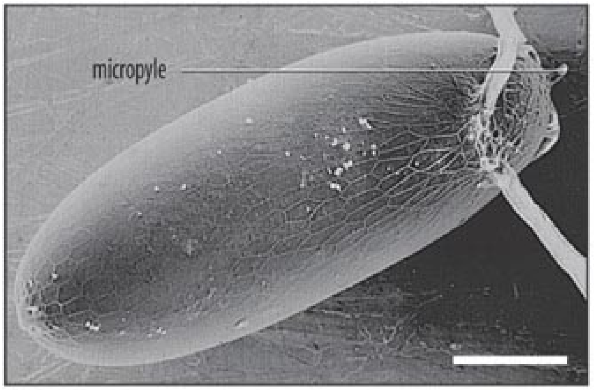
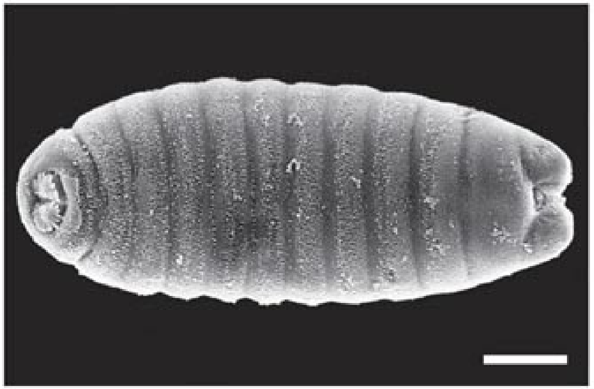
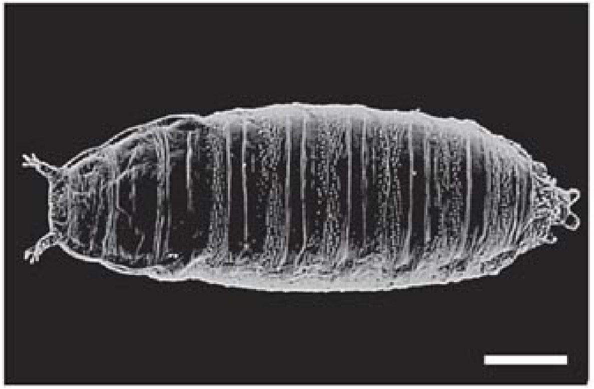
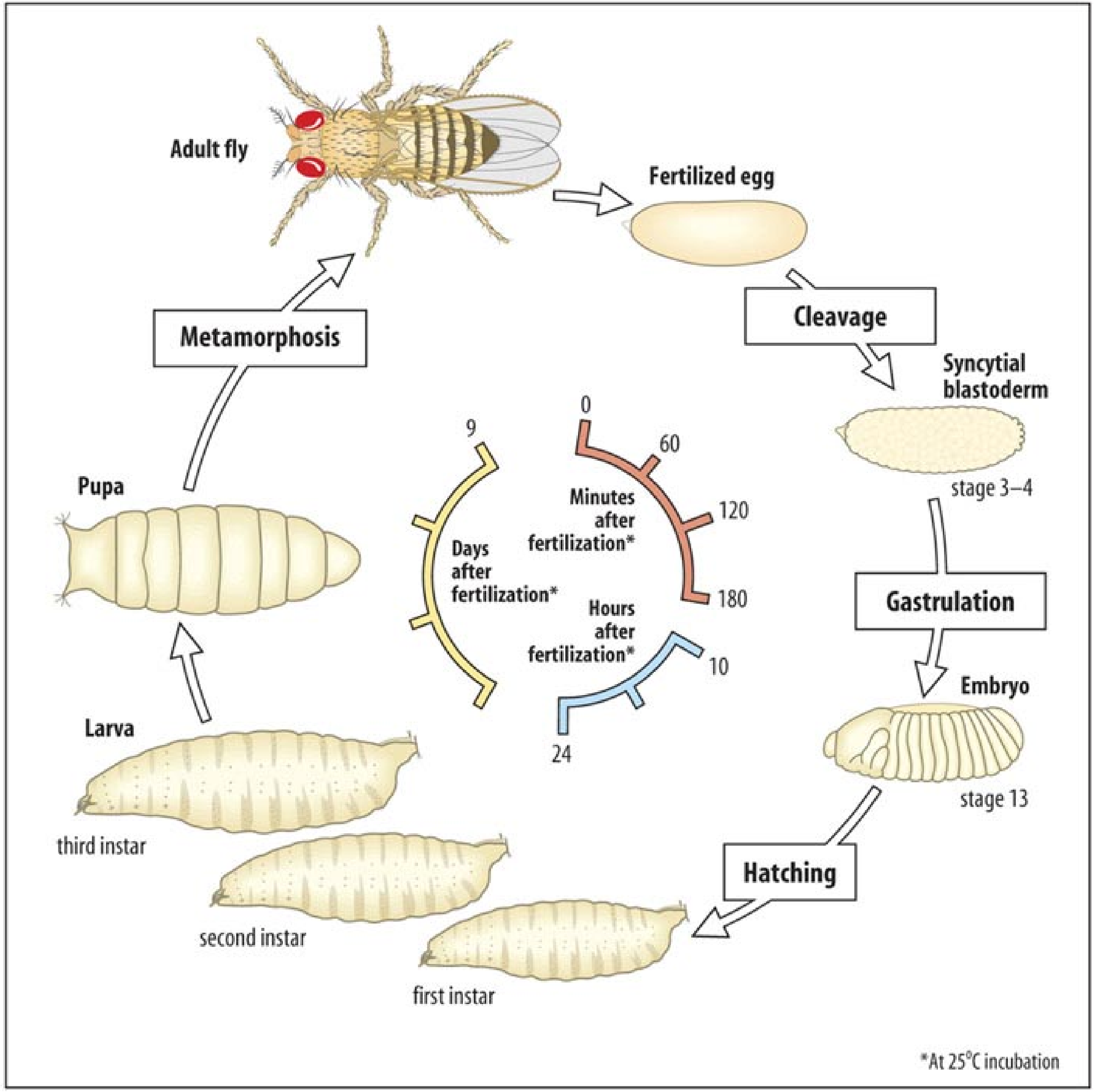
Drosophila Life Cycle



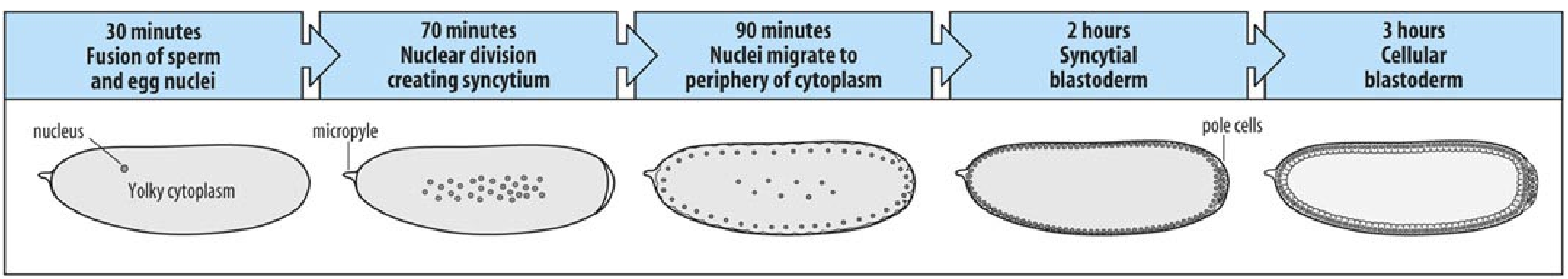




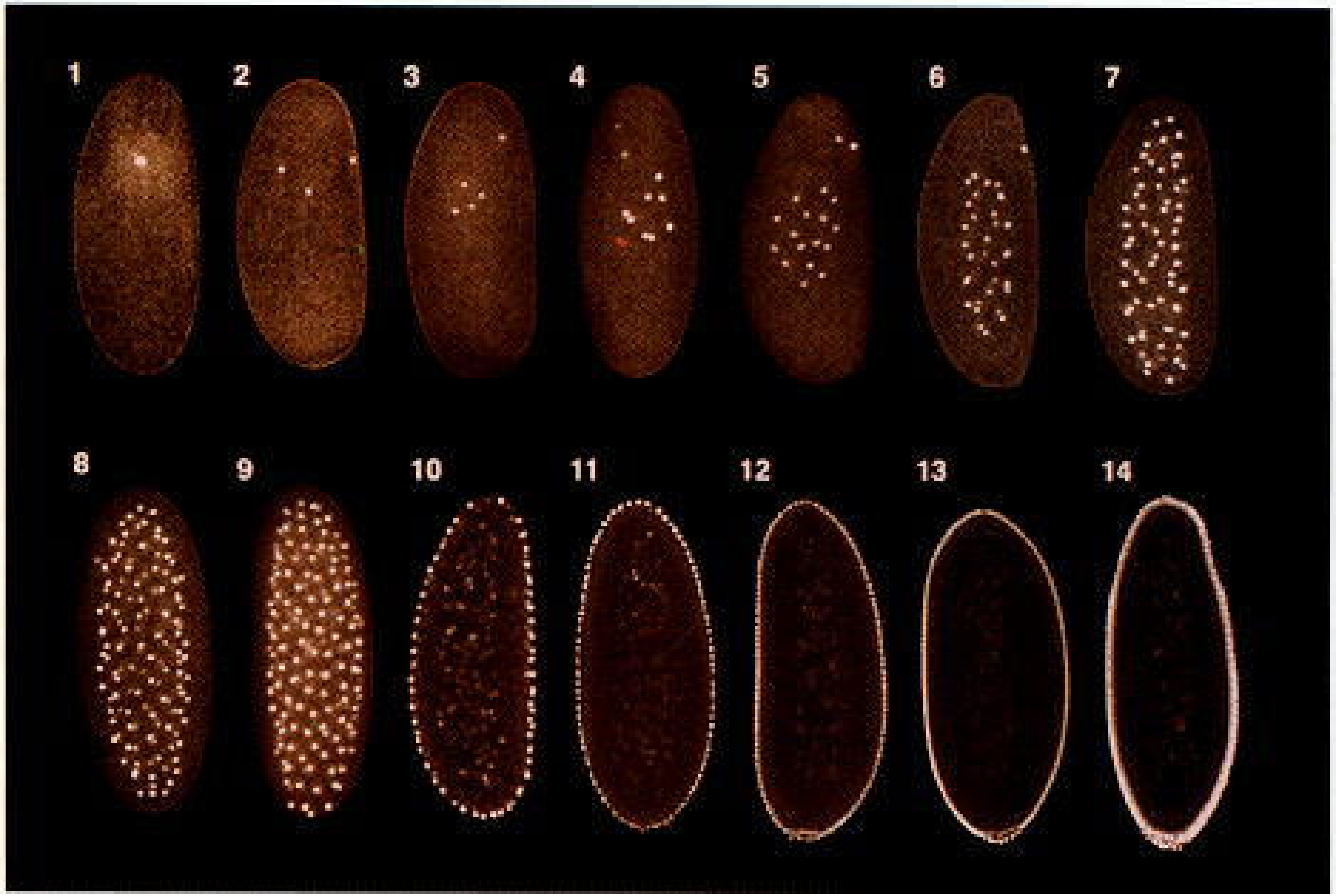


Early Drosophila Cleavage

- Nuclei migrate to periphery after 10 nuclear divisions.
- Cellularization occurs when plasma membrane folds in to divide nuclei into cells.

