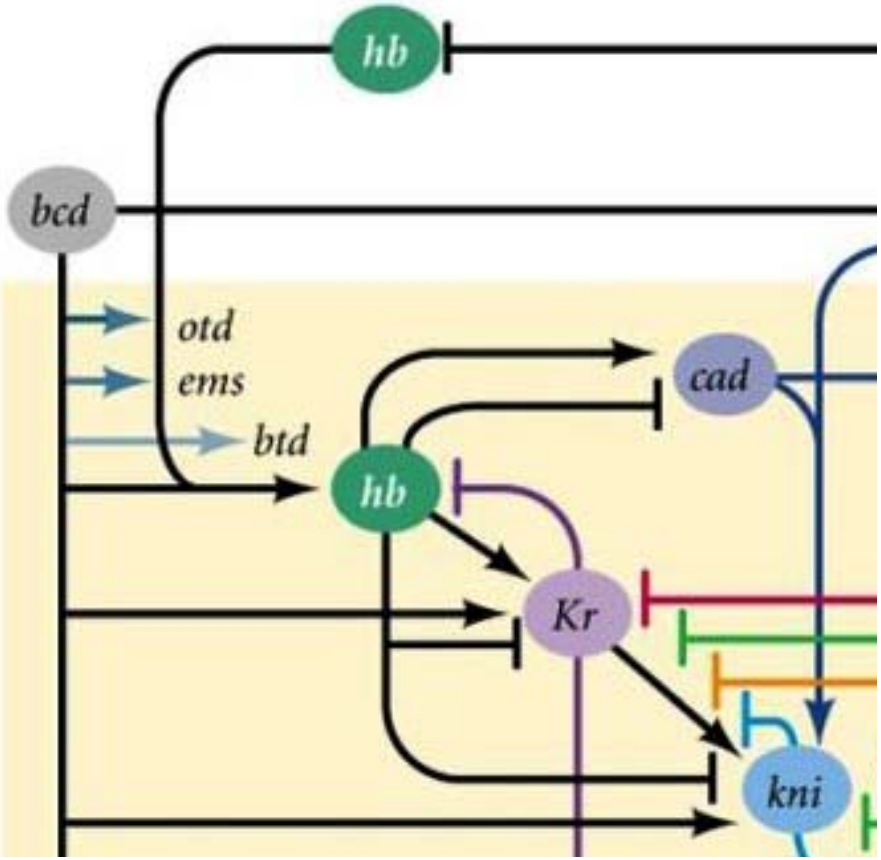
# How does it work?



