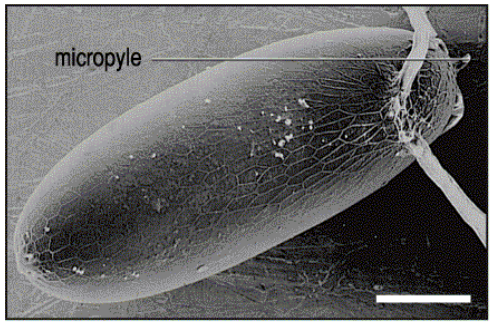
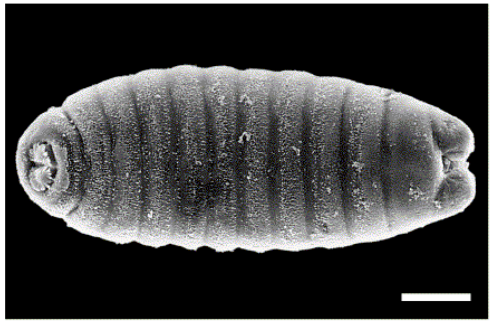
Axis determination in flies

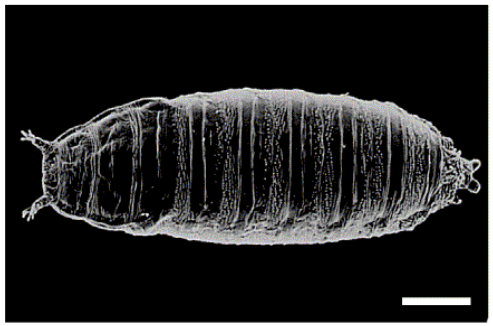
Sem 9.3.B.5

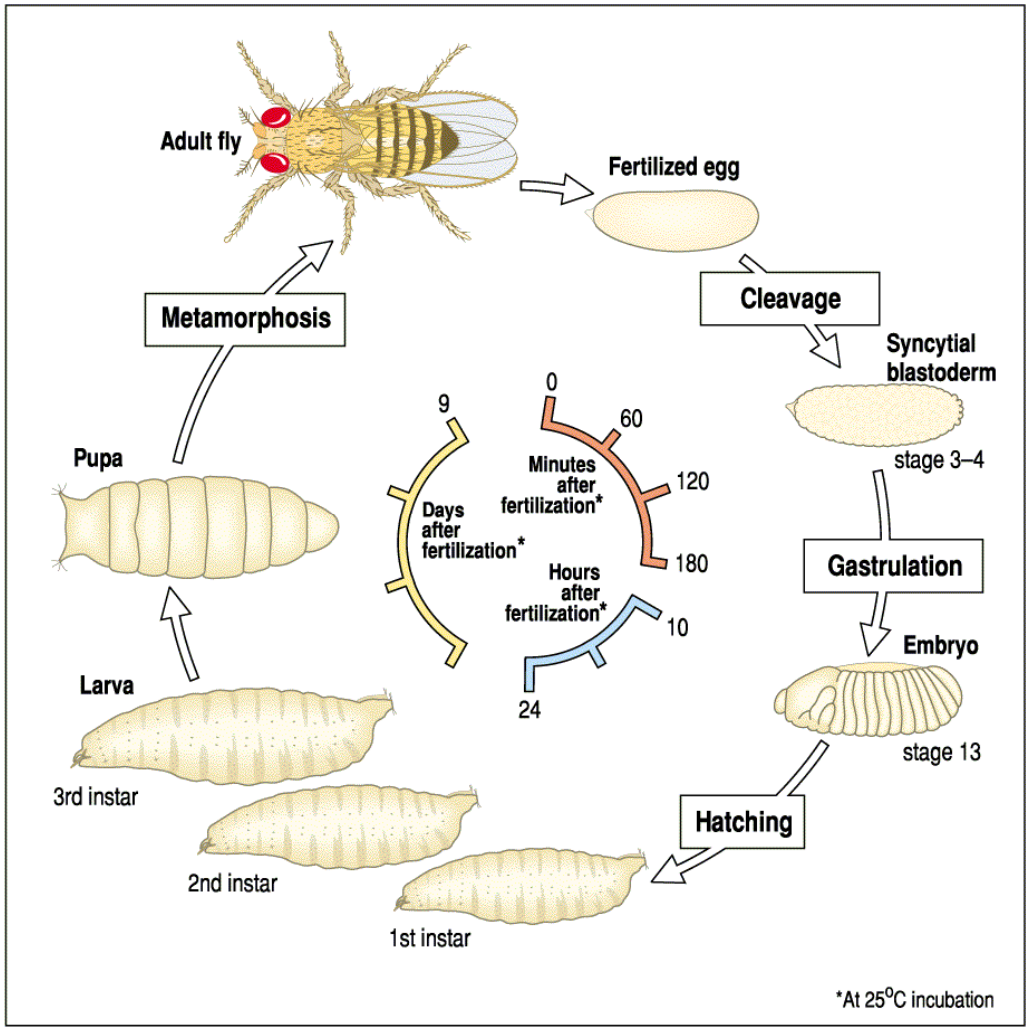
Animal Science

Drosophila life cycle



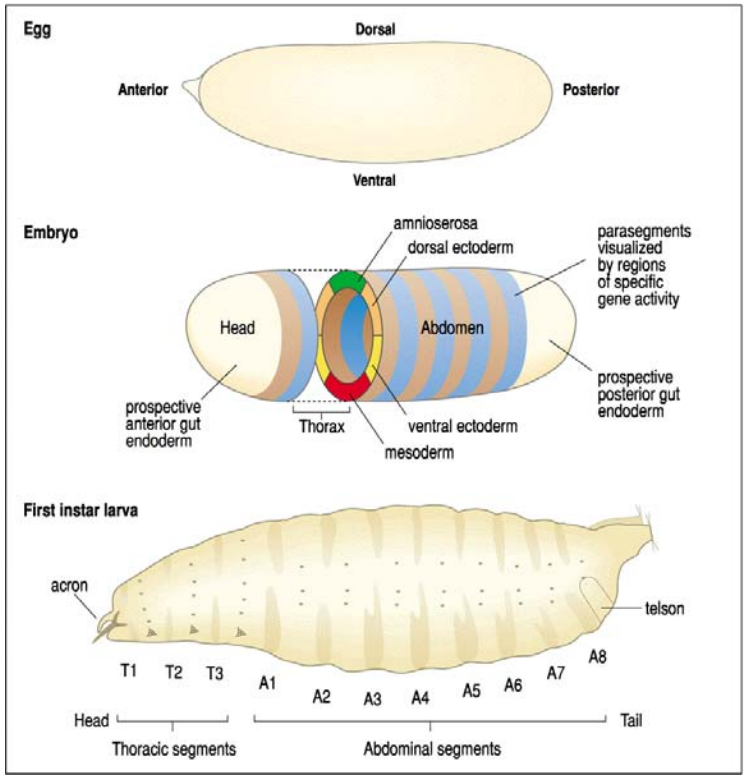


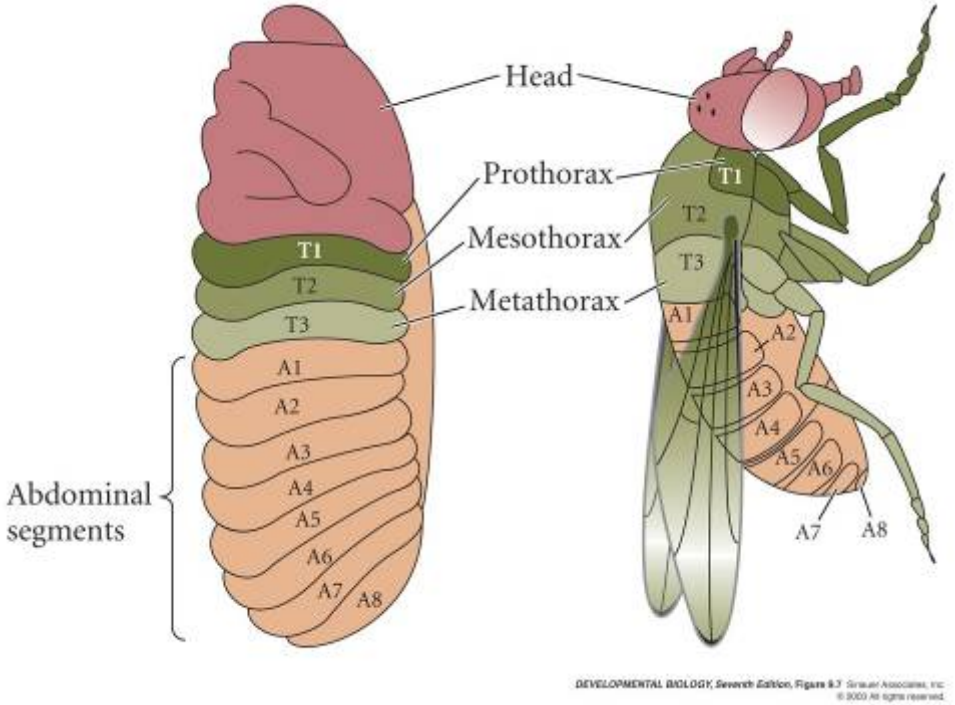




Culture condition: 25^{0}C and 60% humidity

Comparison of Larval and Adult Segmentation in *Drosophila*