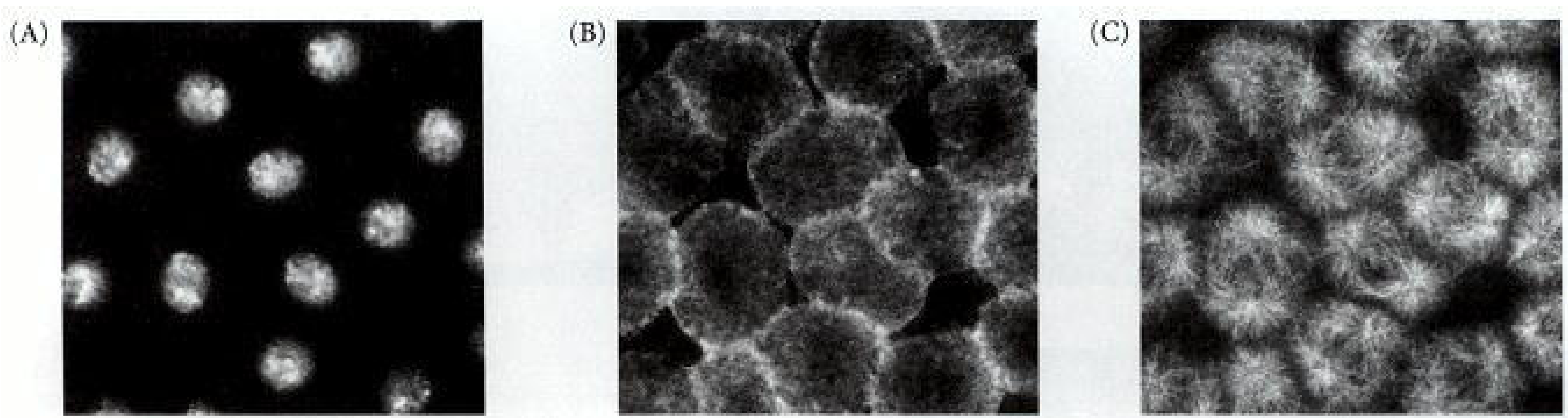


Drosophila Superficial Cleavage

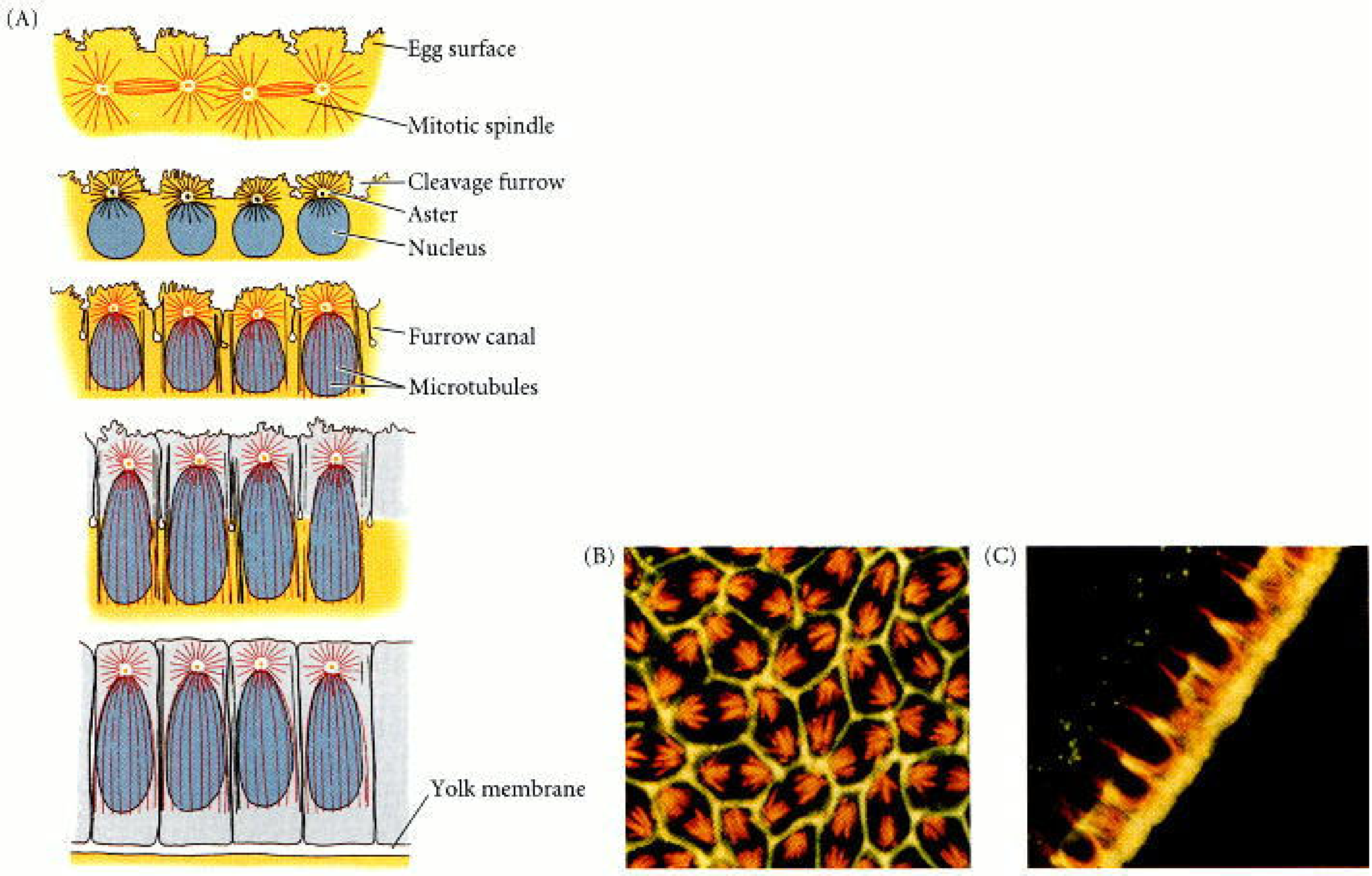


Cytoskeleton Surrounds Nuclei

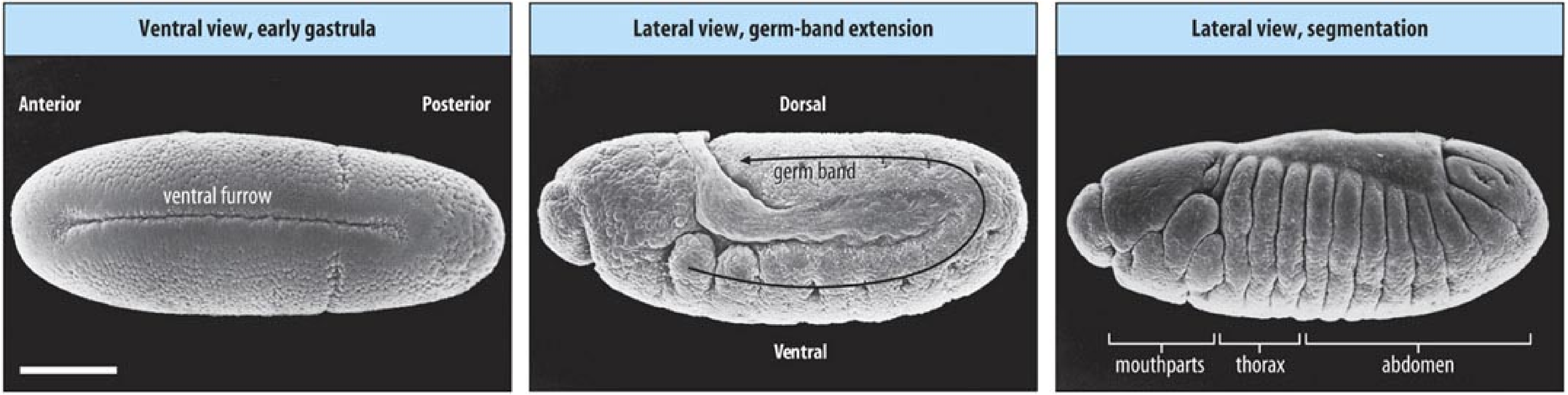
Microtubules and microfilaments direct the formation of individual cells from the syncytial blastoderm.