# *Krüppel*



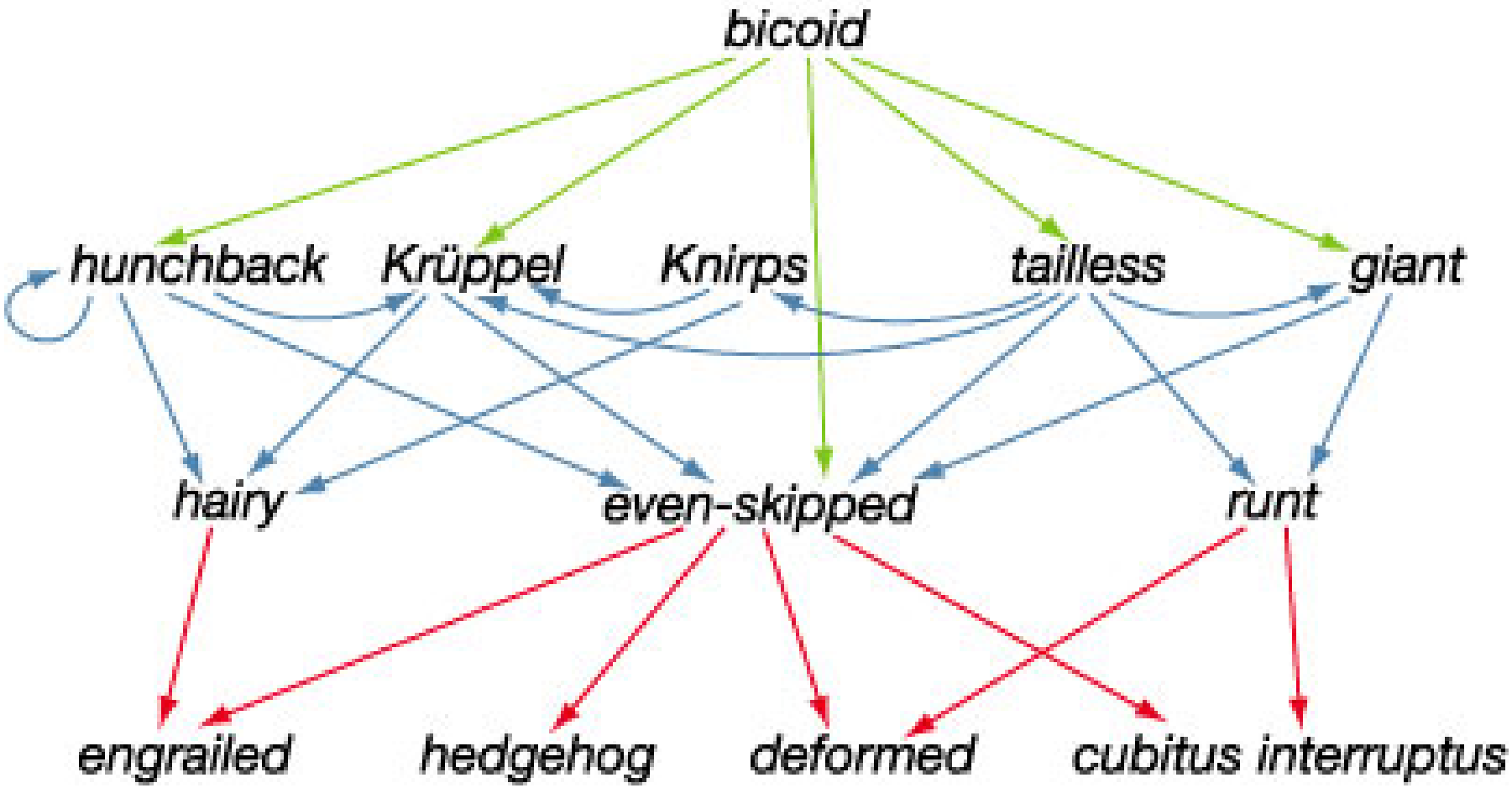

- Very simply:
  - Bicoid activates *Krüppel*
  - Hunchback activates *Krüppel* at low concentrations and represses *Krüppel* at high concentrations
  - Knirps represses *Krüppel*