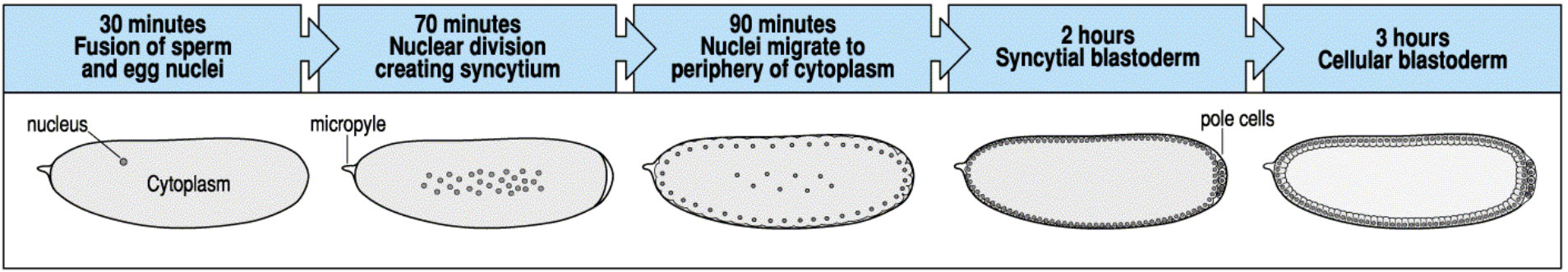




Early development of *Drosophila*

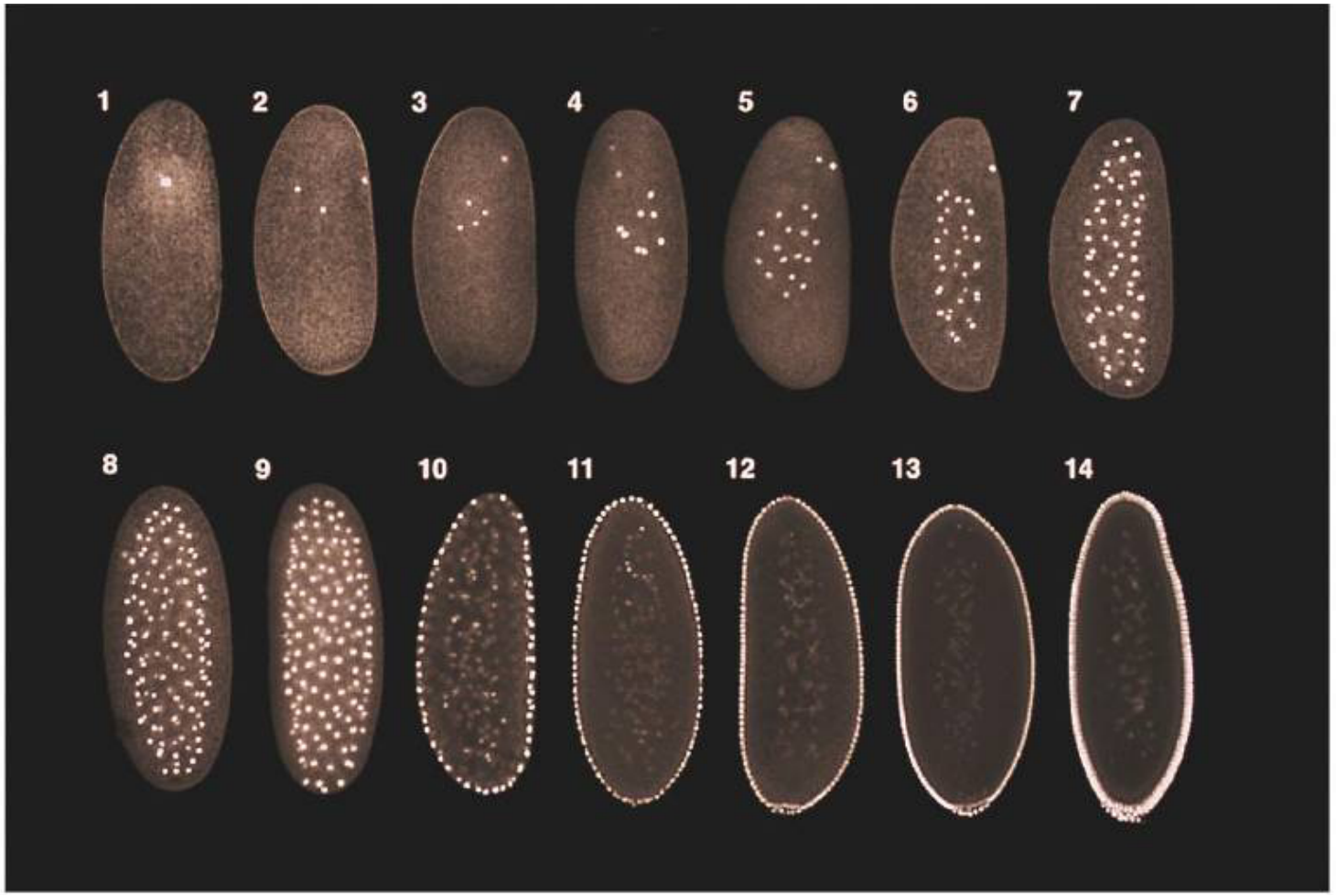
Rapid division
8 mins/division
9 divisions

13 divisions asynchronous

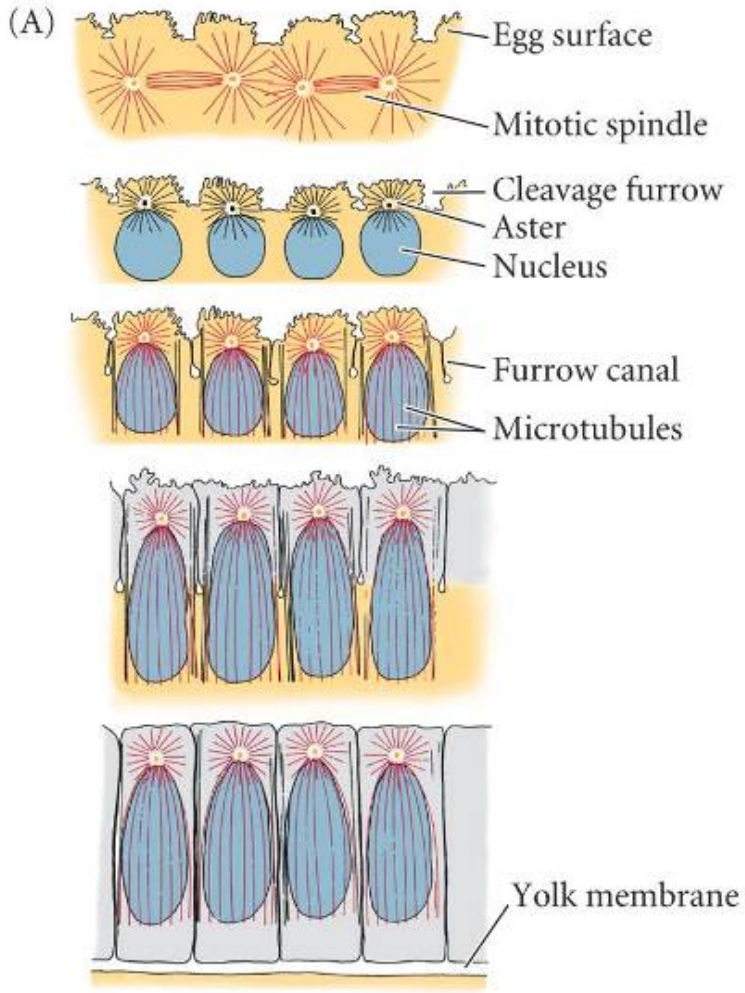


Single cell

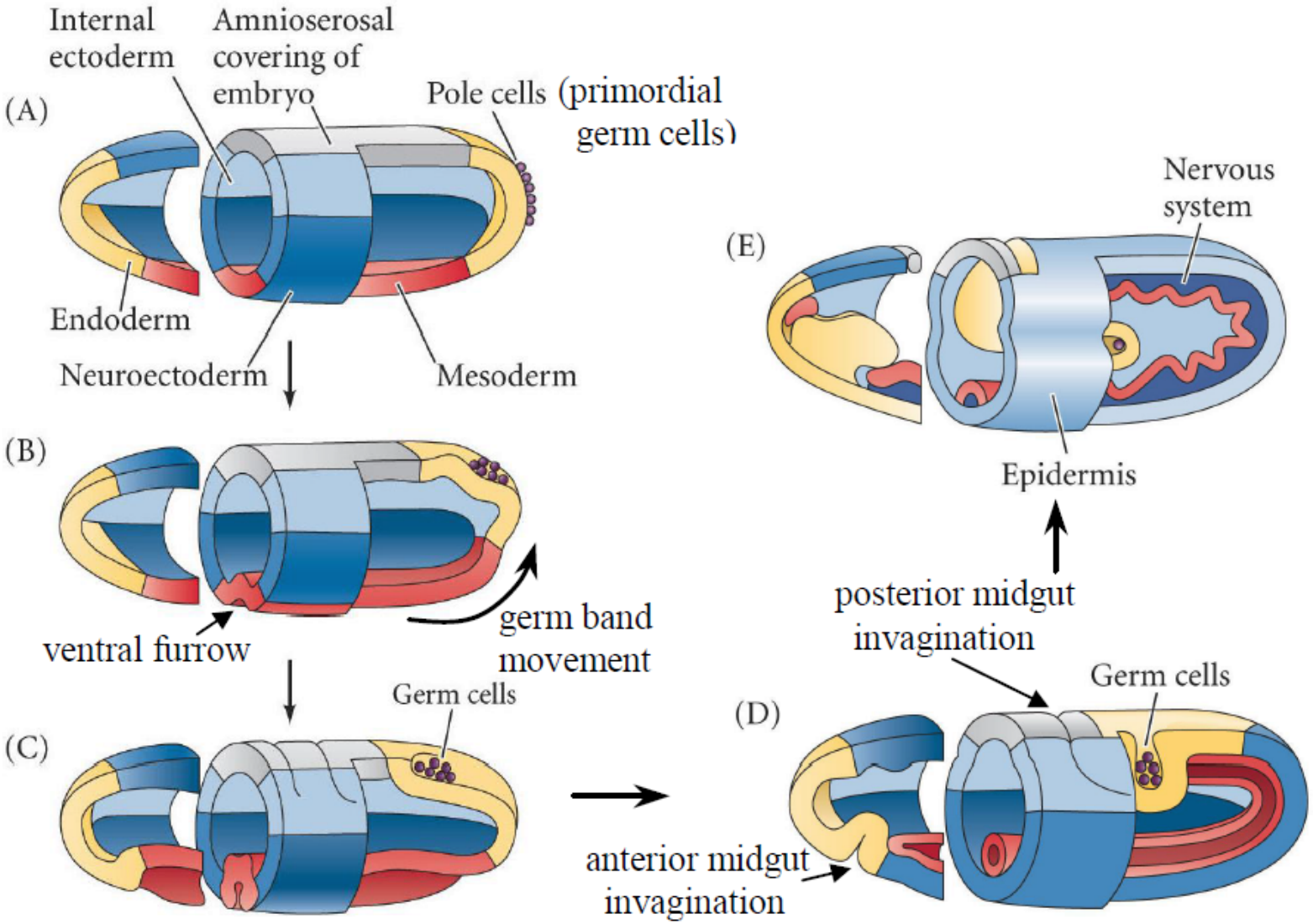
Confocal images of Chromatin Showing Superficial Cleavage in *Drosophila* Embryo



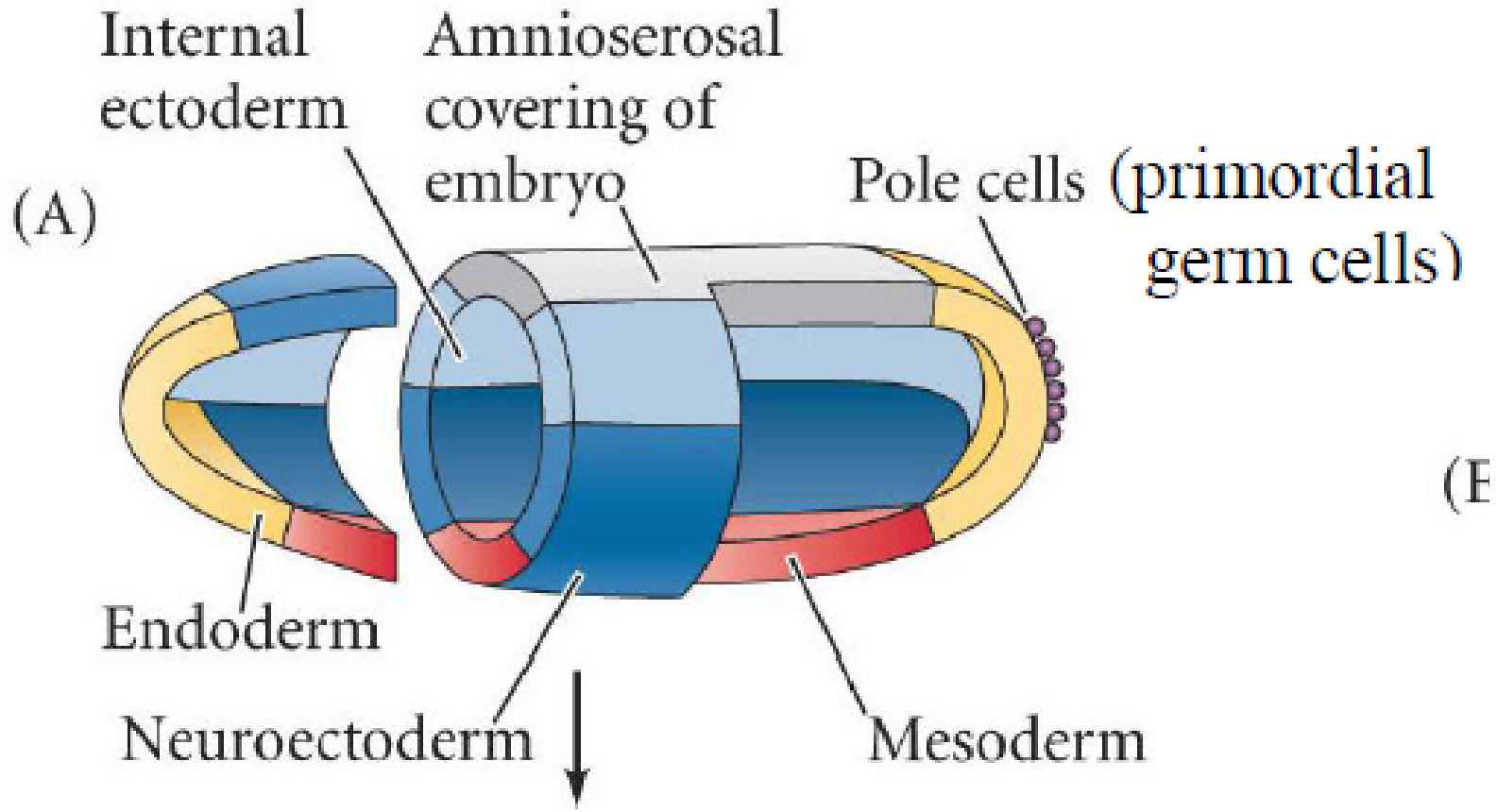
Formation of the Cellular Blastoderm in *Drosophila*