Cellularization of Syncytial Blastoderm

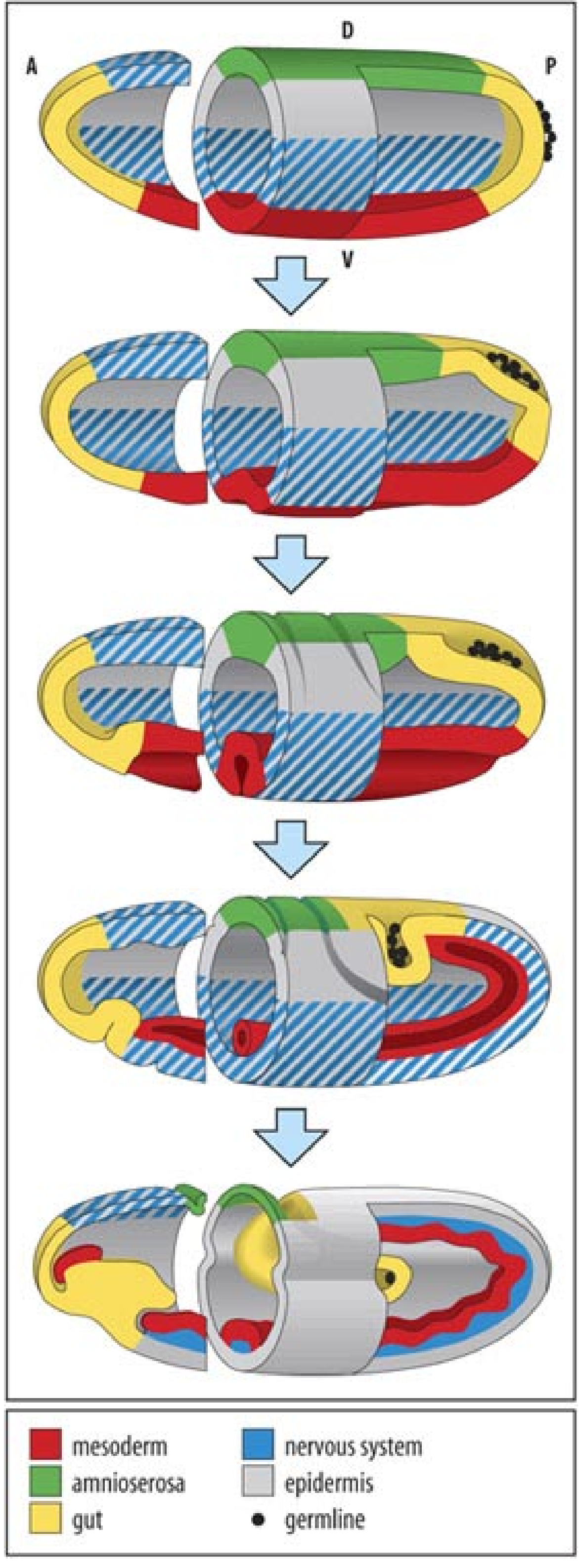


Gastrulation and Germ Band Extension

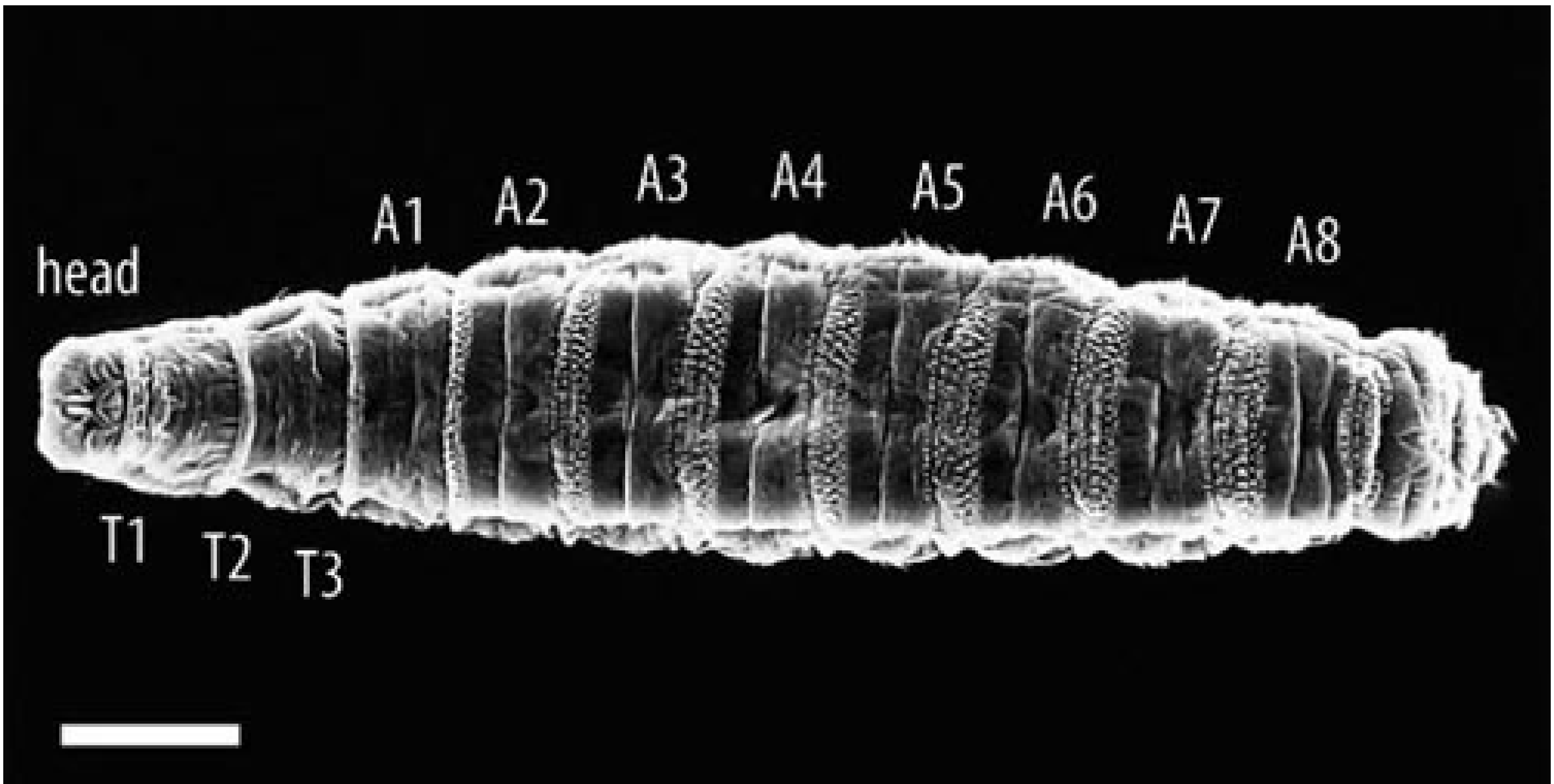


Fate Map During Gastrulation

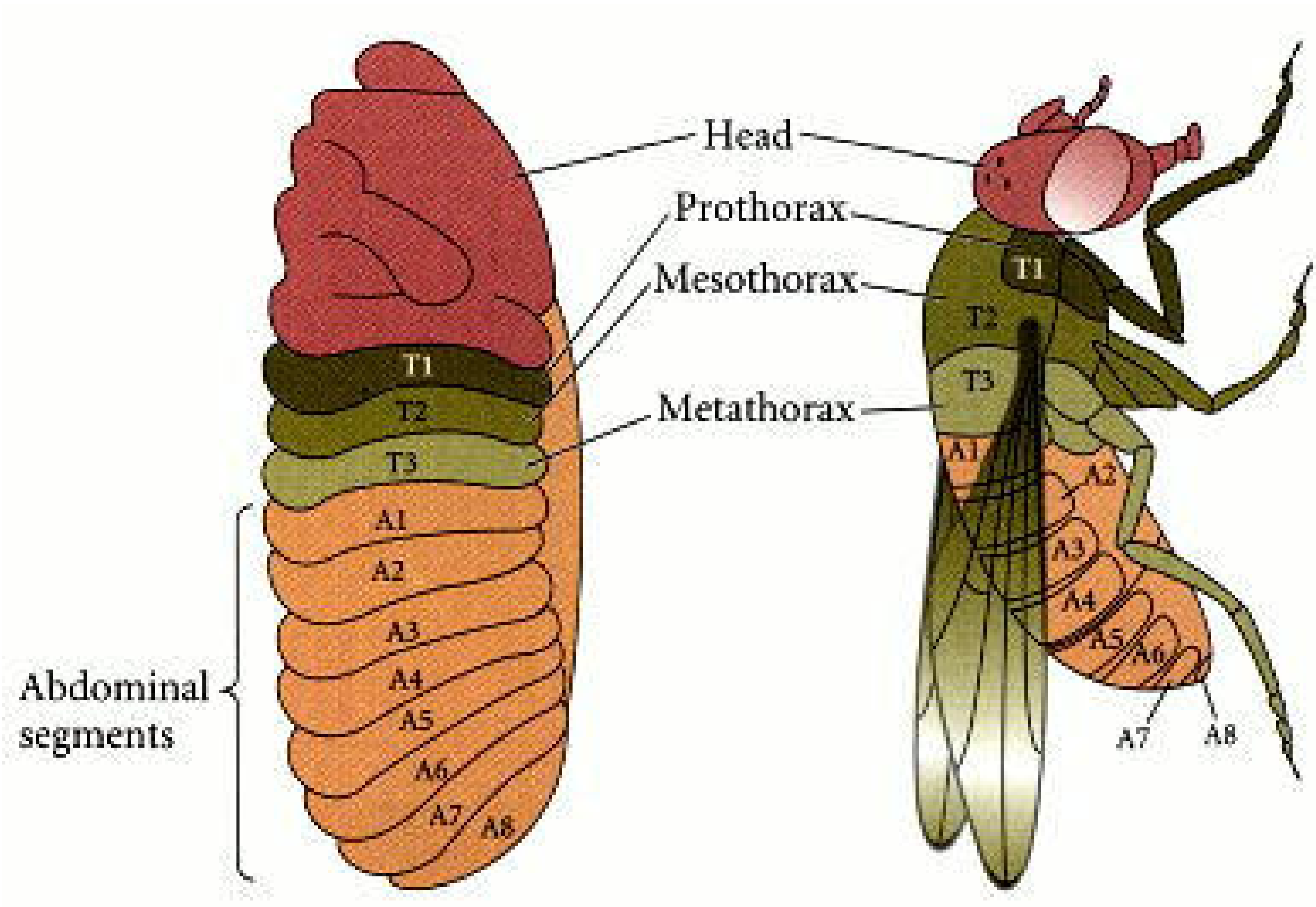
- Ventral furrow folds in to form the mesoderm.
- Lateral to the mesoderm, the neuroectoderm moves ventral to the mesoderm.
- Ectoderm spreads on surface to form the epidermis.
- Endoderm invaginates at anterior and posterior ends.
- Pole cells internalize.