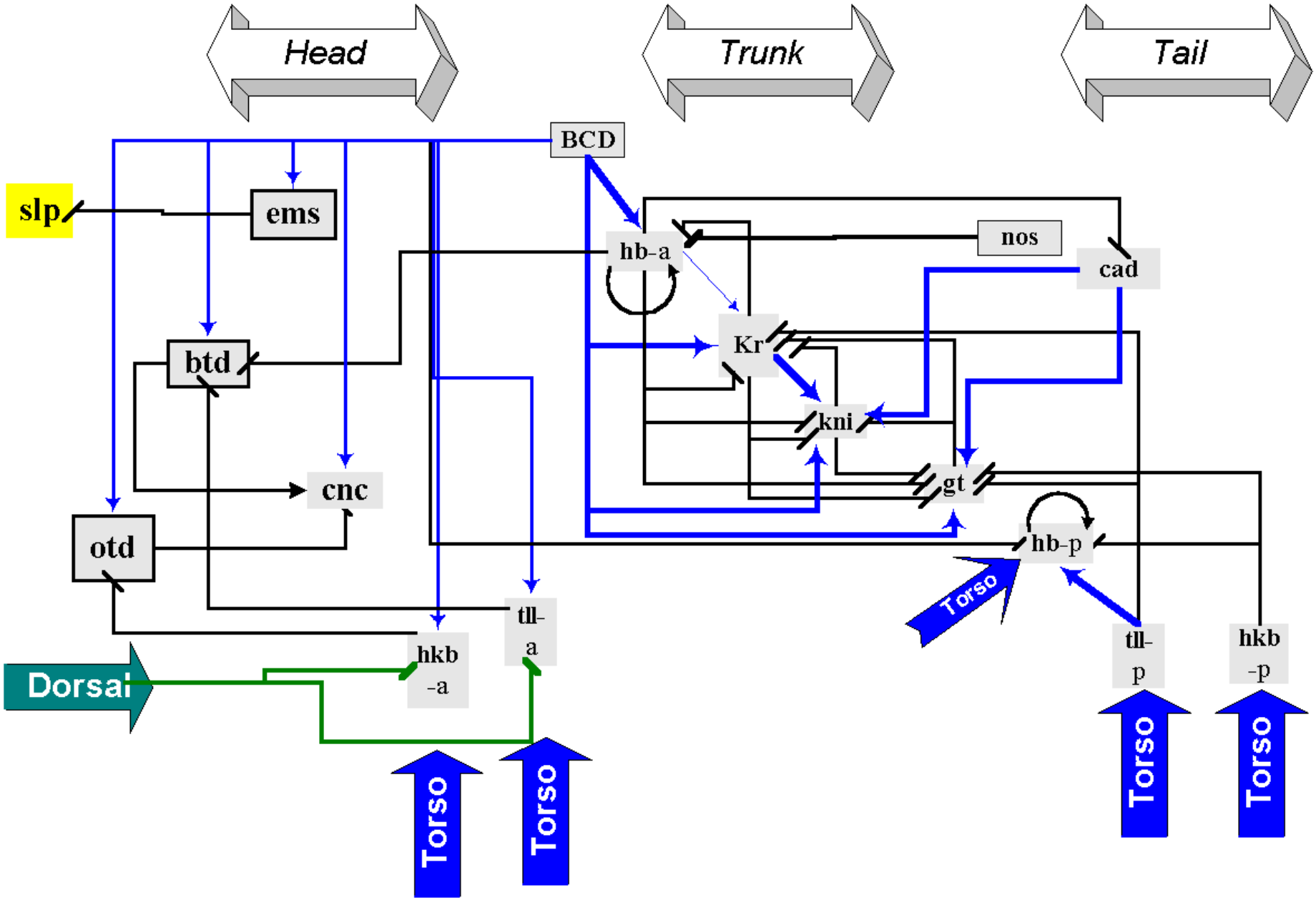
# A little more detailed

- For instance, *Krüppel* gene expression is negatively regulated on its anterior boundary by the Hunchback and Giant proteins and on its posterior boundary by the Knirps and Tailless proteins.
- If Hunchback activity is lacking, the domain of *Krüppel* expression extends anteriorly.
- If Knirps activity is lacking, *Krüppel* gene expression extends more posteriorly.
- The boundaries between the regions of gap gene transcription are probably created by mutual repression.