Gastrulation

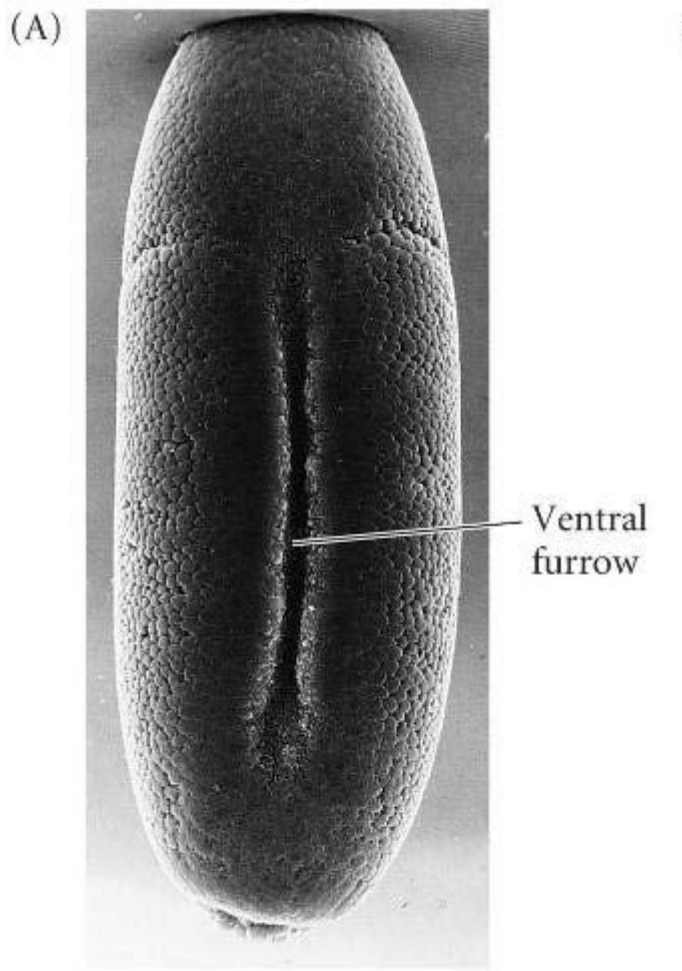


Presumptive cellular distribution

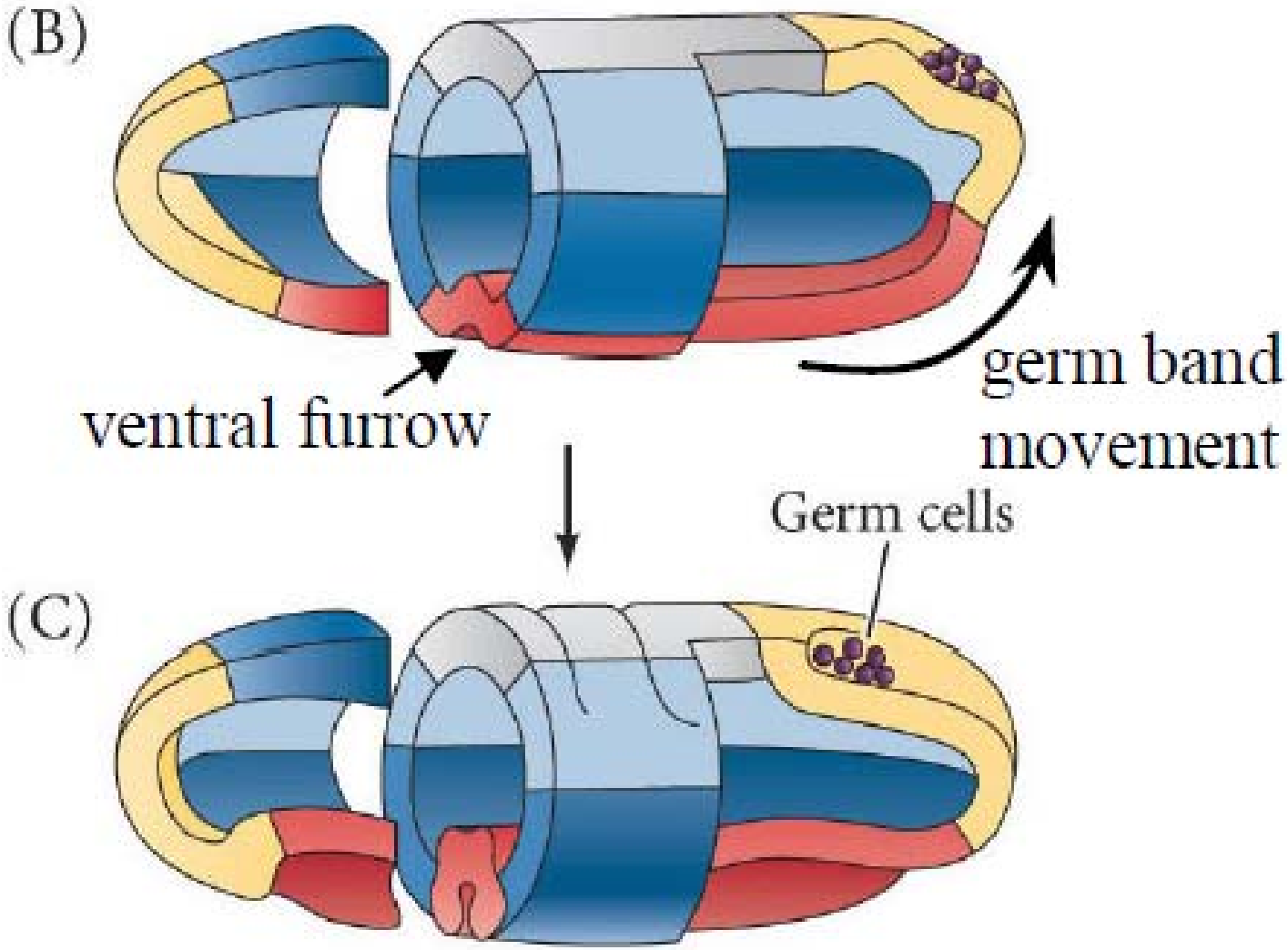


Ventral furrow

- The first movements of *Drosophila* gastrulation segregate the presumptive mesoderm, endoderm, and ectoderm.
- The prospective mesoderm about 1000 cells constituting the ventral midline of the embryo folds inward to produce the **ventral furrow**