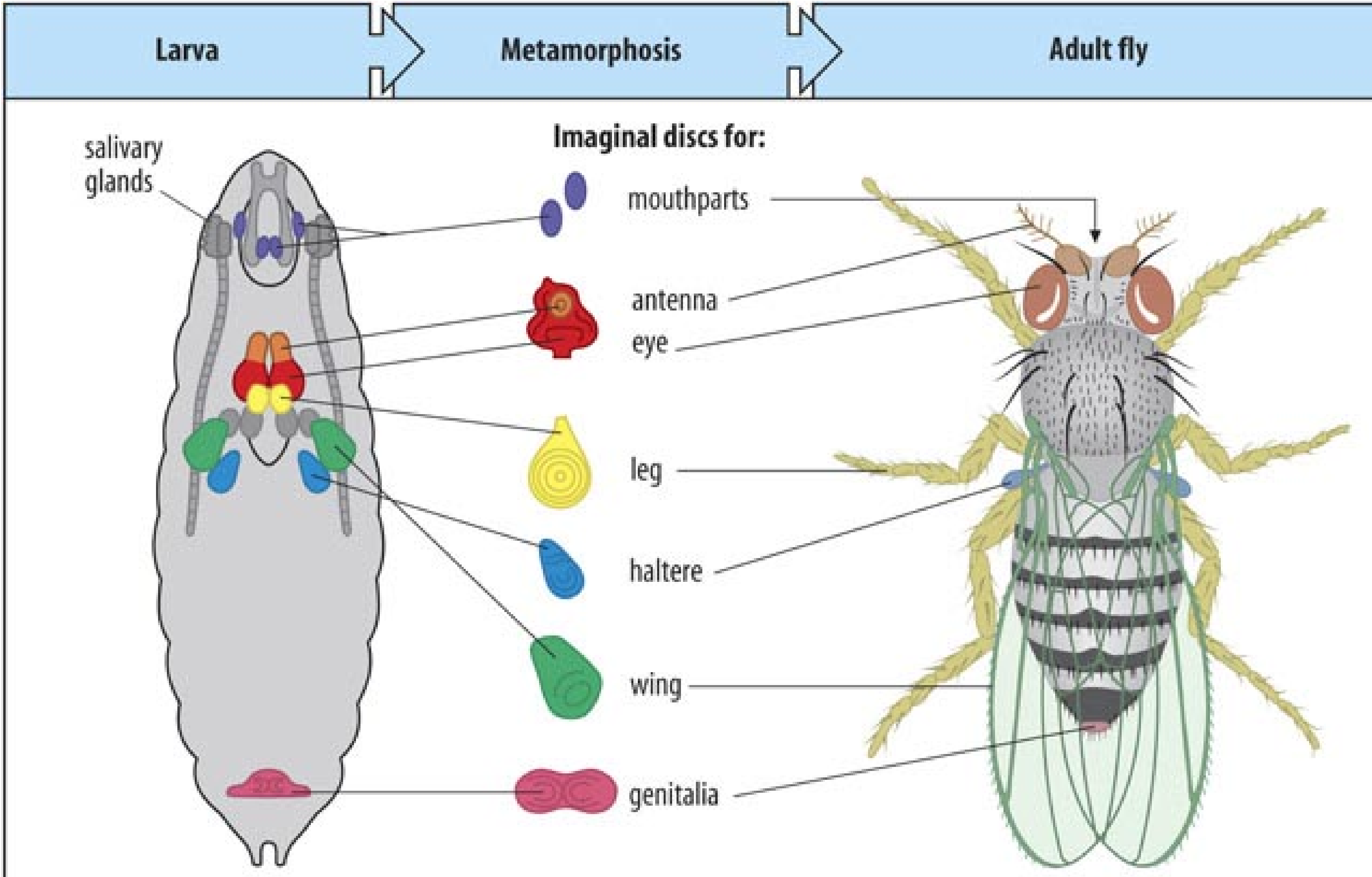
Larvae



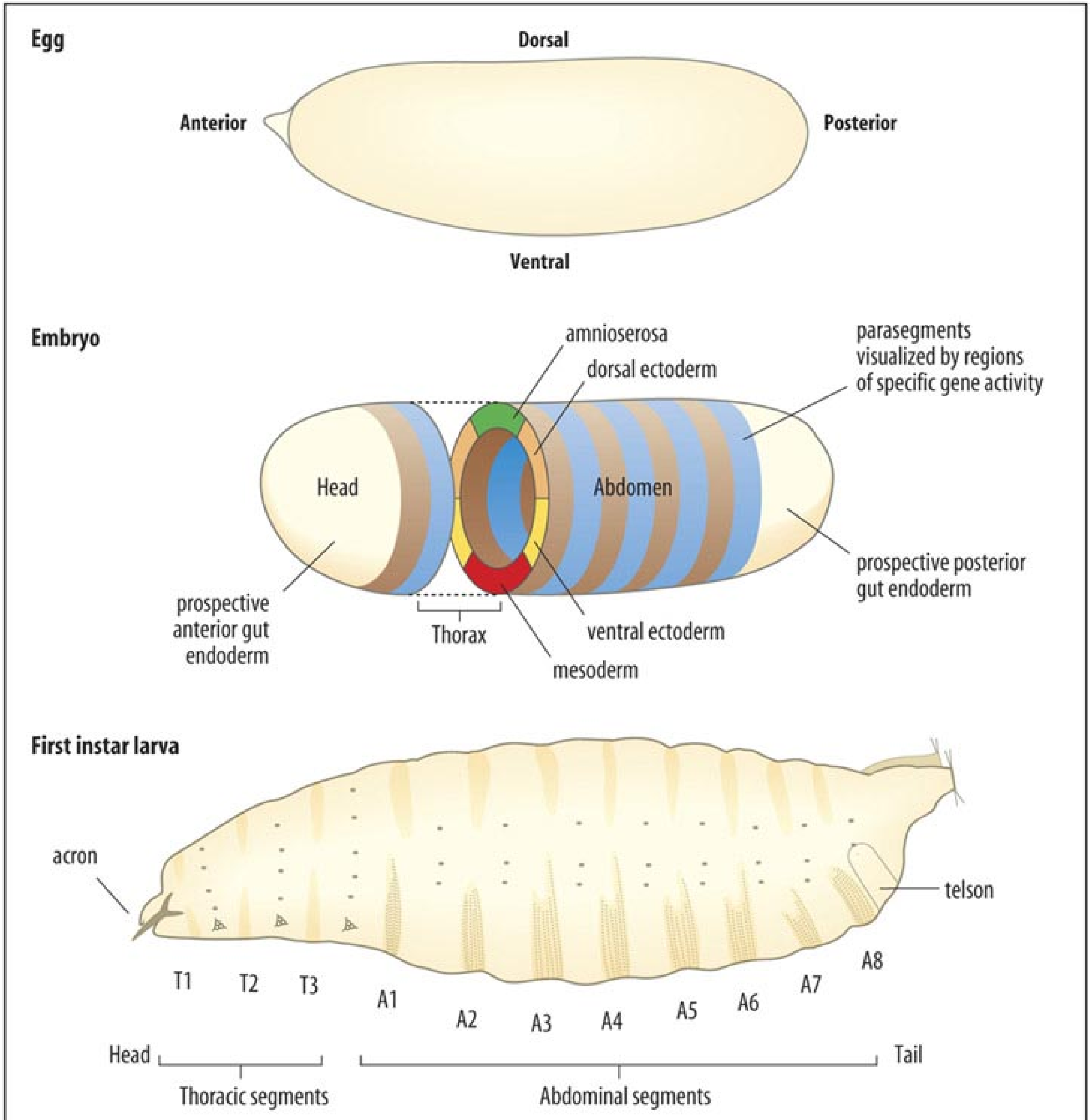
Comparison of Larvae and Adult Segments



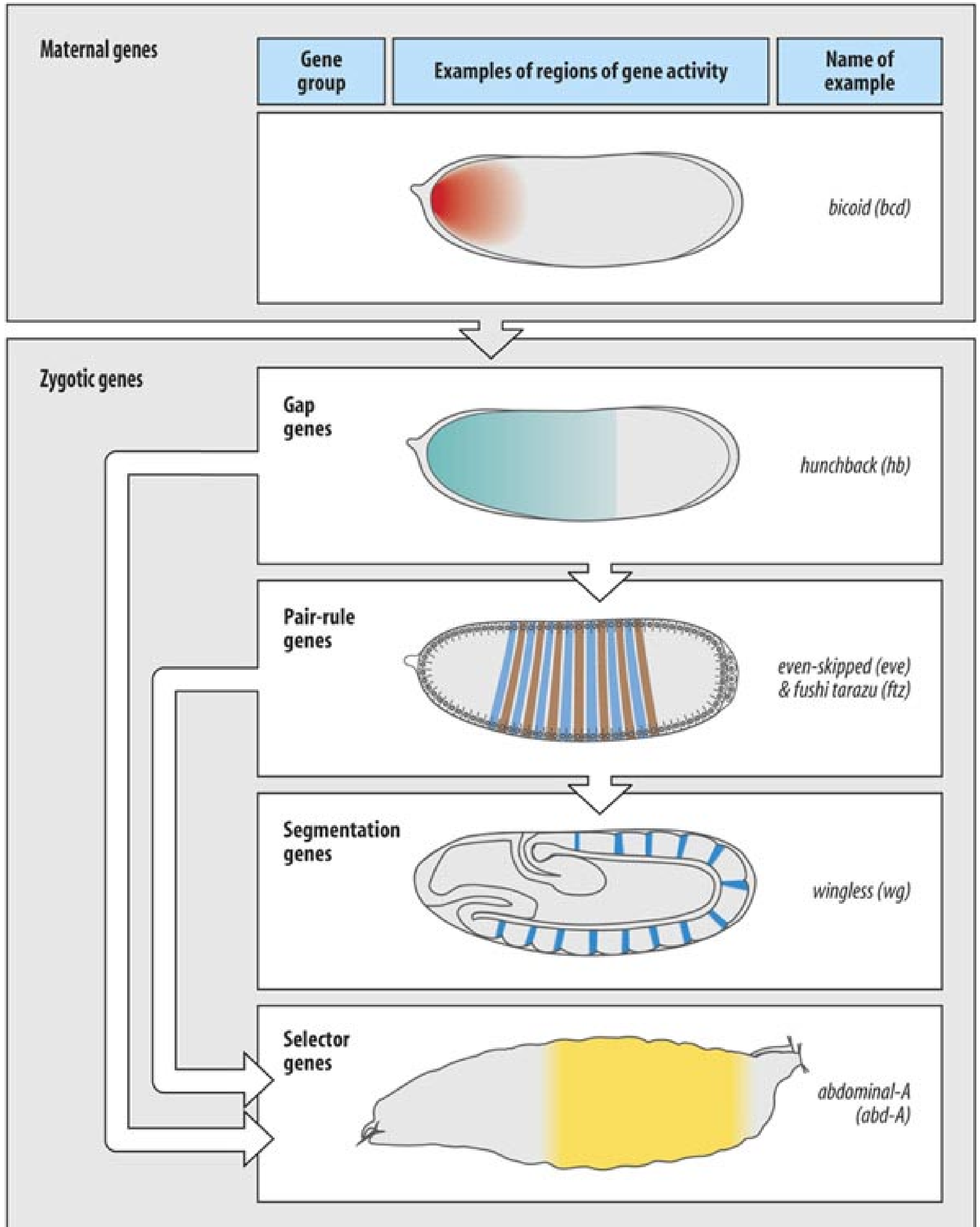
Imaginal Discs

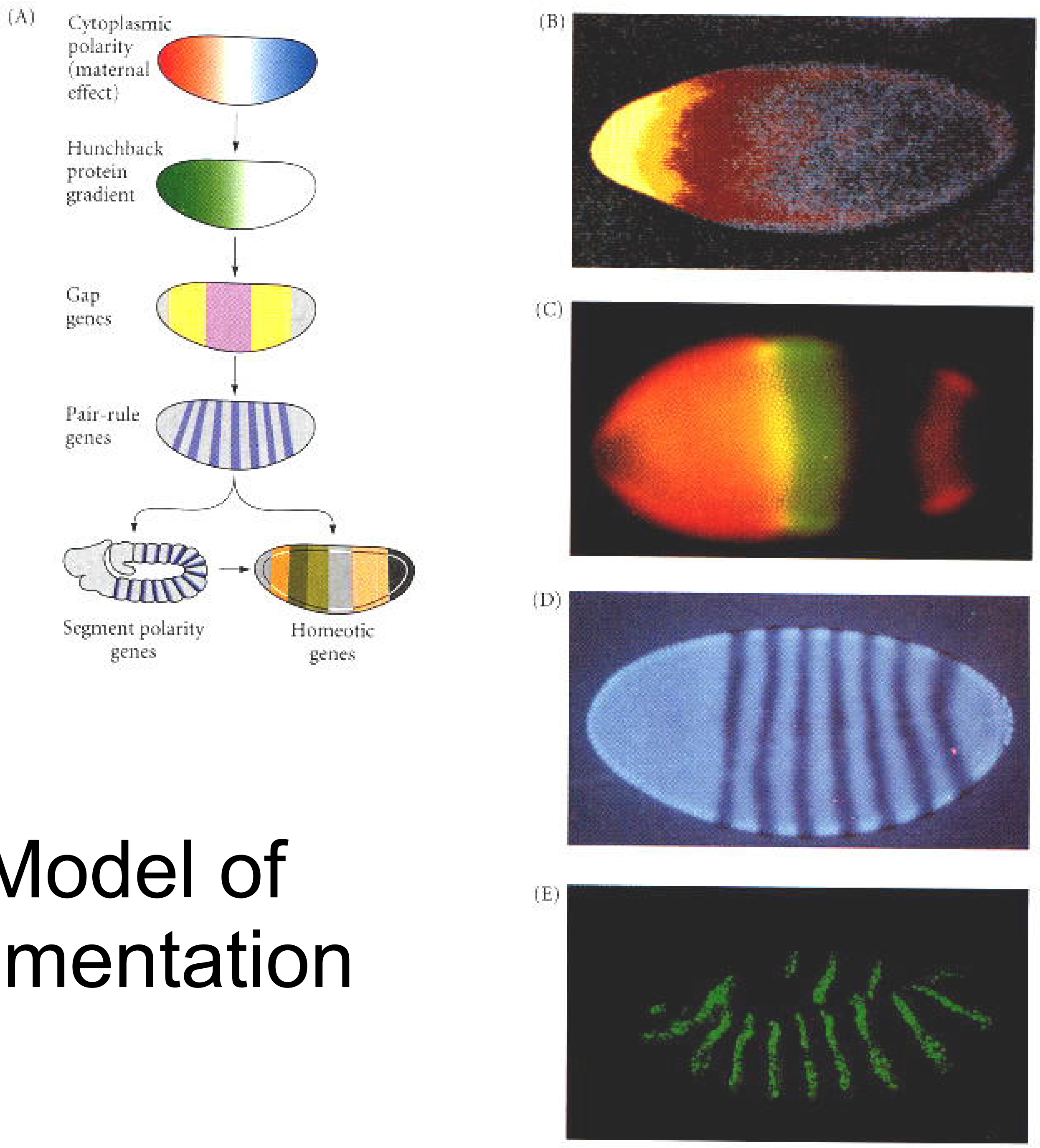


Segmentation



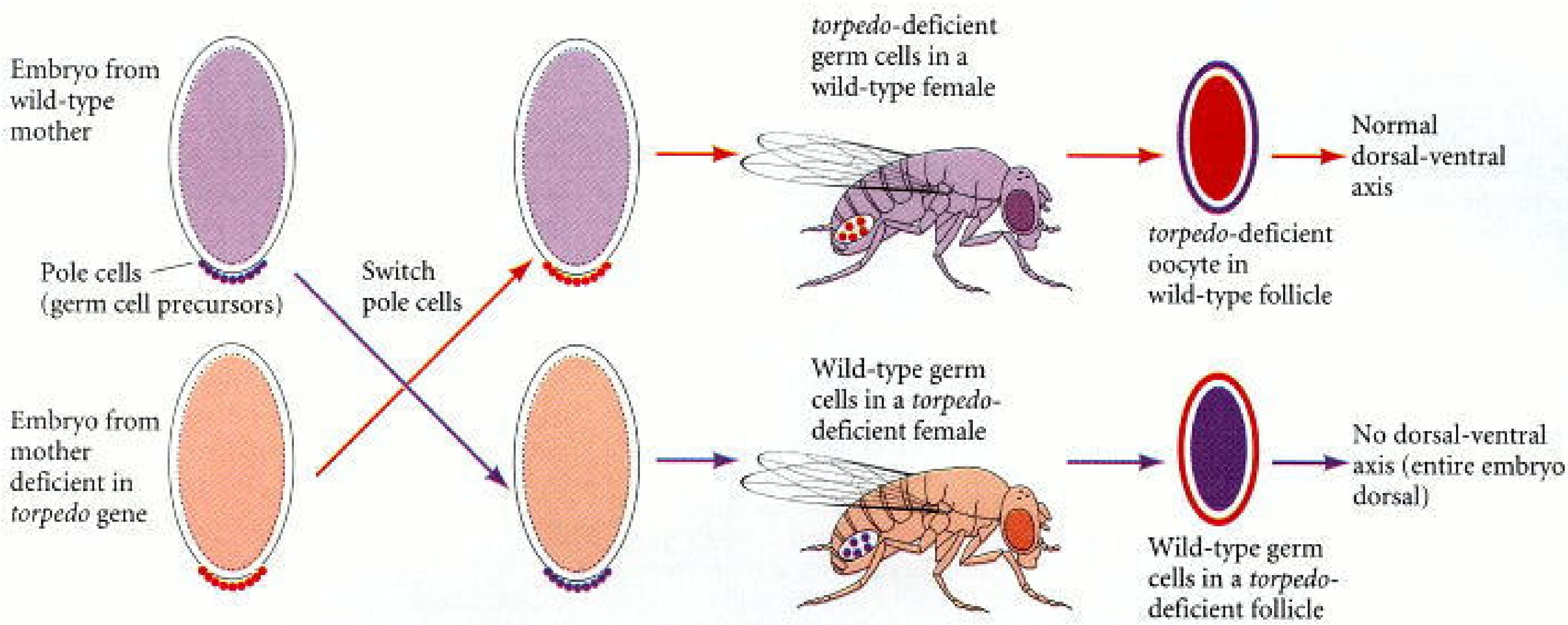
Overview of Pattern Formation



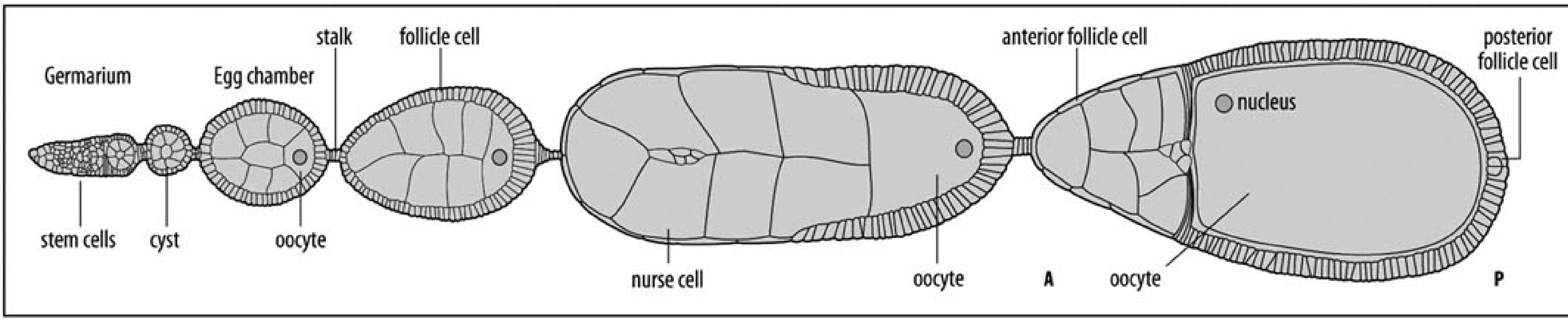


Generalized Model of Drosophila Segmentation

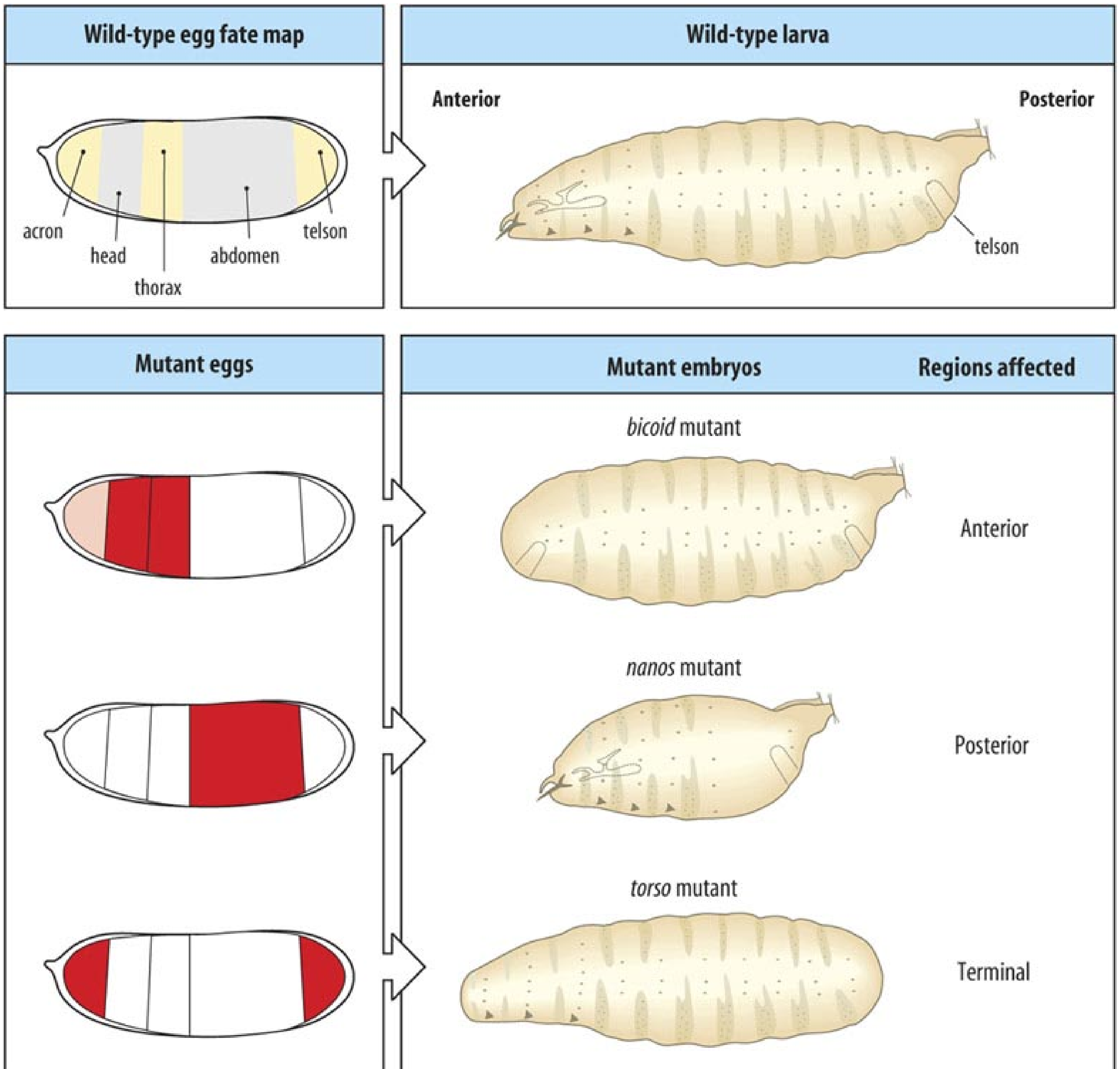
Maternal Effect Genes