- Just as the Giant and Hunchback proteins can control the anterior boundary of *Krüppel* transcription, so Krüppel protein can determine the posterior boundaries of *giant* and *hunchback* transcription.
- If an embryo lacks the *Krüppel* gene, *hunchback* transcription continues into the area usually allotted to *Krüppel*. These boundary-forming inhibitions are thought to be directly mediated by the gap gene products, because all four major gap genes (*hunchback, giant, Krüppel,* and *knirps*) encode DNA-binding proteins that can activate or repress the transcription of other gap genes.

# Is this simpler?



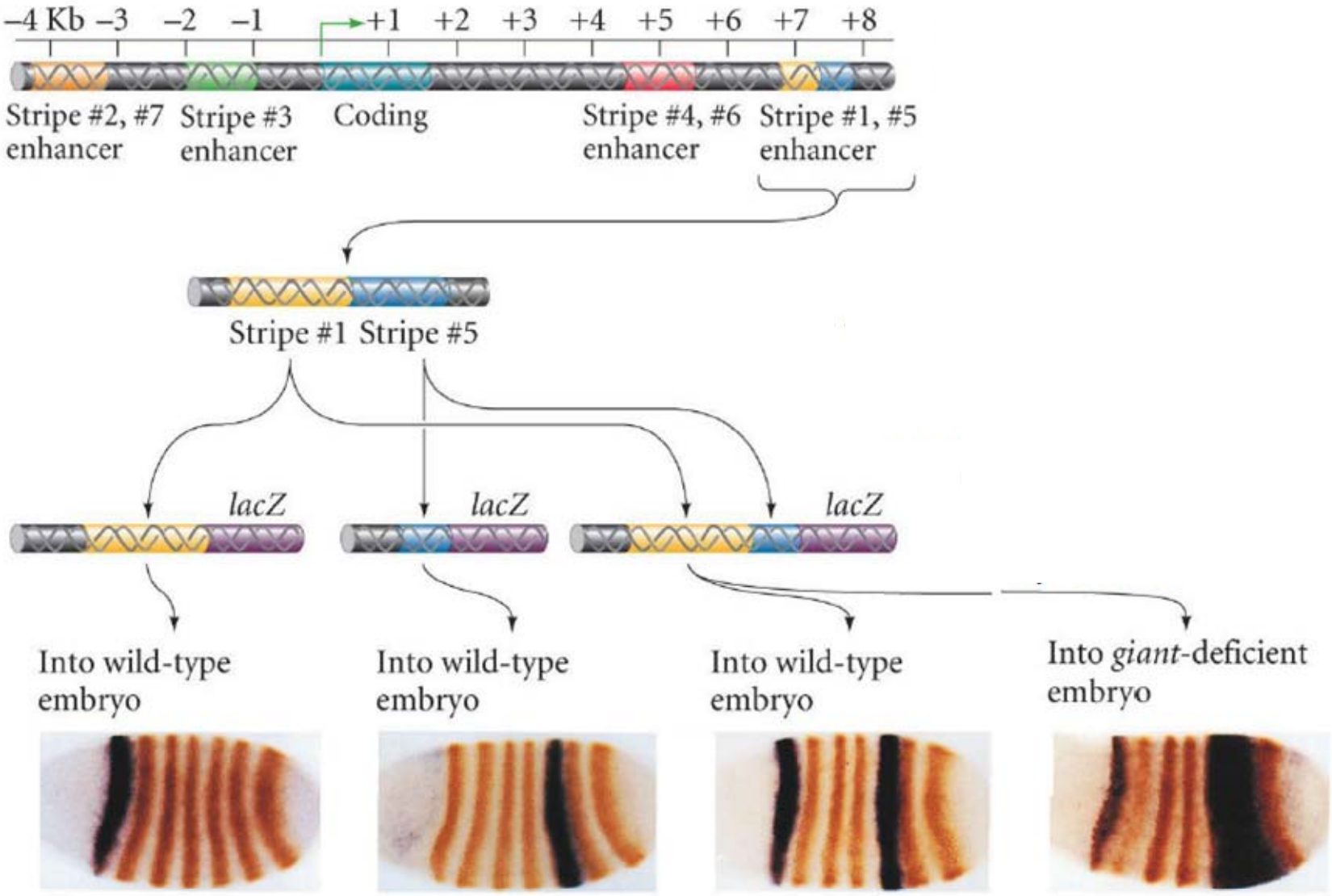
# Or this?