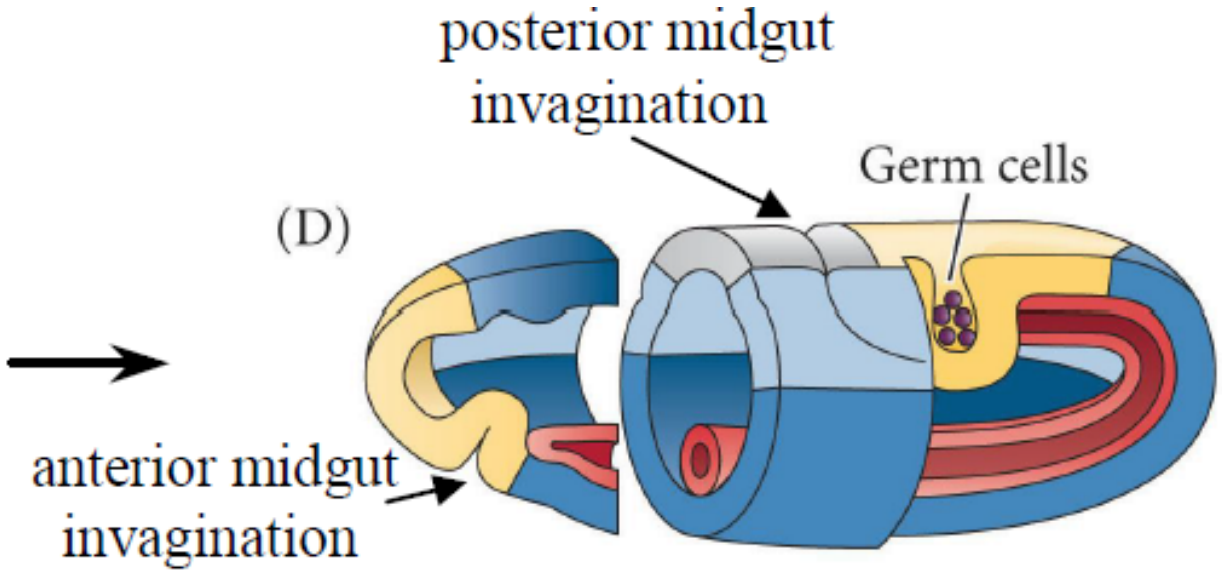


Ventral furrow

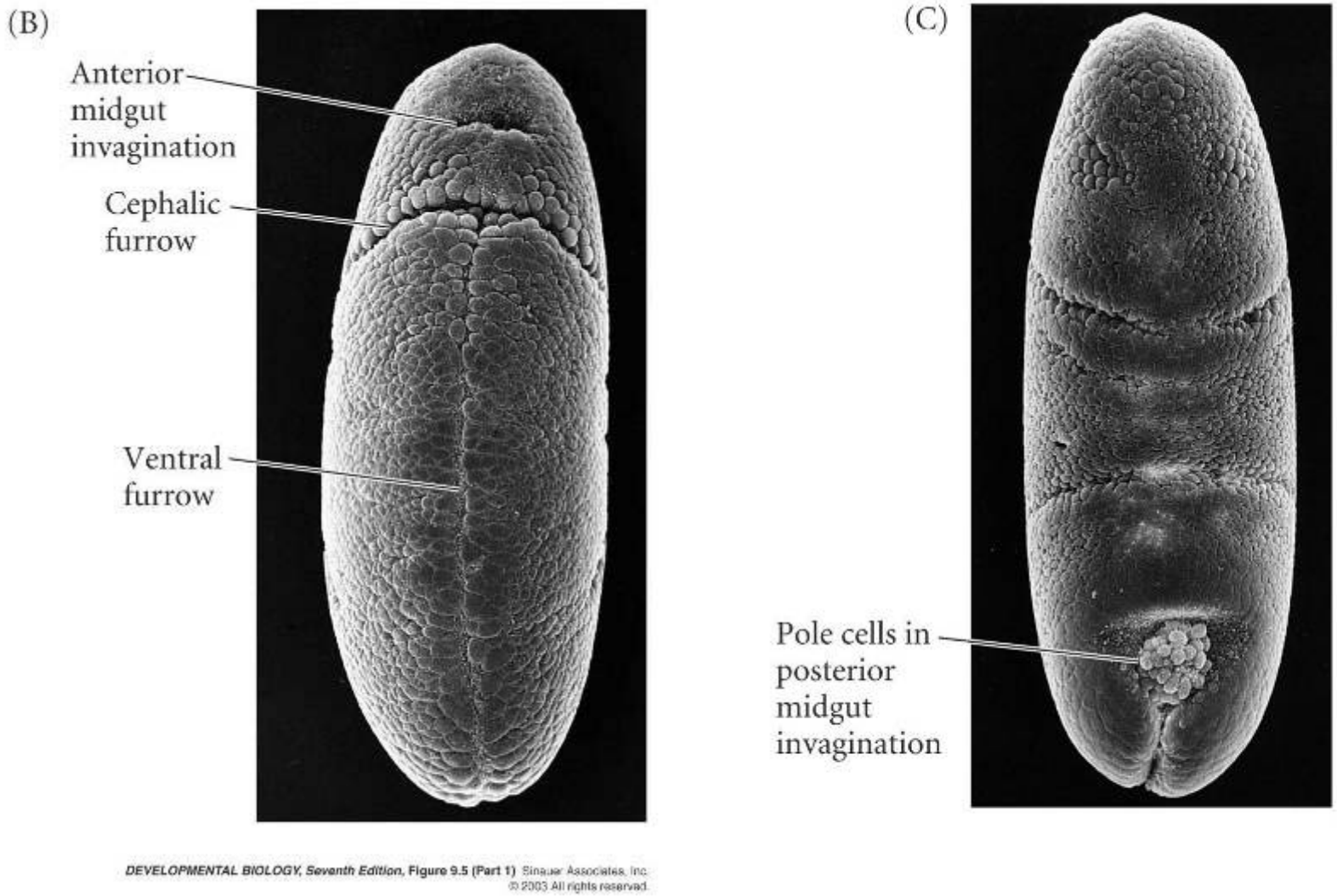


Mesoderm & Endoderm

- This furrow eventually pinches off from the surface to become a ventral tube within the embryo. It then flattens to form a layer of mesodermal tissue beneath the ventral ectoderm.
- The prospective endoderm invaginates as two pockets at the anterior and posterior ends of the ventral furrow. The pole cells are internalized along with the endoderm. At this time, the embryo bends to form the cephalic furrow.



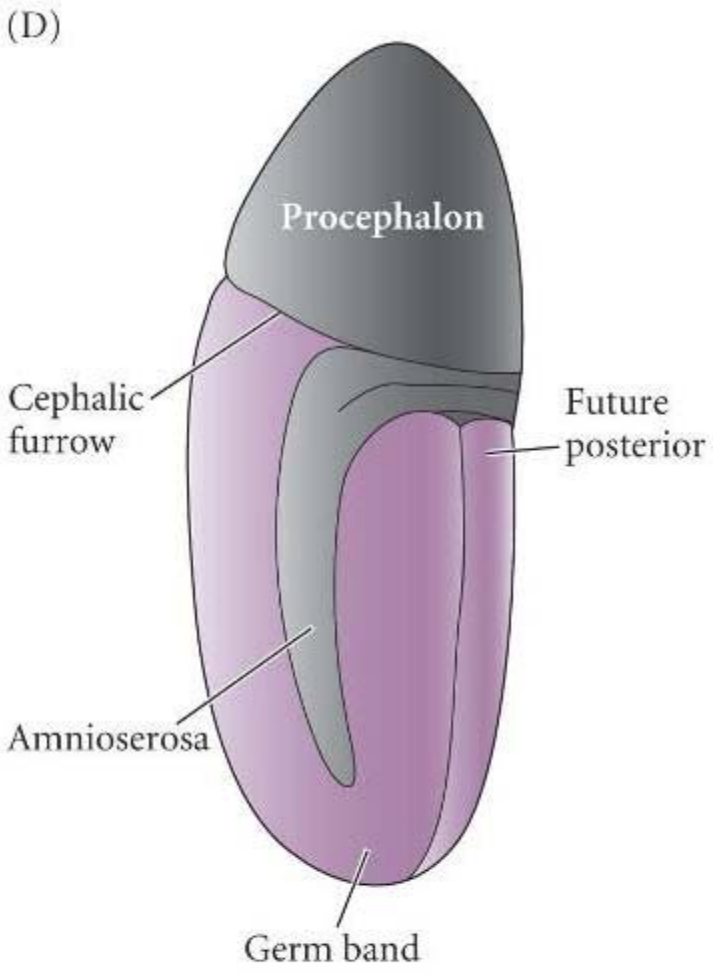
Continued



DEVELOPMENTAL BIOLOGY, Seventh Edition, **Figure 9.5 (Part 1)** Sinauer Associates, Inc.

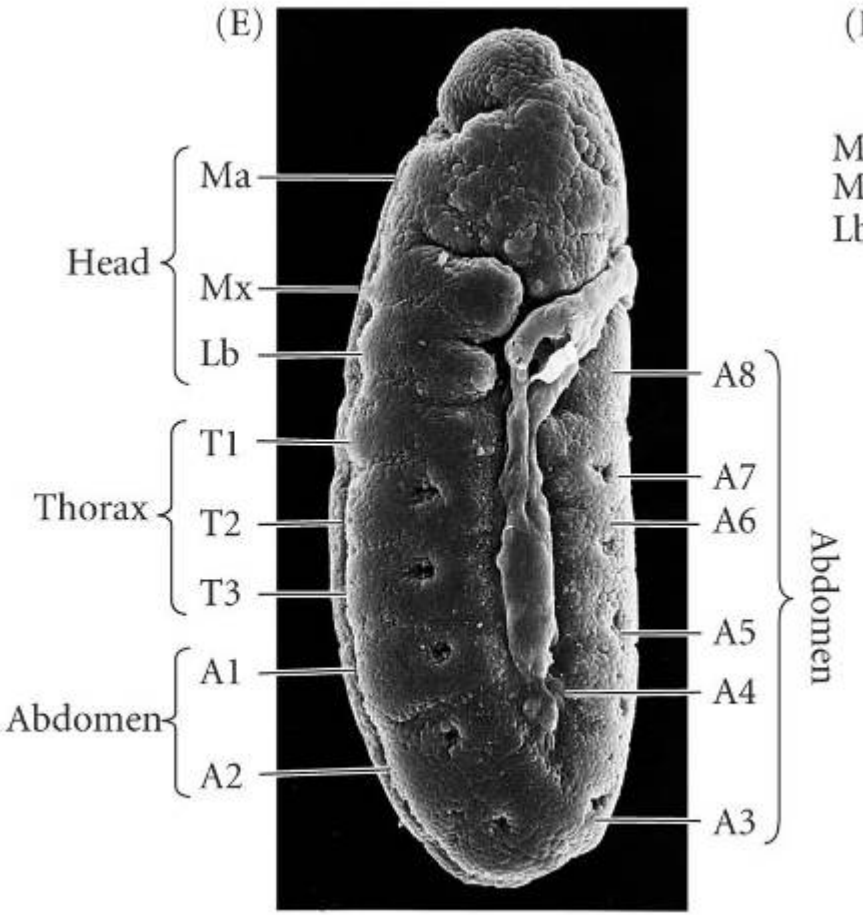
Germ band extension

- The ectodermal cells on the surface and the mesoderm undergo convergence and extension, migrating toward the ventral midline to form the **germ band**, a collection of cells along the ventral midline that includes all the cells that will form the trunk of the embryo.
- The germ band extends posteriorly and, perhaps because of the egg case, wraps around the top (dorsal) surface of the embryo



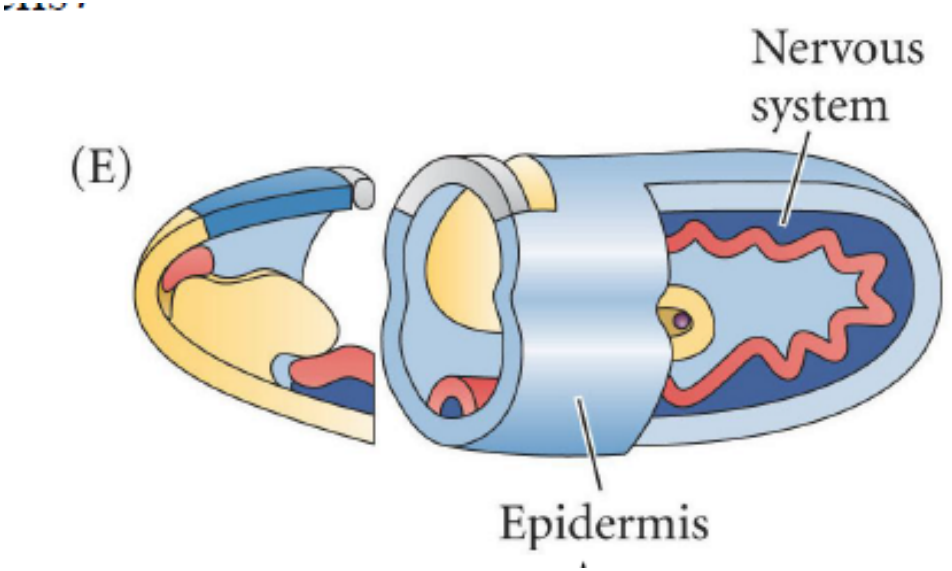
Continued

- While the germ band is in its extended position, several key morphogenetic processes occur: organogenesis, segmentation, and the segregation of the imaginal discs



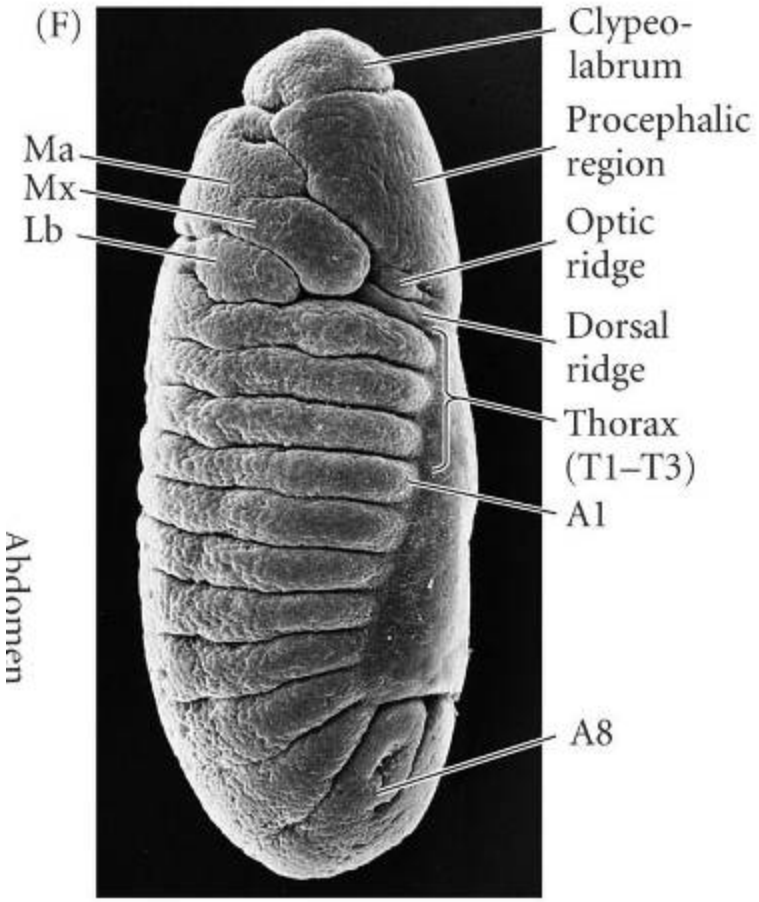
Nervous system

- In addition, the nervous system forms from two regions of ventral ectoderm. Neuroblasts differentiate from this neurogenic ectoderm within each segment (and also from the nonsegmented region of the head ectoderm). Therefore, in insects like *Drosophila,* the nervous system is located ventrally, rather than being derived from a dorsal neural tube as in vertebrates.