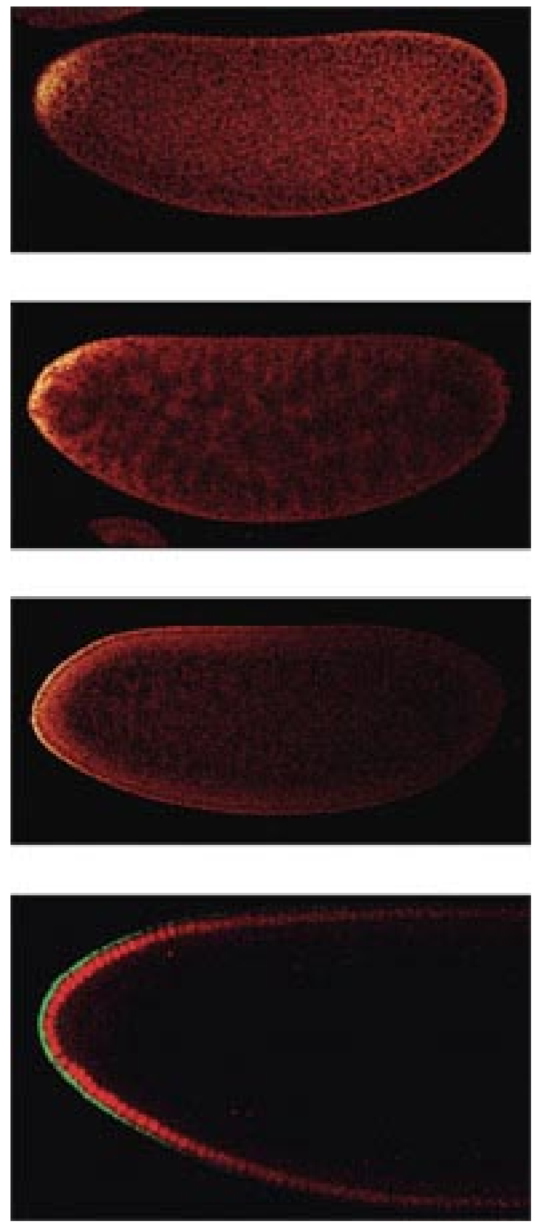
Egg Development



Maternal Effect Genes



Bicoid Gradient



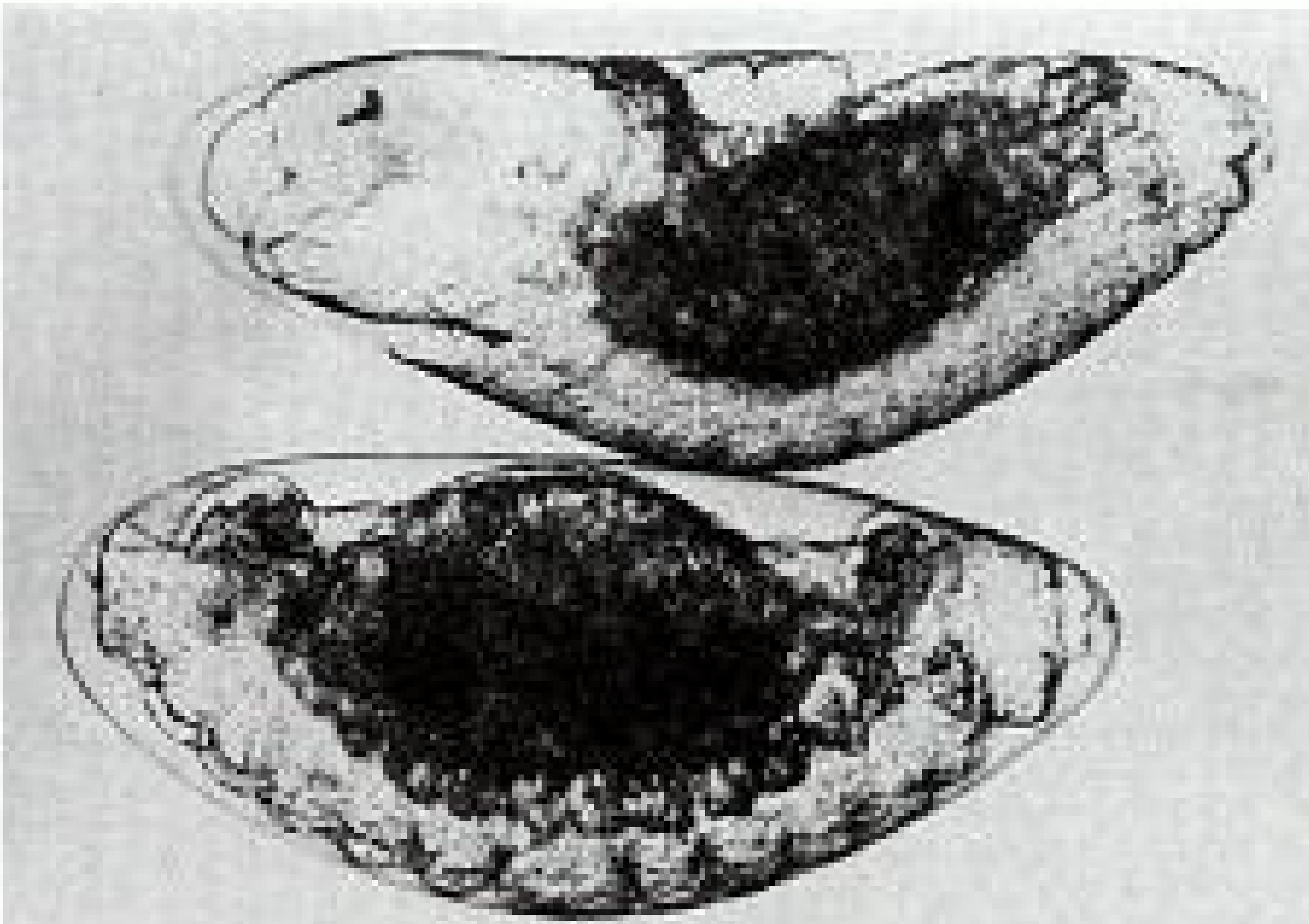
bicoid Gradient

bicoid mRNA secreted by nurse cells

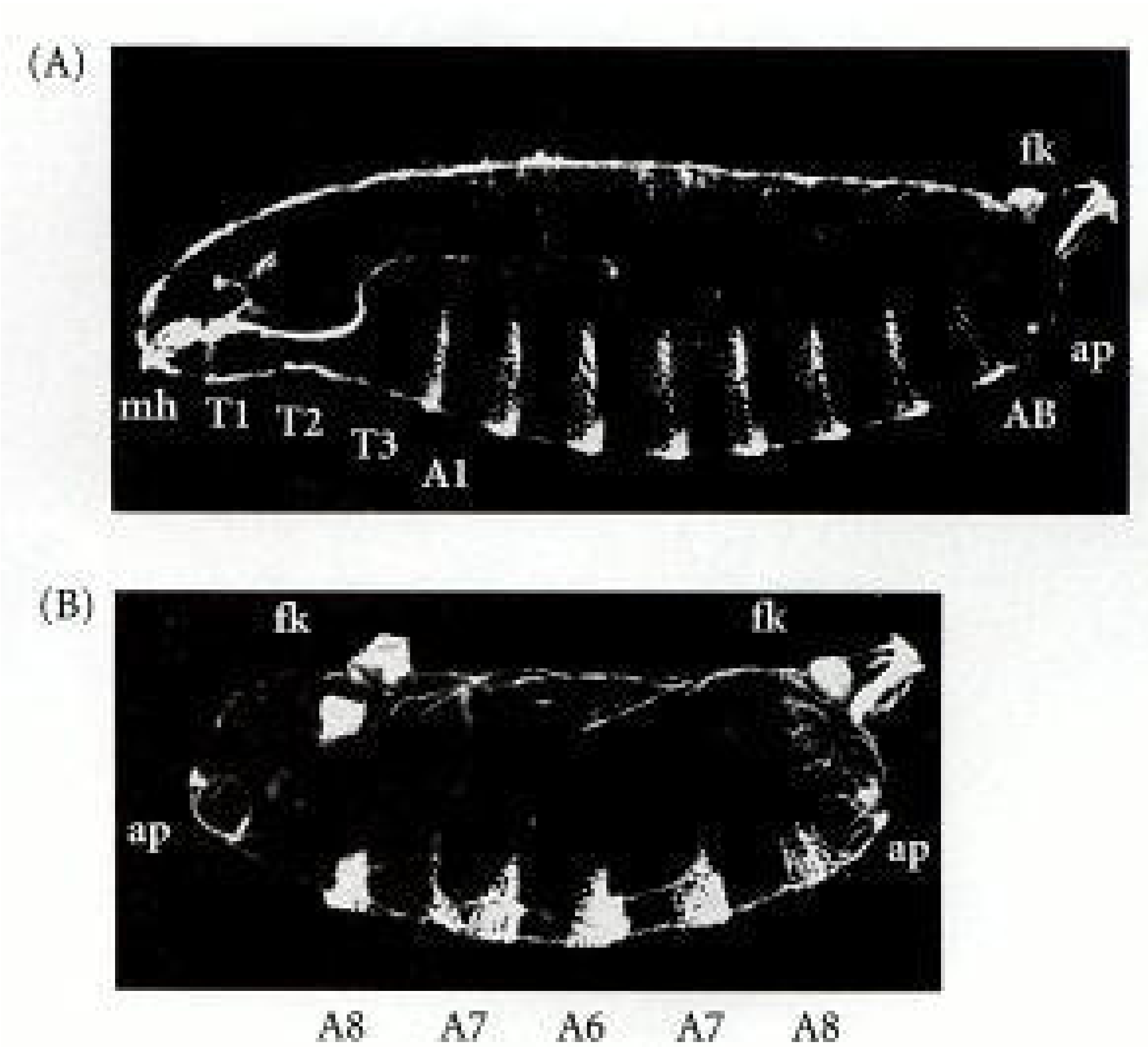
bicoid mRNA binds to dynein – a microtubule motor protein

- *bicoid* mRNA moves toward the non-growing end of the microtubule
- Non-growing end of microtubules becomes anterior end
- *bicoid* mRNA translated into *bicoid* protein during syncytial blastoderm stage