Head
Trunk
Tail
BCD
slp
ems
hb-a
nos
cad
btd
Kr
kni
gt
cnc
otd
hb-p
tll-
a
hkb
-a
Dorsal
Torso
tll-
p
hkb
-p
Torso
Torso
Torso
Torso

# Pair rule genes

- The embryo is divided into large regions by the gap genes, which activate transcription of the pair-rule genes in seven stripes of expression.
- The activation of the pair-rule genes in striped patterns by the maternal coordinate genes and gap genes is the first sign of segmentation in the embryo.
- Pair-rule gene expression patterns determine the position of the parasegments.
- How is this co ordinated? It turns out there is a hierarchy within the pair-rule gene class. The primary pair-rule genes *(even-skipped, hairy, and runt)* are activated directly by the gap genes and they regulate the expression of the secondary pair-rule genes *(fushi-tarazu, odd-skipped, odd-paired, paired,* and others) .

# How does it work?

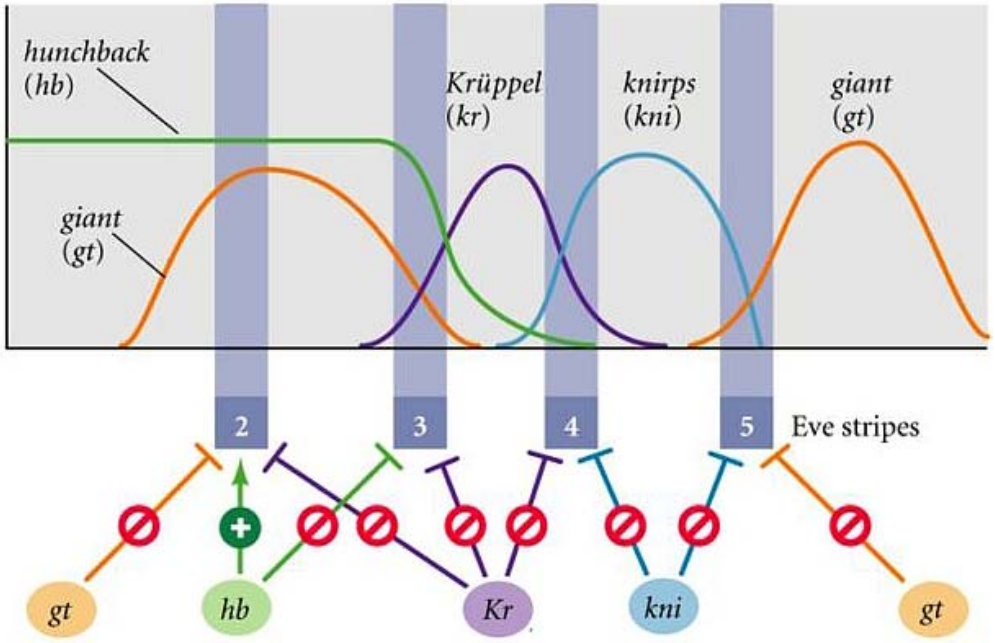


# Or

How are the primary pair-rule genes regulated? It turns out that there is a separate enhancer controlling the expression of each stripe of gene expression in each of the primary pair-rule genes. The first evidence for this came from regulatory mutations that altered the transcription of specific stripes of expression. This was shown conclusively when the regulatory regions were analyzed using transgenes driving lacZ expression. It was discovered that there were regulatory elements for each stripe of expression. So upstream of *even-skipped* there are 7 independent regulatory elements - one for each stripe. The three primary pair-rule genes are expressed in 7 stripes each for a total of 21 over-lapping domains of gene expression. In principle, the way each of these stripes of expression is established is no different from the way the gap gene expression patterns are established through a combination of positive and negative inputs.

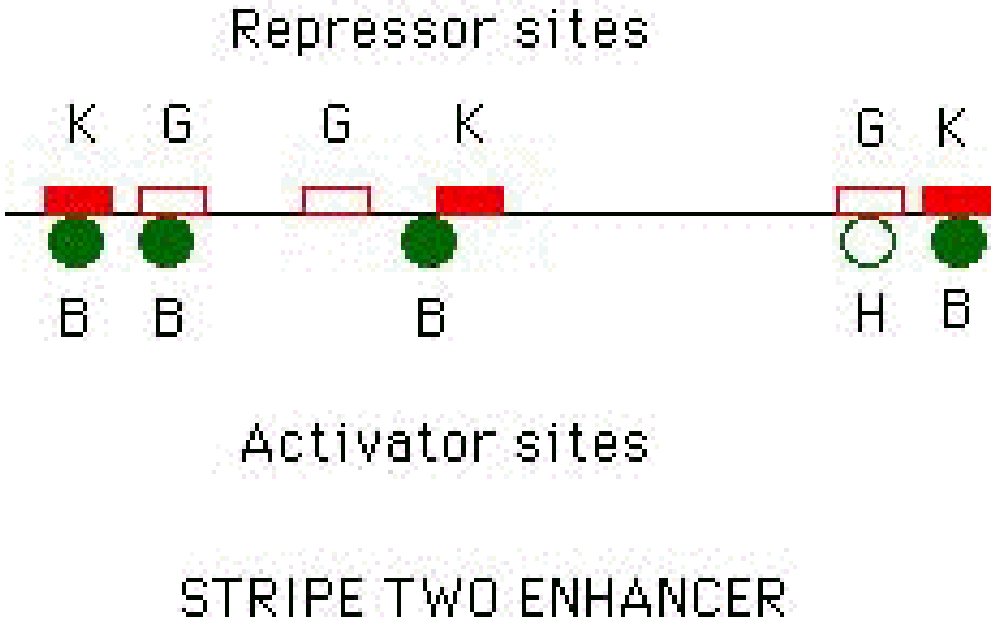
# *even-skipped*

- Let's look at for example what is happening with the expression of *even-skipped* in the second stripe from the anterior.
- Bicoid and Hunchback activate *even-skipped* stripe 2 expression. *even-skipped* stripe 2 is repressed on the anterior side by the gap gene *giant* and on the posterior side by Krüppel.



# Still at it

Upstream of the *even-skipped* gene is a 430 bp enhancer element that controls just the expression of *even-skipped* in the stripe 2 region (it is aptly named the "stripe 2 enhancer"!). This enhancer has 12 known factor binding sites, including 6 activator and 6 repressor sites. The 6 activator sites include 5 Bicoid binding sites and one Hunchback site. There are 3 binding sites for each of Giant and Krüppel.