Germ band retraction

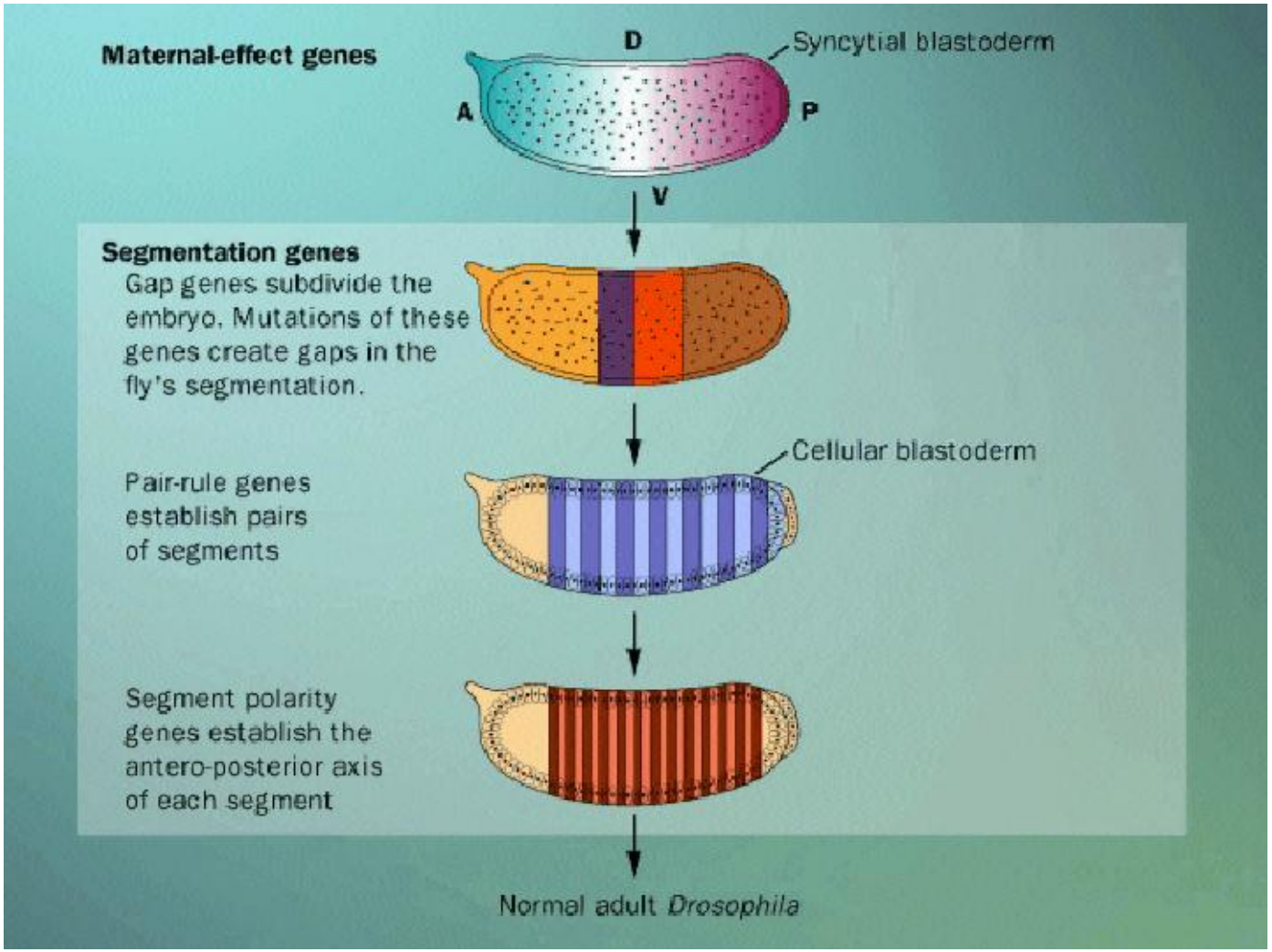
- Thus, at the end of germ band formation, the cells destined to form the most posterior larval structures are located immediately behind the future head region. At this time, the body segments begin to appear, dividing the ectoderm and mesoderm.
- The germ band then retracts, placing the presumptive posterior segments into the posterior tip of the embryo



DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 9.5 (Part 3) Sinauer Associates, Inc.

How does the cells know who they are and where they belong?

All in the cytoplasm, at least in the beginning



Maternal affect genes

- The anterior-posterior polarity of the embryo, larva, and adult has its origin in the anterior-posterior polarity of the egg
- The **maternal effect genes** expressed in the mother's ovaries produce messenger RNAs that are placed in different regions of the egg (mostly).
- These messages generally encode transcriptional and translational regulatory proteins that diffuse through the syncytial blastoderm and activate or repress the expression of certain zygotic genes.

The whole list, so far

Gene	Mutant phenotype	Proposed function and structure
AnteriroGroup		
bicoid (*bcd*)	Head and thorax deleted, replaced by inverted telson	Graded anterior morphogen; contains homeodomain; represses caudal
exuperantia (*exu*)	Anterior head structures deleted	Anchors bicoid mRNA
swallow (*swa*)	Anterior head structures deleted	Anchors bicoid mRNP
PosteriroGroup		
nanos (*nos*)	No abdomen	Posterior morphogen; represses hunchback
tudor (*tud*)	No abdomen, no pole cells	Localization of Nanos
oskar (*osk*)	No abdomen, no pole cells	Localization of Nanos
vasa (*vas*)	No abdomen, no pole cells; oogenesis defective	Localization of Nanos
valois (*val*)	No abdomen, no pole cells; cellularization defective	Stabilization of the Nanos localization complex
pumilio (*pum*)	No abdomen	Helps Nanos protein bind hunchback message
caudal (*cad*)	No abdomen	Activates posterior terminal genes
TerminalGroup		
torso (*tor*)	No termini	Possible morphogen for termini
trunk (*trk*)	No termini	Transmits Torsolike signal to Torso
fs(1)*Nasrat*[*fs*(1)*N*]	No termini; collapsed eggs	Transmits Torsolike signal to Torso
fs(1)*polehole*[*fs*(1)*ph*]	No termini; collapsed eggs	Transmits *Torsolike* signal to *Torso*

Bicoid

- A transcription factor, homebox family of protein
- *bicoid* mRNA Synthesized in nurse cells dumped into oocyte
- The protein cannot be detected in oocytes, indicating translation of *bicoid* is inhibited prior to fertilization. Zygotic translation can be detected shortly after egg deposition, and immediately after fertilization, at the anterior tip of the embryo. As a consequnce of the anterior localization of RNA, a gradient of Bicoid protein becomes established prior to cellularization
- The anterior-posterior gradient of Bicoid plus its partner Hunchback are required to either activate or inhibit transcription of a variety of zygotic genes, including *hunchback*, gap genes such as *empty spiracles, Krüppel* and *knirps*, pair rule genes like *even-skipped* and *runt*, and even some homeotic and Polycomb group genes.

Hunchback

- Belongs to zinc finger protein family
- Hunchback can operate both as a transcription activator or repressor
- mRNA is distributed evenly along the embryo
- *bicoid* influences the Hunchback protein expression higher at anterior
- Hunchback acts both to activate anterior gap gene function as a co-activator with Bicoid, and to shift the effective morphogenetic activity of Bicoid toward the posterior, thus extending the effective range of Bicoid
- Hunchback's main role is as a repressor of posterior gap gene expression in the anterior. *Krüppel* expression in the middle of the embryo is regulated by HB. *knirps* and *giant* are expressed in the posterior, but these genes are repressed in the anterior by Hunchback

Nanos

- Another zinc finger binding protein
- In mature eggs and the developing zygote *nos* is required to specify posterior identity. *nos* mRNA is localized to the pole plasm, a specialized cytoplasm later incorporated into pole cells, the precursors of the fly's reproductive system
- For its role in specifying posterior identity, *nos* is required briefly but immediately after fertilization.
- Translation of both bicoid and hunchback targets is inhibited by the binding of NOS protein to the 3' untranslated region of their mRNAs at the posterior

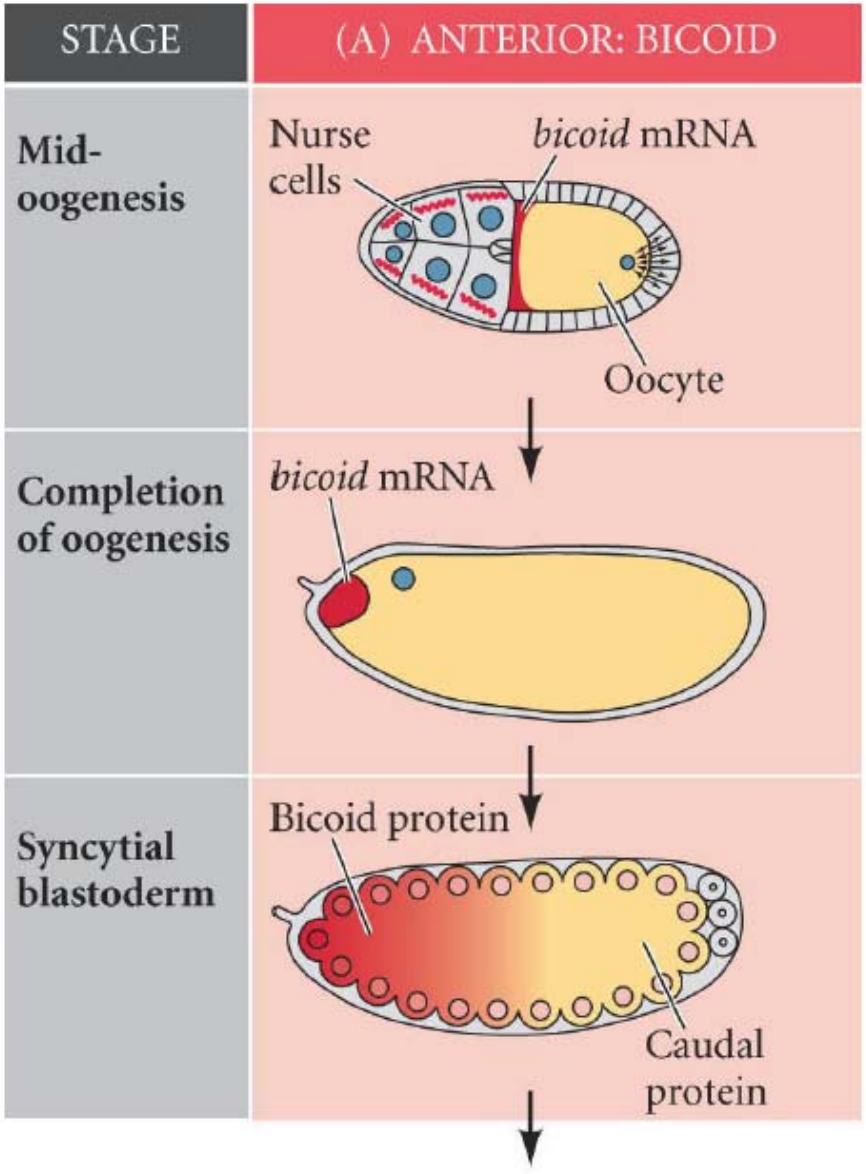
Caudal

- Another homebox family transcription factor
- The mRNA is found throughout the egg and embryo
- Bicoid limits the expression of Cad to the posterior
- *caudal* has been shown to be essential for invagination of the hindgut primordium and for further specification and development of the hindgut