- *bicoid* protein diffuses toward posterior end

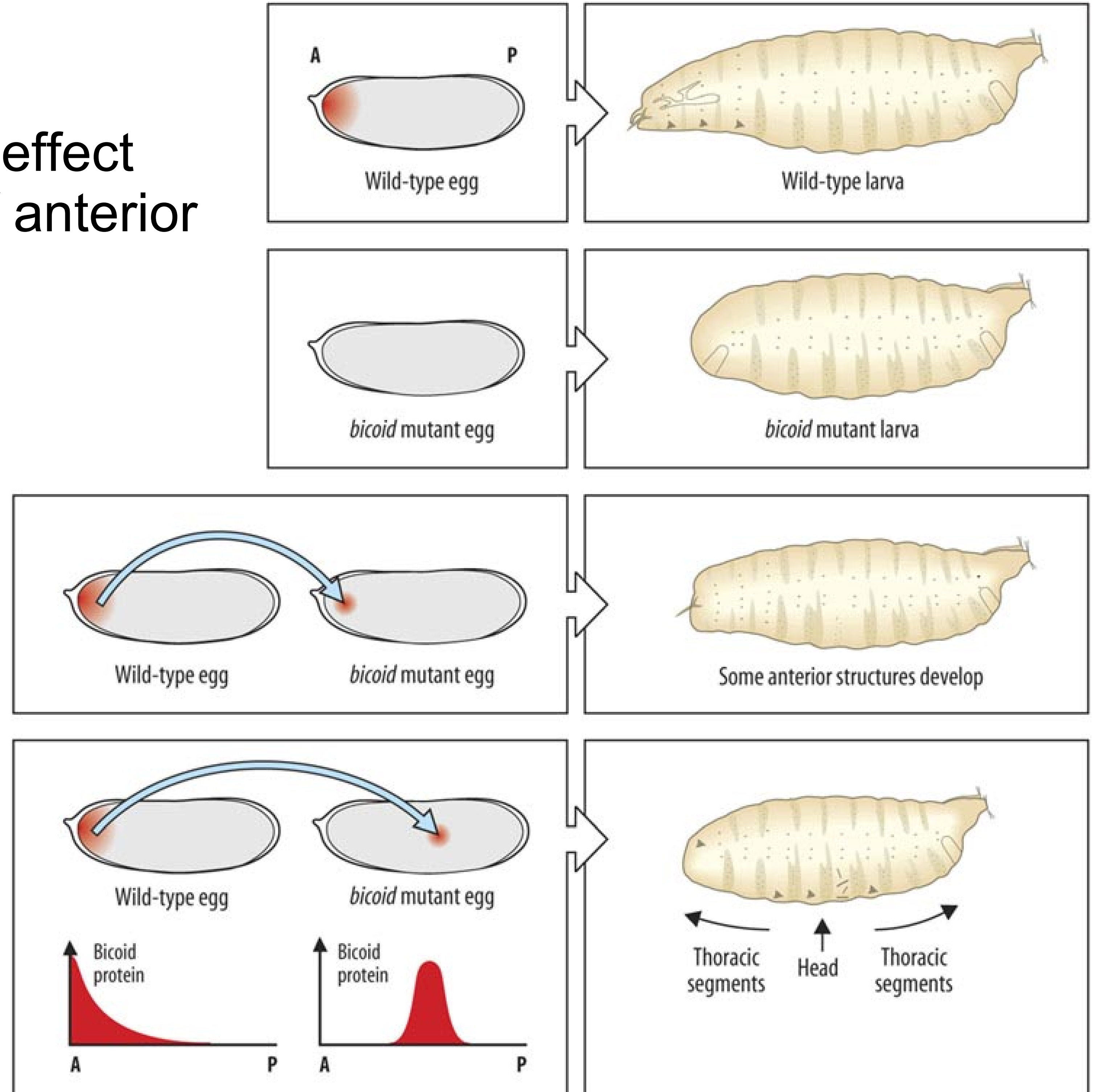
UV-irradiation of anterior end



bicoid mutants



Bicoid gradient effect development of anterior end



Posterior Group Genes

mRNA's that specify the posterior end

- *oskar* – localizes nanos to posterior end
- *nanos* – blocks translation of *hunchback*
- *caudal* – translation inhibited by *bicoid* protein

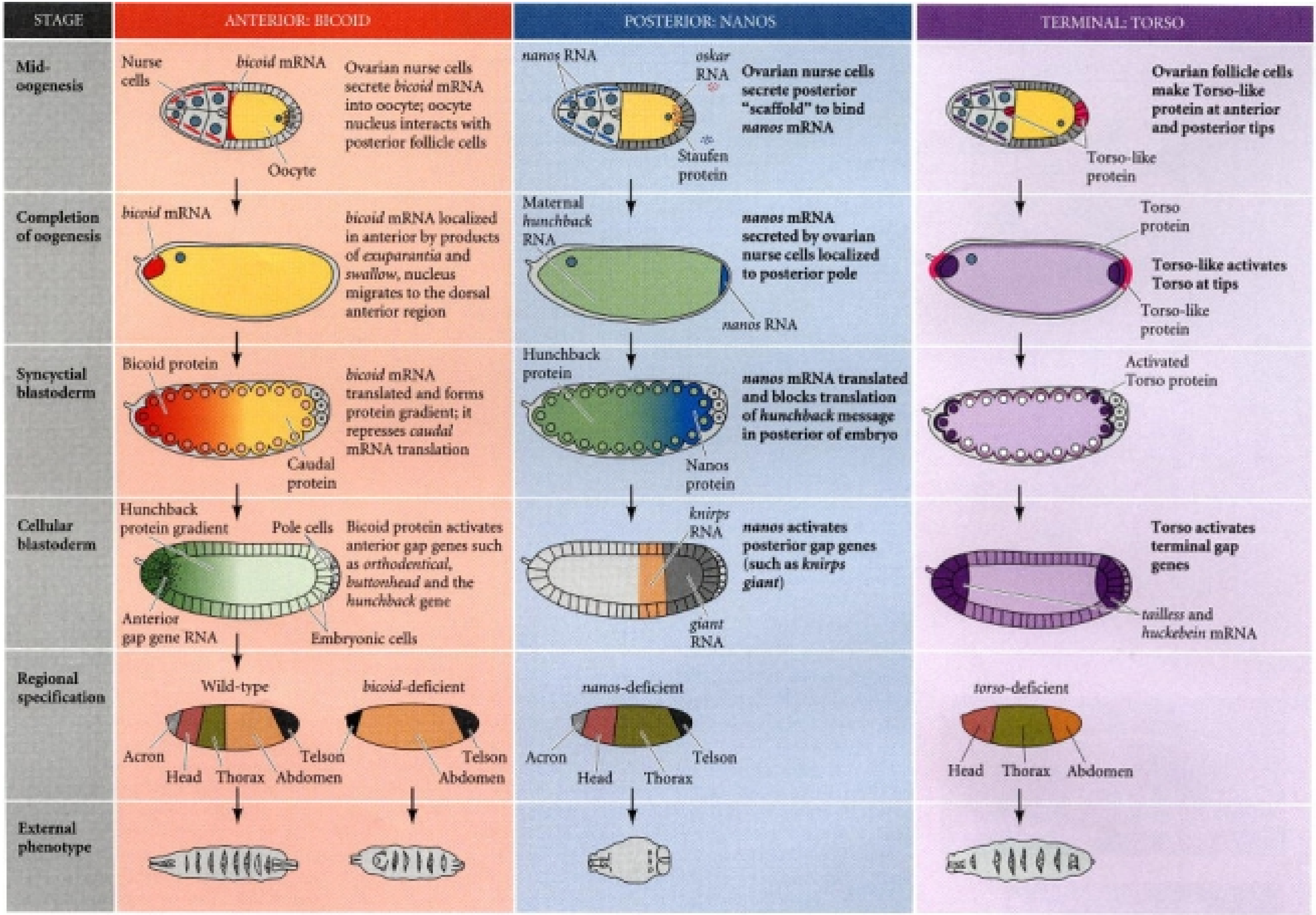
nanos Gradient

How does nanos gradient form?