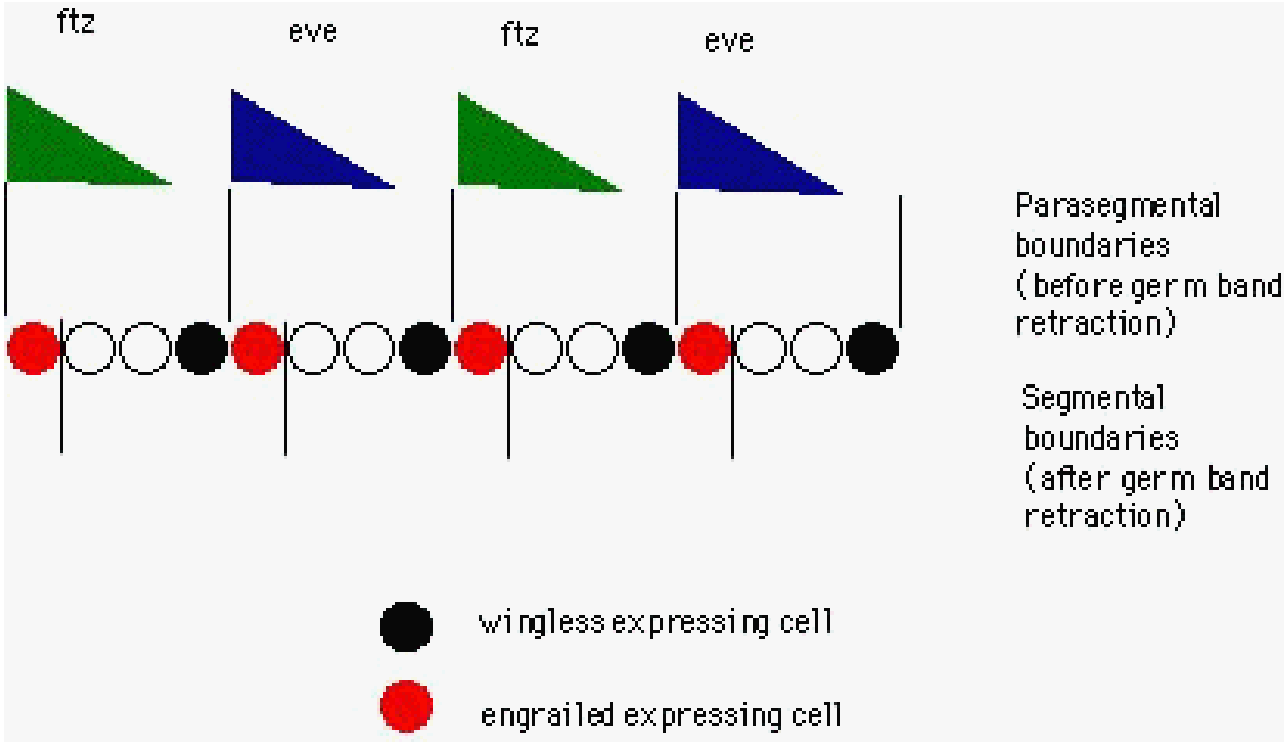


# Secondary pair-rule gene

- Once initiated by the gap gene proteins, the transcription pattern of the primary pair-rule genes becomes stabilized by their interactions among themselves. The primary pair-rule genes also form the context that allows or inhibits the expression of the later-acting secondary pair-rule genes. One such secondary pair-rule gene is *fushi tarazu* (*ftz*; Japanese, "too few segments;“).
- Early in cycle 14, *ftz* RNA and protein are seen throughout the segmented portion of the embryo. However, as the proteins from the primary pair-rule genes begin to interact with the *ftz* enhancer, the *ftz* gene is repressed in certain bands of nuclei to create interstripe regions. Meanwhile, the Ftz protein interacts with its own promoter to stimulate more transcription of the *ftz* gene.

# The segment polarity genes

The pair-rule genes are already expressed in a periodic pattern, so it is easy to imagine how they establish the segment polarity gene expression in every parasegment. The expression patterns of the segment polarity genes *engrailed (en)* and *wingless (wg)* are established through positive and negative transcriptional regulation by the pair-rule genes. For example, the expression of *en* is activated by either Ftz or Eve in each parasegment, whereas *wingless* is repressed by Ftz or Eve in each parasegment.



# Cell to cell signaling-1

- The regulation of the segment polarity genes by the pair-rule genes is only the first stage of regulation. There are two problems that must be overcome. First, the expression of the coordinate, gap and pair-rule genes fades away and new mechanisms for regulating *wingless* and *engrailed* are required. Furthermore, cellularization of the embryo has occurred by this stage and it turns out that the mechanism for maintaining *wingless* and *engrailed* expression is based on cell to cell communication. This is why not all of the segment-polarity genes are transcription factors.
  So, how are the patterns of *wingless* and *engrailed* maintained?