Torso

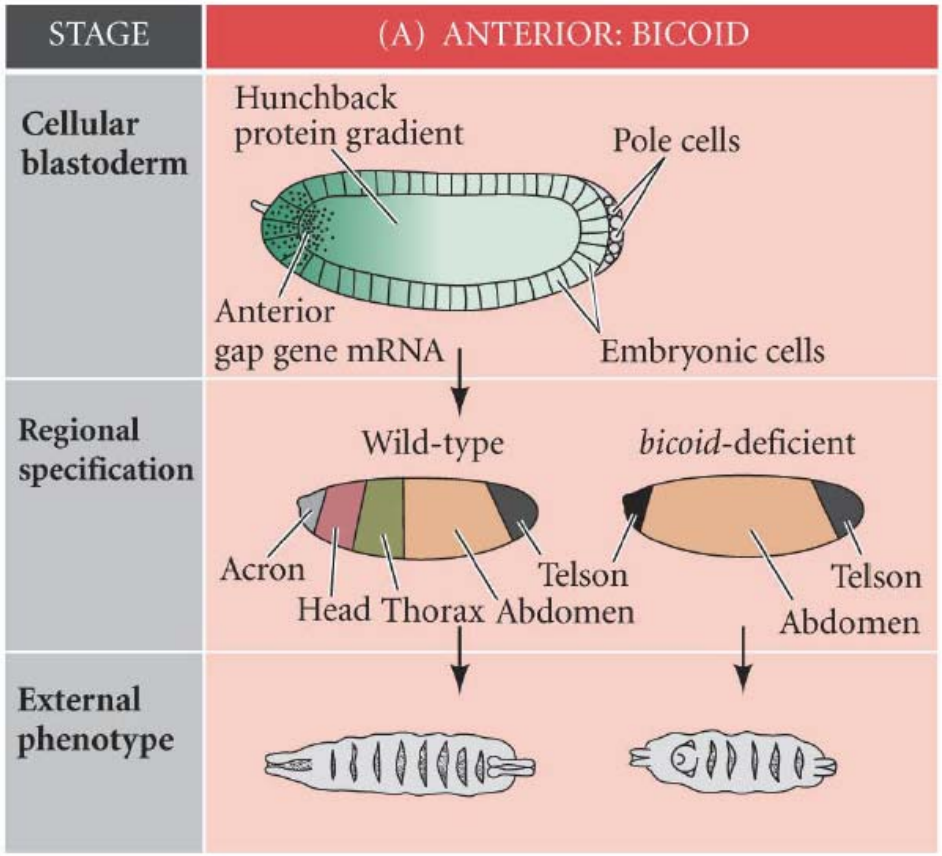
- All the genes involved in the activation of Torso and its targets make up the so-called terminal group. The synergistic network of interactions and influences involving these genes is known as the terminal pathway.
- It is a Receptor Tyrosine Kinase (RTK), a membrane receptor
- Activation of the downstream targets of Torso involves a phosphorylation cascade. Upstream, a protein molecule (ligand), probably Trunk, triggers Torso signaling locally
- The targets of Torso are members of the Ras pathway
- The resultant phosphorylation cascade activates *tailless* and *huckebein,* two targets of the terminal system. *tailless* is held in a state of repression by *grainyhead/NTF-1* until Torso pathway signals inactivate the repression

Anterior specification 1

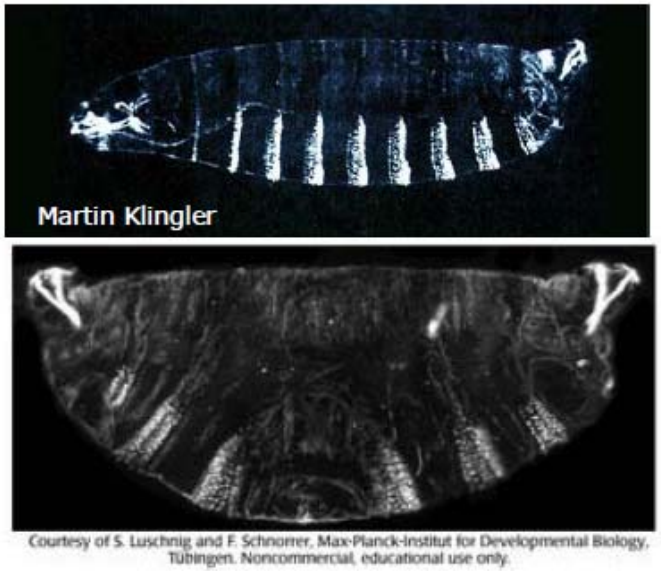


- Lets look at *bicoid* distribution
- In each egg *bicoid* mRNA are localized to the anterior
- So the fertilized eggs have bicoid expressed at anterior
- This is called maternal contribution
- Remember at this time the zygotic transcription is yet to start
- All the examples are transcription factors, until *torso* protein

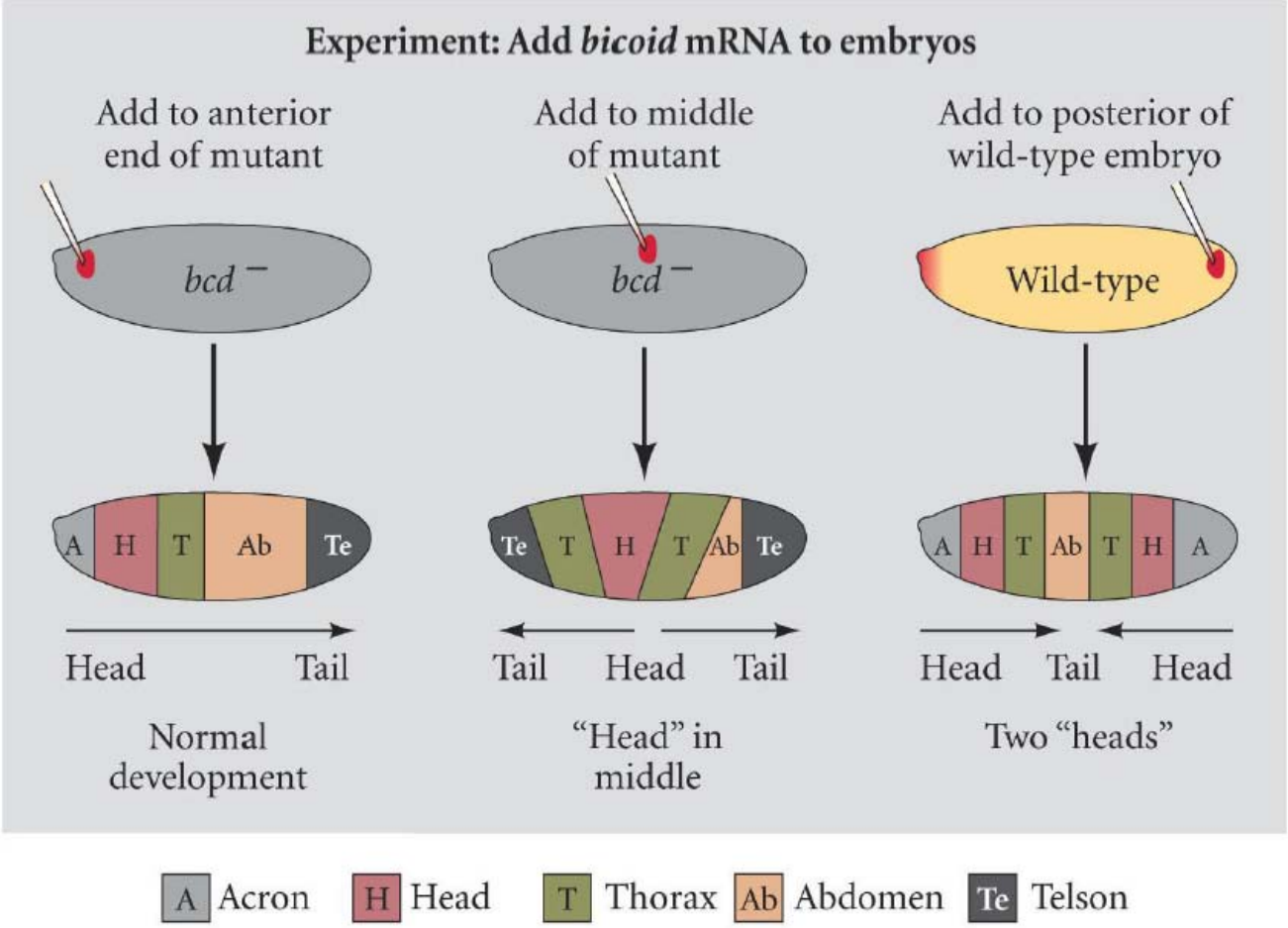
Anterior specification 2



- Hunchback is another example of anterior specification protein
- So what happens if you loose *bicoid*?