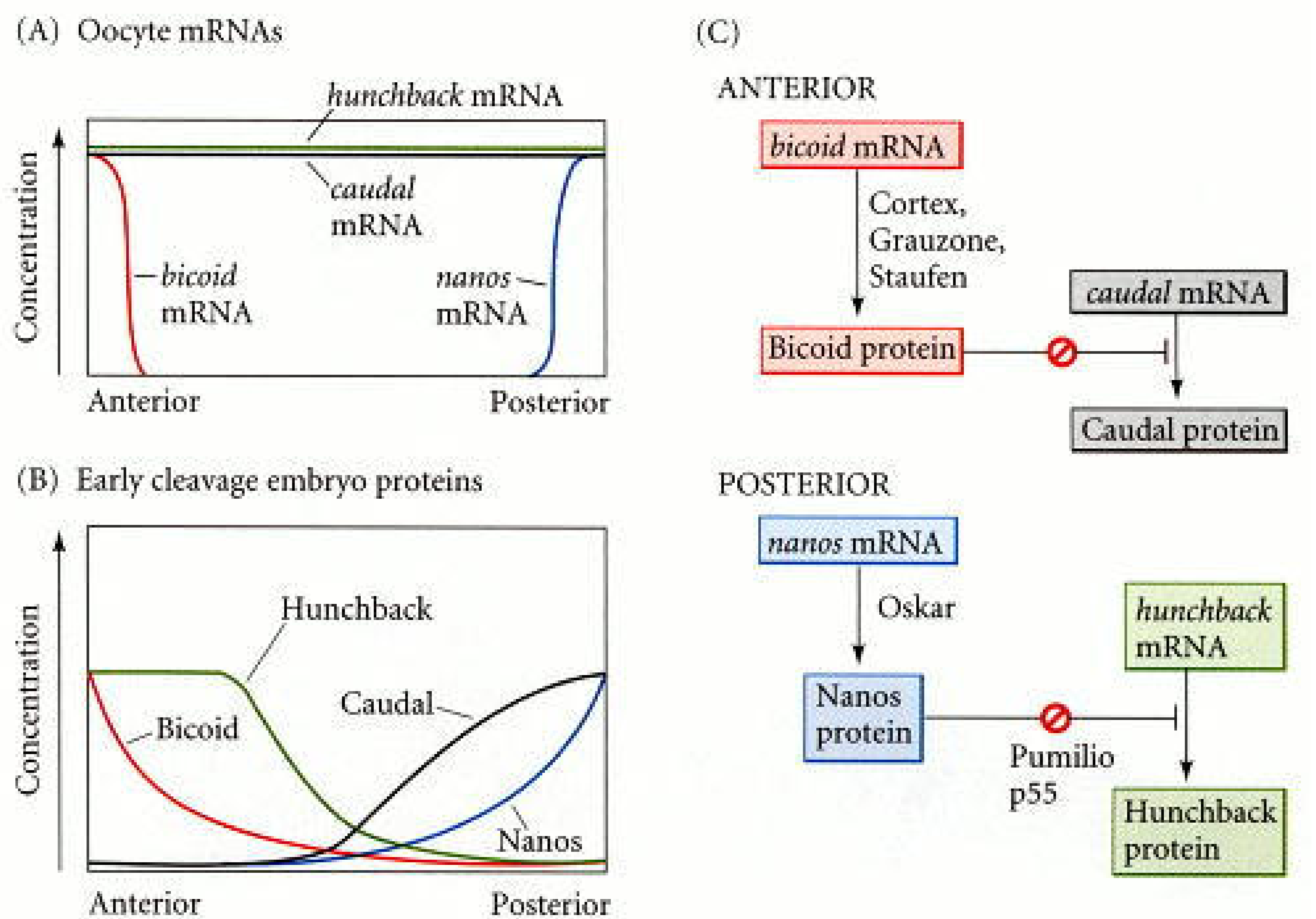
oskar rides along growing microtubules by binding to *kinesin I*

Growing end of microtubules becomes posterior end

nanos passively diffuses and gets trapped by *oskar* at posterior end.

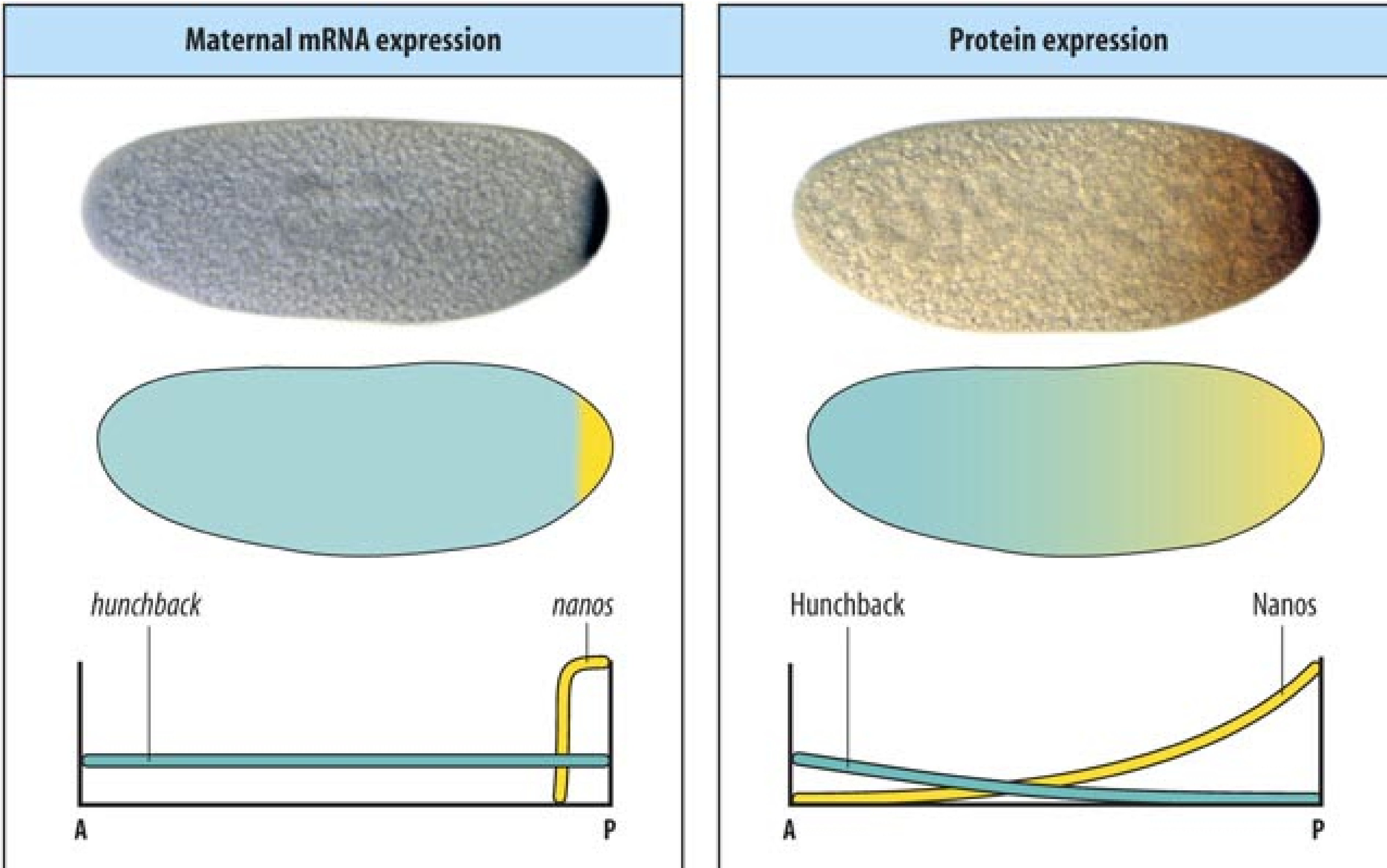


STAGE
ANTERIOR: BICOID
POSTERIOR: NANOS
TERMINAL: TORSO
Mid-oogenesis
Nurse cells
bicoid mRNA
Oocyte
Ovarian nurse cells secrete bicoid mRNA into oocyte; oocyte nucleus interacts with posterior follicle cells
nanos RNA
oskar RNA
Staufen protein
Ovarian nurse cells secrete posterior "scaffold" to bind nanos mRNA
Ovarian follicle cells make Torso-like protein at anterior and posterior tips
Torso-like protein
Completion of oogenesis
bicoid mRNA
bicoid mRNA localized in anterior by products of exuparantia and swallow, nucleus migrates to the dorsal anterior region
Maternal hunchback RNA
nanos RNA
nanos mRNA secreted by ovarian nurse cells localized to posterior pole
Torso protein
Torso-like activates Torso at tips
Torso-like protein
Syncytial blastoderm
Bicoid protein
Caudal protein
bicoid mRNA translated and forms protein gradient; it represses caudal mRNA translation
Hunchback protein
Nanos protein
nanos mRNA translated and blocks translation of hunchback message in posterior of embryo
Activated Torso protein
Cellular blastoderm
Hunchback protein gradient
Pole cells
Anterior gap gene RNA
Embryonic cells
Bicoid protein activates anterior gap genes such as orthodenticle, buttonhead and the hunchback gene
knirps RNA
giant RNA
nanos activates posterior gap genes (such as knirps giant)
Torso activates terminal gap genes
tailless and huckebein mRNA
Regional specification
Wild-type
bicoid-deficient
Acron
Head
Thorax
Abdomen
Telson
Telson
Abdomen
nanos-deficient
Acron
Head
Thorax
Telson
torso-deficient
Head
Thorax
Abdomen
External phenotype

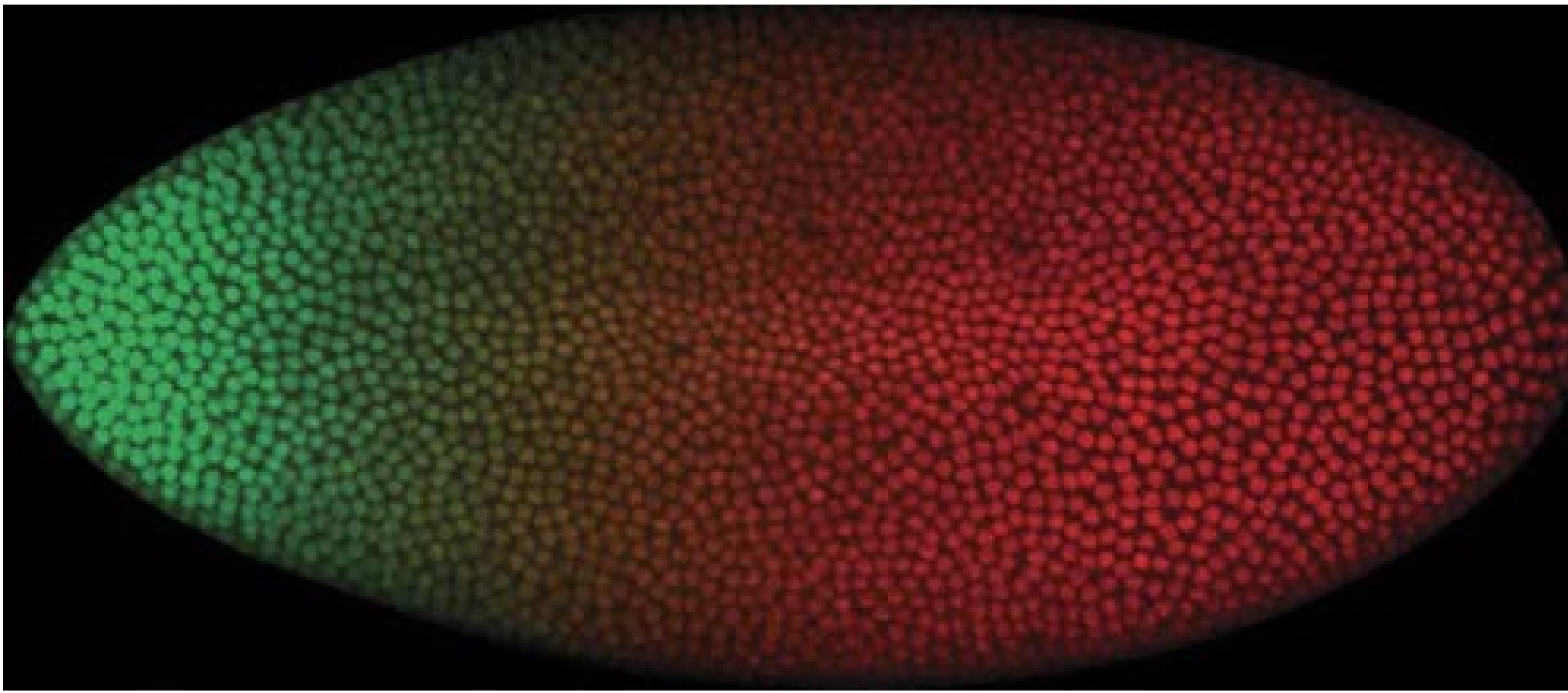


(A) Oocyte mRNAs
hunchback mRNA
Concentration
caudal mRNA
bicoid mRNA
nanos mRNA
Anterior
Posterior
(B) Early cleavage embryo proteins
Concentration
Hunchback
Caudal
Bicoid
Nanos
Anterior
Posterior
(C)
ANTERIOR
bicoid mRNA
Cortex, Grauzone, Staufen
Bicoid protein
caudal mRNA
Caudal protein
POSTERIOR
nanos mRNA
Oskar
Nanos protein
hunchback mRNA
Pumilio p55
Hunchback protein