# Cell to cell signaling-2

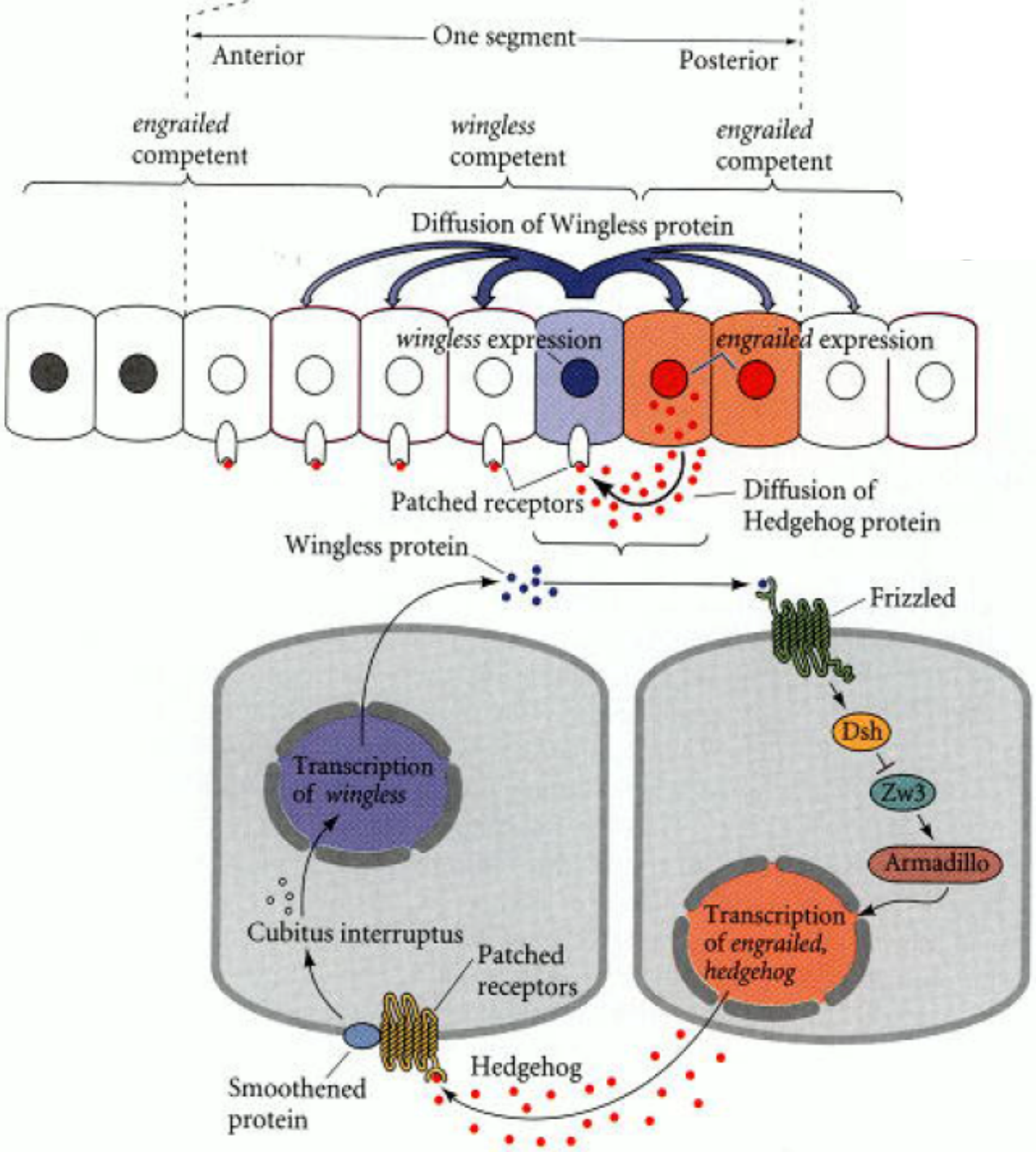
- Genetic experiments showed that *en* and *wg* are required for each other's expression: Wg disappears in *en* mutant embryos; En disappears in *wg* embryos. This suggests that Wg and En maintain each other's expression. However, they are expressed in completely different cells, so cell- cell communication must occur.
- *engrailed* encodes a homeodomain protein; *wingless* encodes a secreted peptide, a member of the WNT family. Wingless is secreted from the cells which make it. When the Wingless protein binds to its receptor on posterior cells, the signal is transduced to the nucleus and maintains the transcription of *engrailed*.

# Cell to cell signaling-3

- A second signal must be invoked to explain the maintenance of *wingless* expression by *engrailed* expression. Since Engrailed is a transcription factor, it cannot be directly responsible for the signal from the posterior *engrailed* expressing cells. *wingless* and *engrailed* expression are also lost in mutants for *a*nother segment polarity gene is called *hedgehog*. *hedgehog* is expressed in the same cells as *engrailed* and its expression is lost in *wingless* and *engrailed* mutants. These results imply that *hedgehog* is part of the genetic circuit linking *wingless* and *engrailed* expression. *hedgehog* encodes a secreted factor that is responsible for the signal from the *engrailed* expressing cells back to the *wingless* expressing cells. *hedgehog* binds to its receptor on the *wingless* expressing cells and this results in the maintenance of *wingless* transcription.