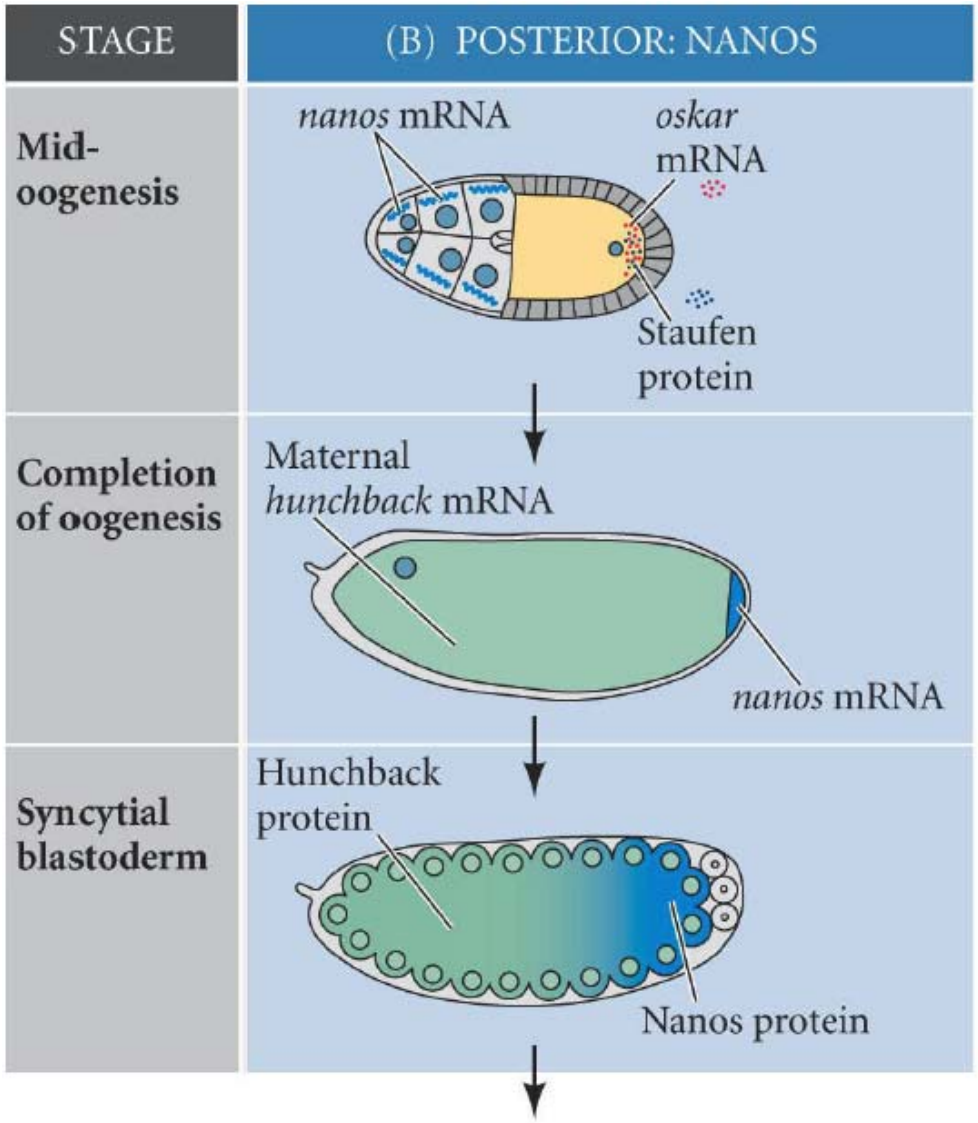


bicoid manipulation



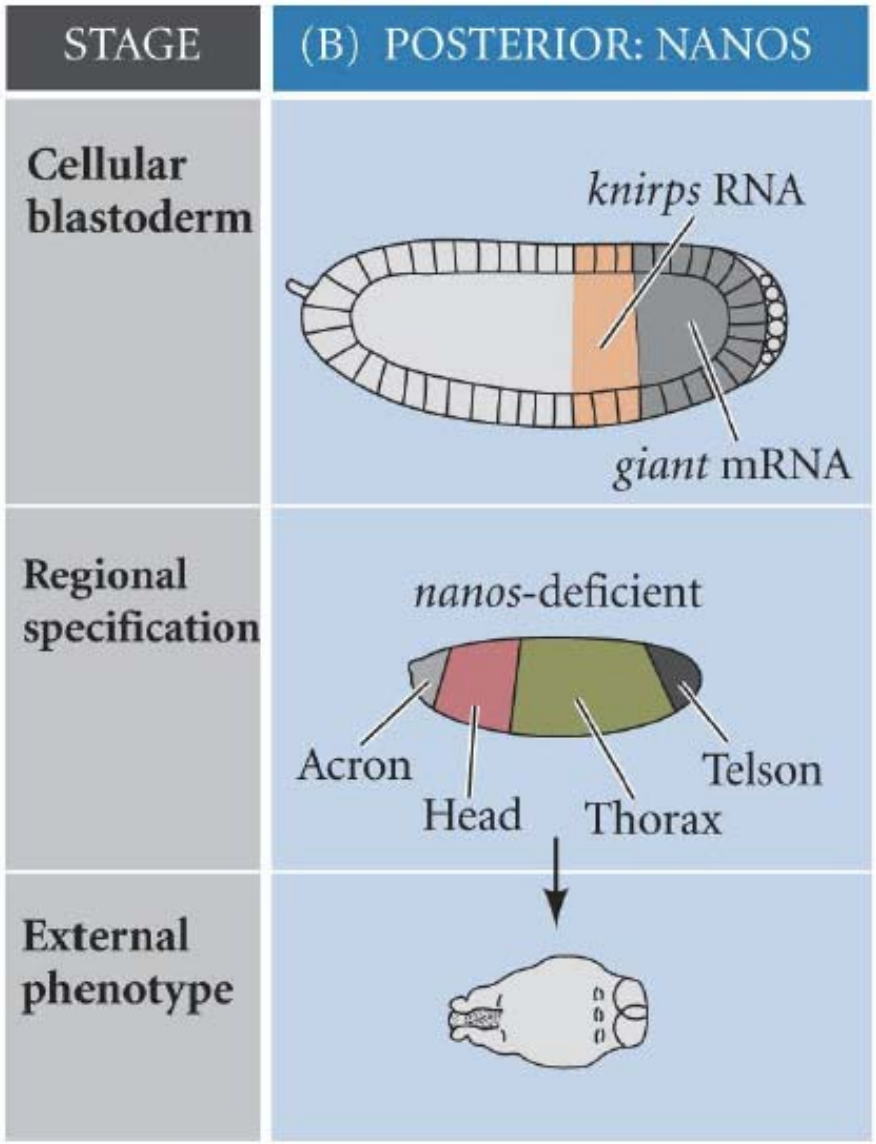
- So if *bicoid* defines anterior, then we can have anterior wherever we want, right?

Posterior specification 1



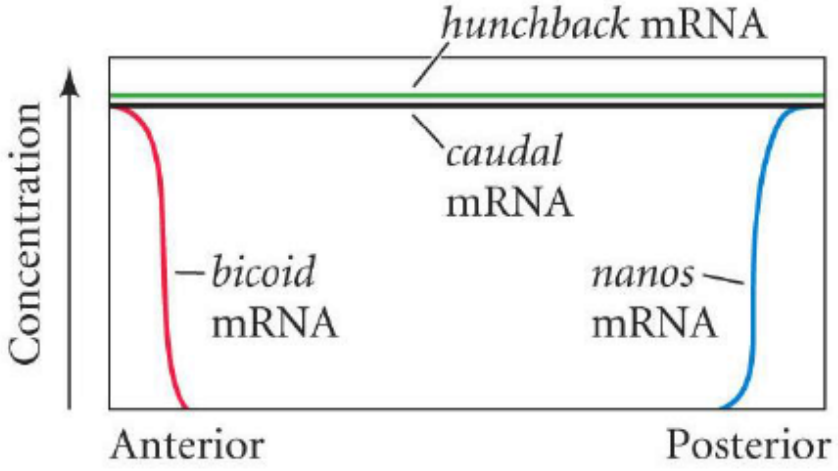
- *nanos* specify posterior
- Similar to *bicoid* it is maternally provided
- It is also a transcription factor
- Only it is expressed at the posterior

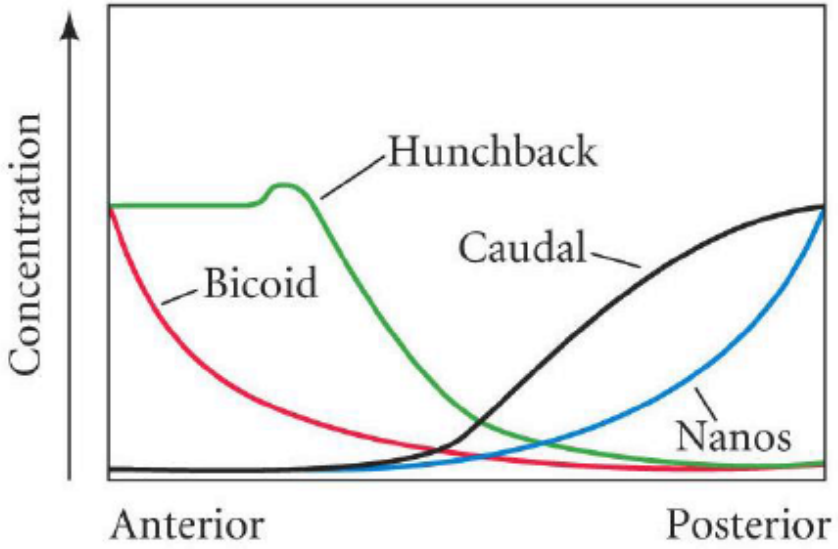
Posterior specification 2



- *knirps* and *giant* are other two factors that specify posterior
- Without *nanos* the embryo develops into abdomen-less larvae
- So the question is how do they regulate?

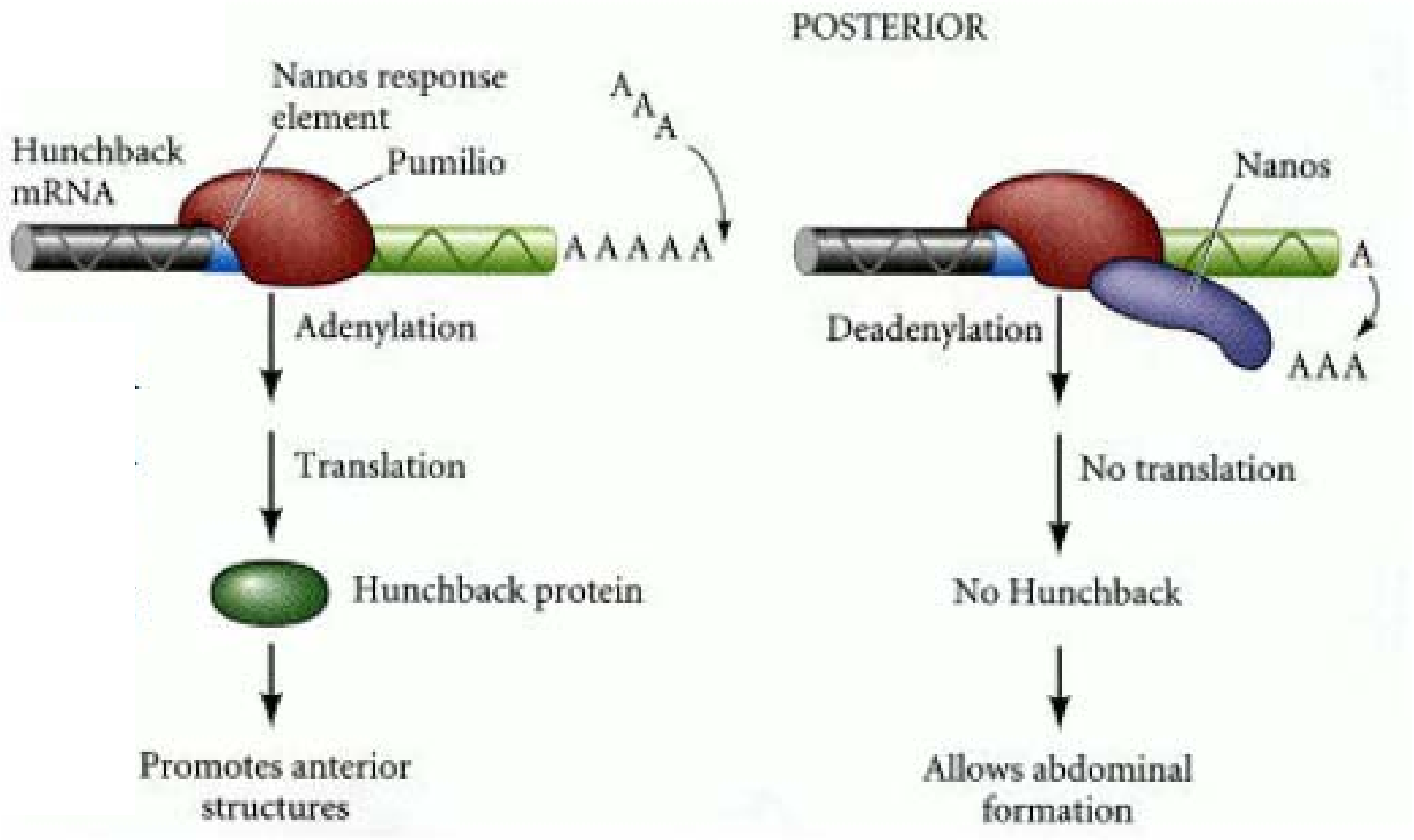
Model of anterior-posterior (AP) patterning



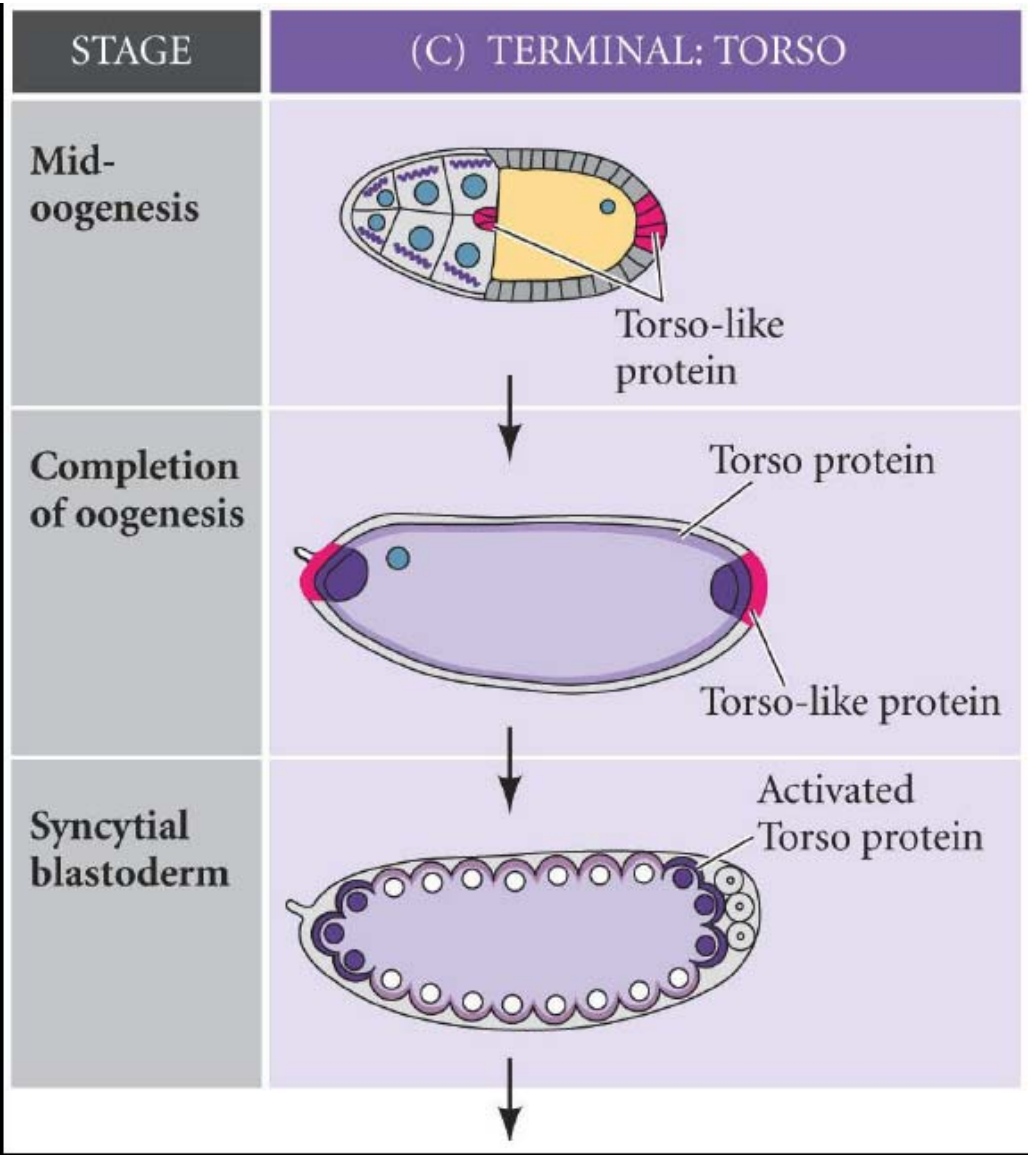


- At the start *bicoid* and *nanos* have definite distribution
- But *hunchback* and *caudal* is evenly distributed
- *bicoid* would stop *caudal* expression at anterior and enhance hunchback expression at anterior
- And *nanos* would do the same to *hunchback*
- So now we have uneven distribution if them as well

At molecular level

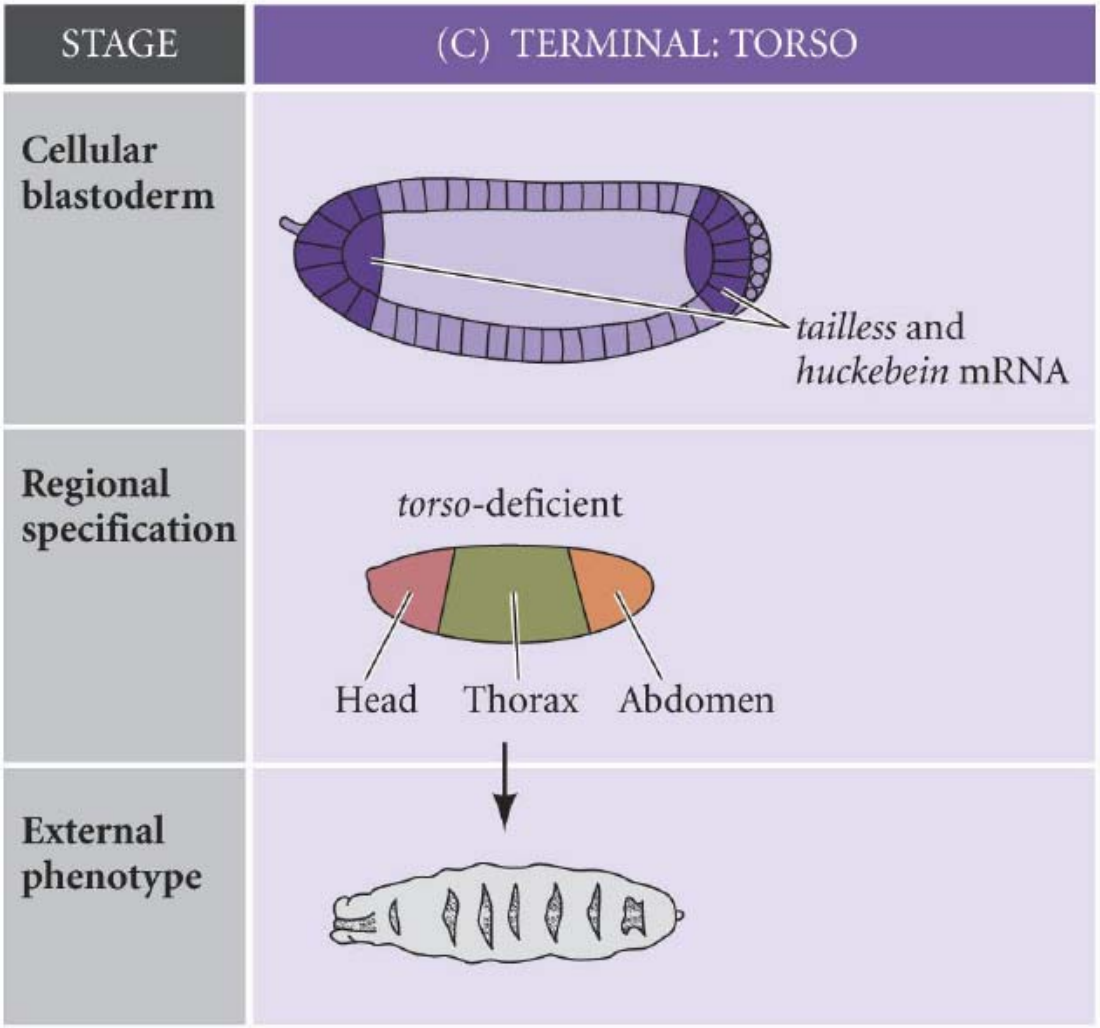


Terminal specification 1



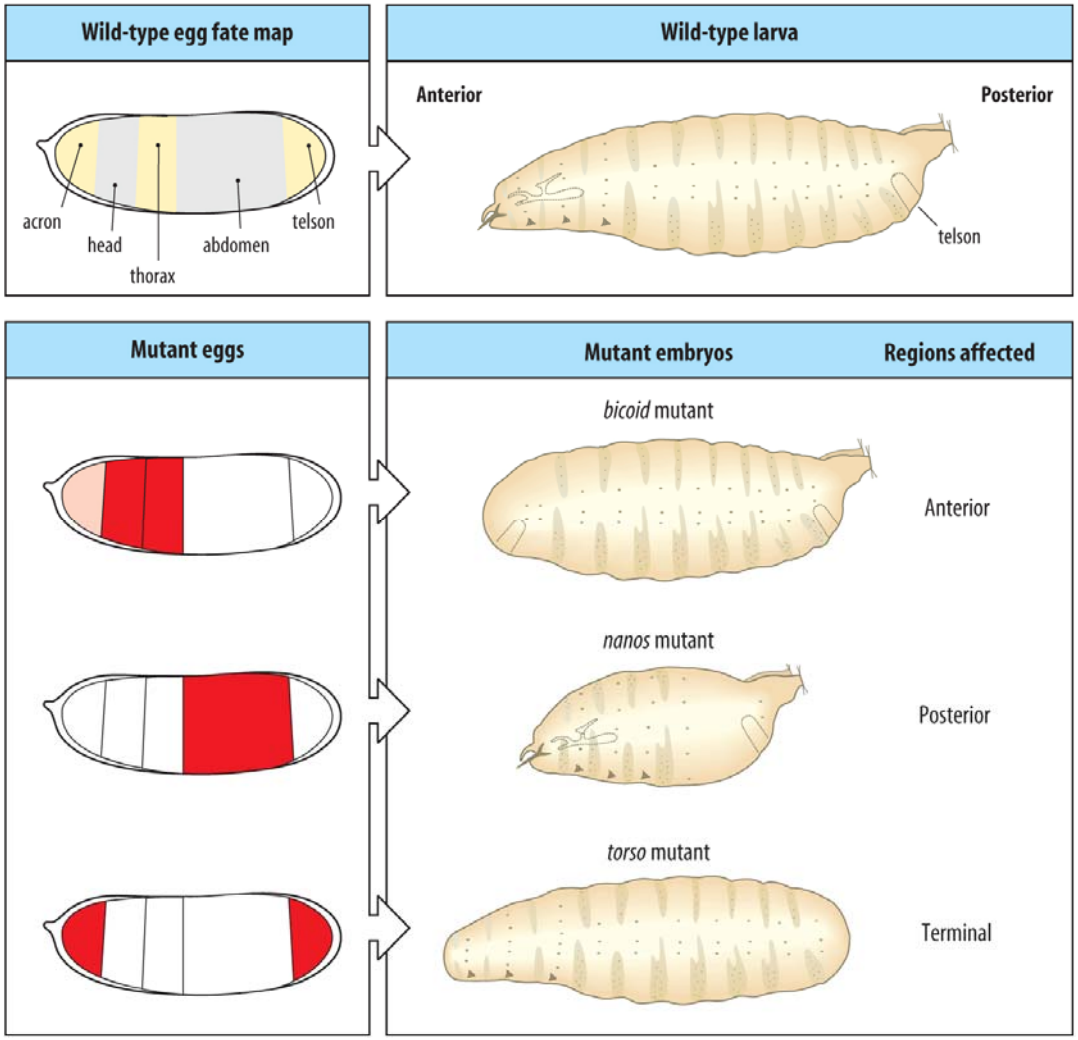
- We must not forget *torso*
- It defines the extreme AP
- Unlike *bicoid* and *nanos* it is a RTK and the distribution is even
- *torso* is activated at both ends by Torso-like protein (maternally provided protein)
- Mutant of *torso (*constitutively active*)* ends in a larvae with only head and tail

Terminal specification 2



- It is a negative regulator of a negative regulator of *tailless* and *huckebein*
- So the expression of *tailless* would create telson (tail)
- Expression of *huckebein* at the anterior with *bicoid* would create acron (head)
- Nobel Prize for Nüsslein-Volhard and Eric Wieschaus, in 1995

To sum up


Wild-type egg fate map
acron
head
thorax
abdomen
telson
Wild-type larva
Anterior
Posterior
telson
Mutant eggs
Mutant embryos
Regions affected
bicoid mutant
Anterior
nanos mutant
Posterior
torso mutant
Terminal

Developmental biology
Scott F. Gilbert