# Really?

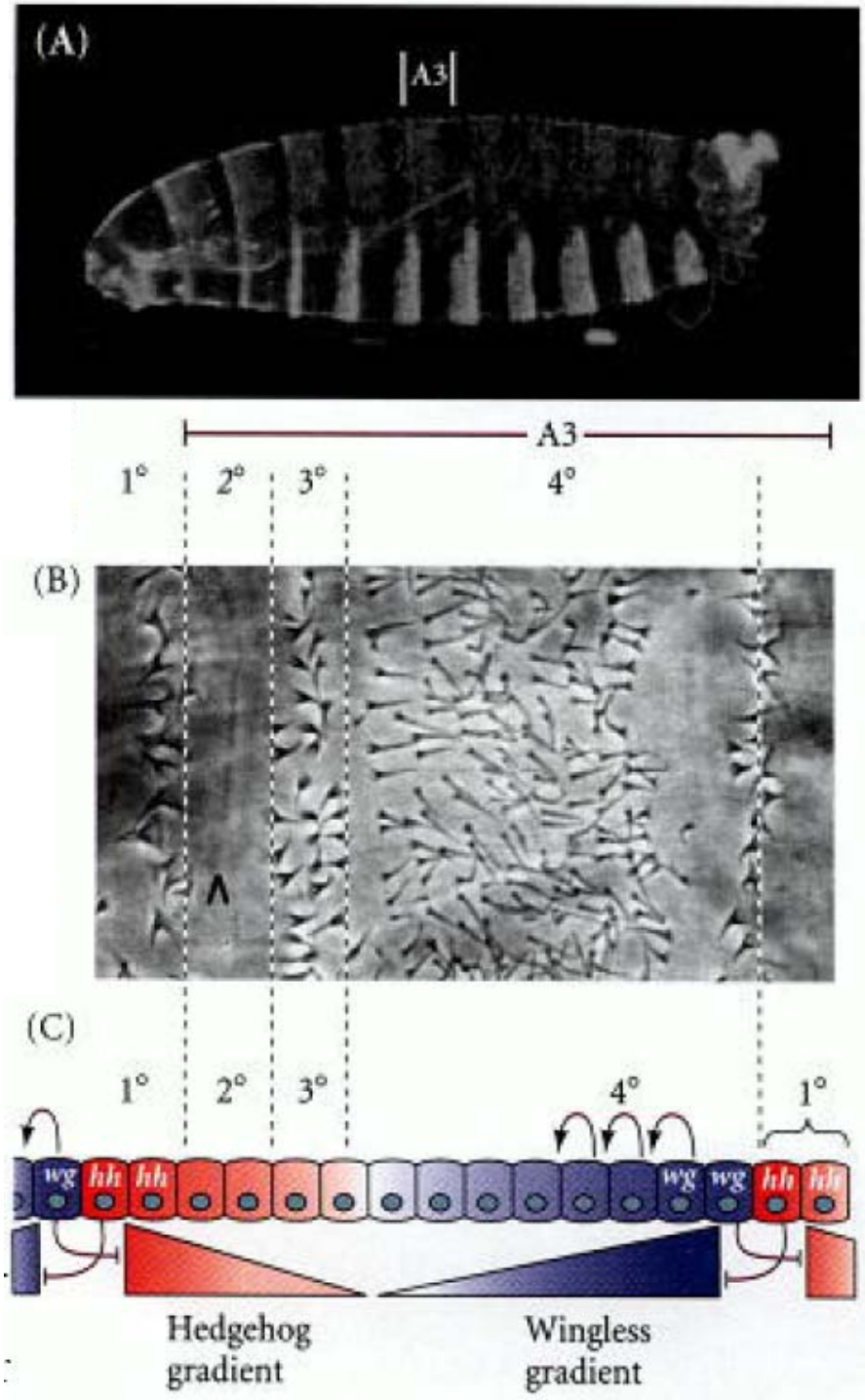
YES


One segment
Anterior
Posterior
engrailed competent
wingless competent
engrailed competent
Diffusion of Wingless protein
wingless expression
engrailed expression
Patched receptors
Diffusion of Hedgehog protein
Wingless protein
Frizzled
Dsh
Zw3
Armadillo
Transcription of wingless
Transcription of engrailed, hedgehog
Cubitus interruptus
Patched receptors
Hedgehog
Smoothened protein

# How do we know?

This process can be seen in the dorsal epidermis, where the rows of larval cells produce different hairs depending on their position within the segment. The 1° row consists denticles. Posterior to denticles, the 2° row produces a smooth cuticle. The next two cell rows have a 3° fate, small, thick hairs, and these are followed by several rows of cells that adopt the 4° fate, producing fine hairs

And you change all this by changing hedgehog and wingless expression.



# Developmental biology
Scott F. Gilbert