Hunchback Gradient



caudal restricted to posterior end by *bicoid*



Green *bicoid*
Anterior end

Red *caudal*
Posterior end

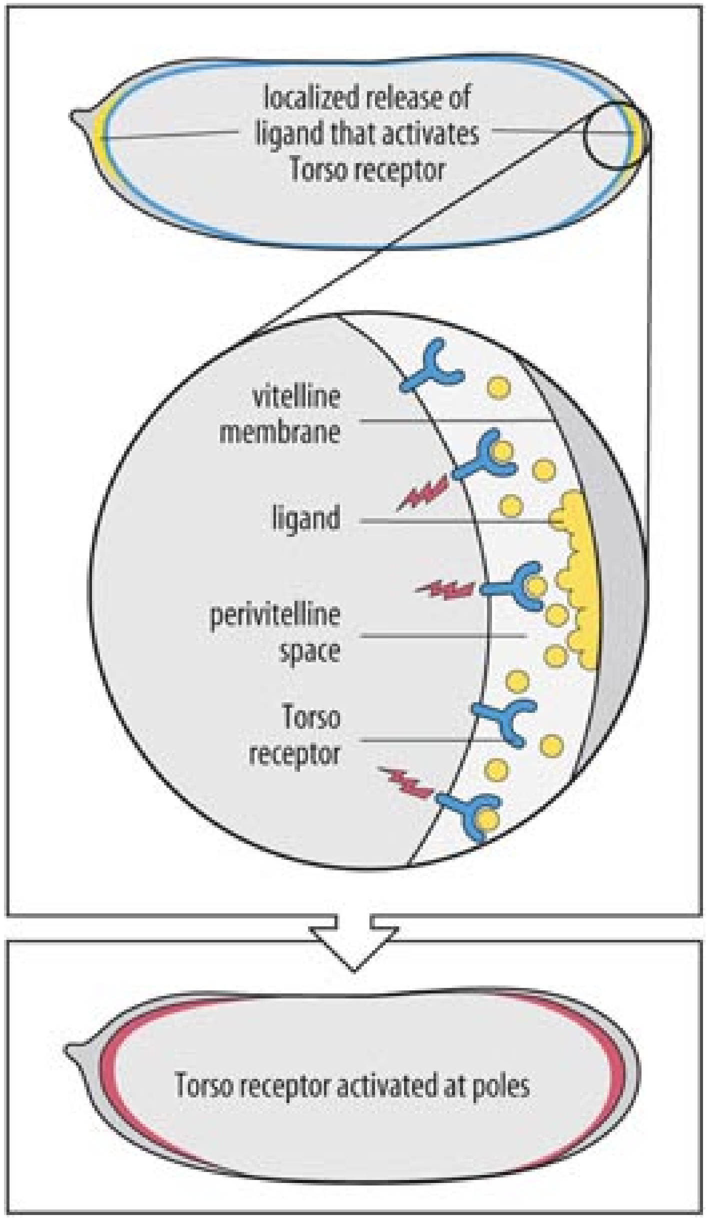
Terminal Group Genes

Necessary for the specification of the ends of the embryo

- Acron – anterior
- Telson – posterior

torso-like protein localized to the anterior and posterior tips

torso-like activates *torso* protein



What determines acron or telson?

If bicoid is present the acron will form.

If bicoid is not present telson will form.

Summary

bicoid gradient promotes *hunchback* translation inhibits *caudal* translation at anterior end

- *bicoid*/*hunchback* gradient forms
- High concentrations of *bicoid* and *hunchback* together activate the anterior gap genes.

nanos gradient promotes *caudal* translation inhibits *hunchback* translation at posterior end

- *caudal* gradient forms
- High concentration of *caudal* activates posterior gap gene transcription

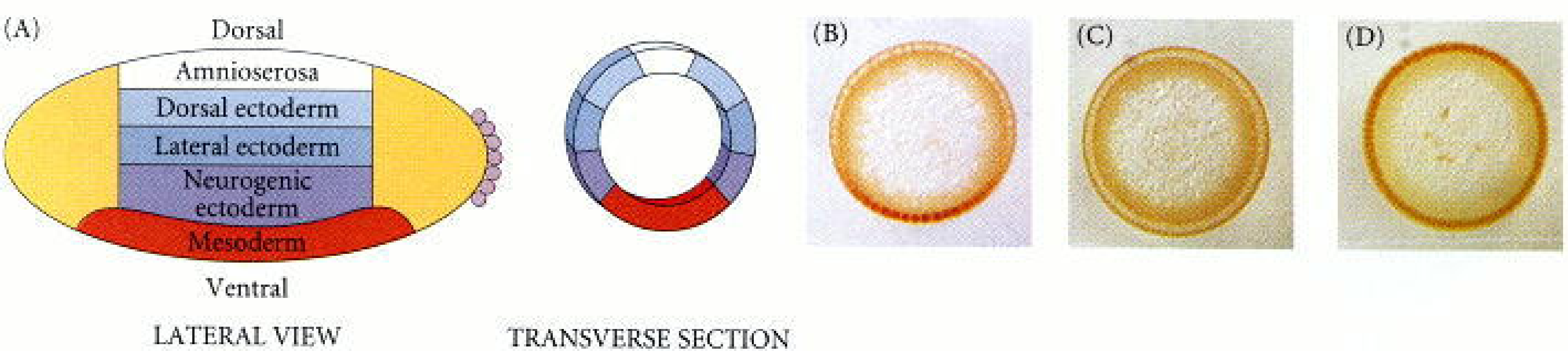
torso-like expressed at ends of embryo activates *torso*

- Acron develops where bicoid and torso are present
- Telson develops where torso alone is present

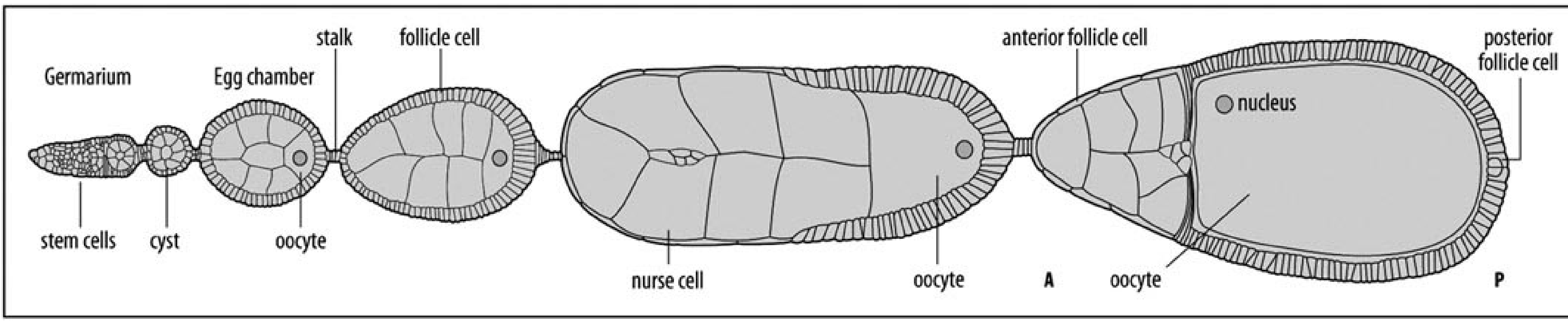
Dorsal-Ventral Polarity

Dorsal localized to ventral cells during oogenesis.

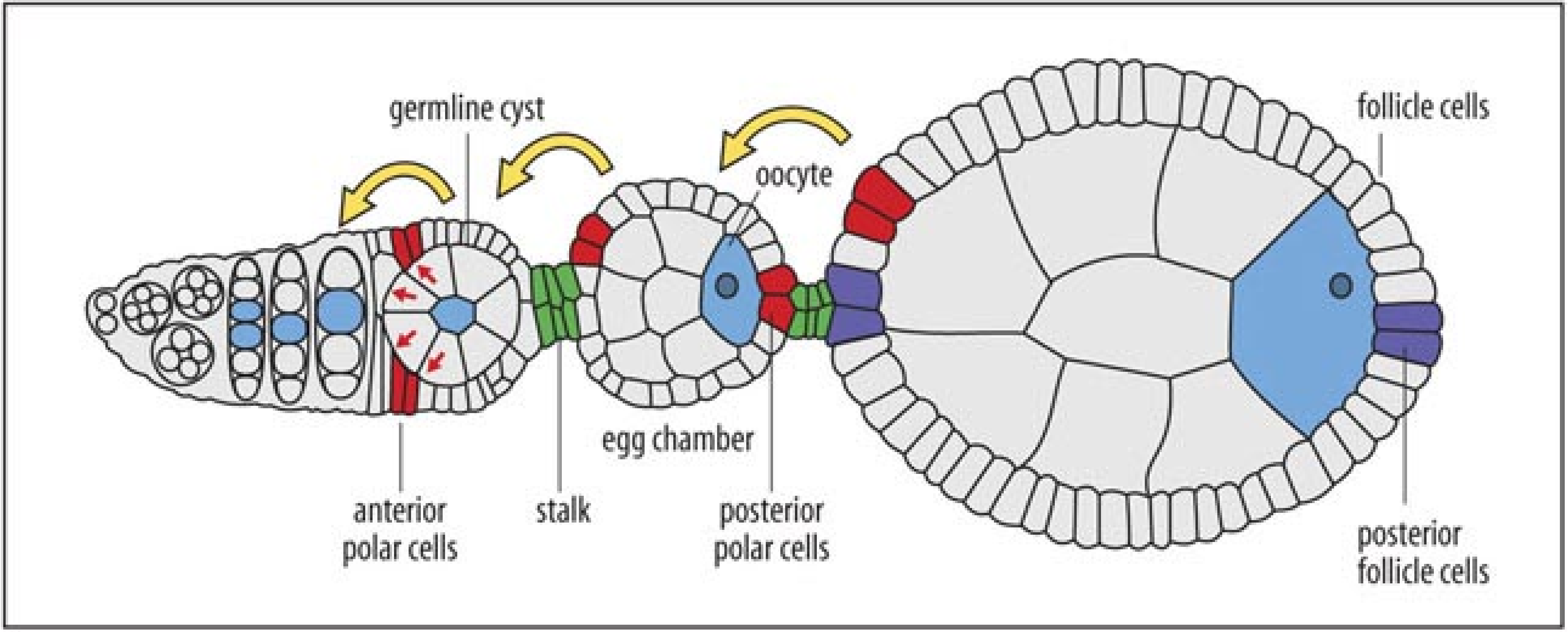
Forms *Dorsal* protein gradient.



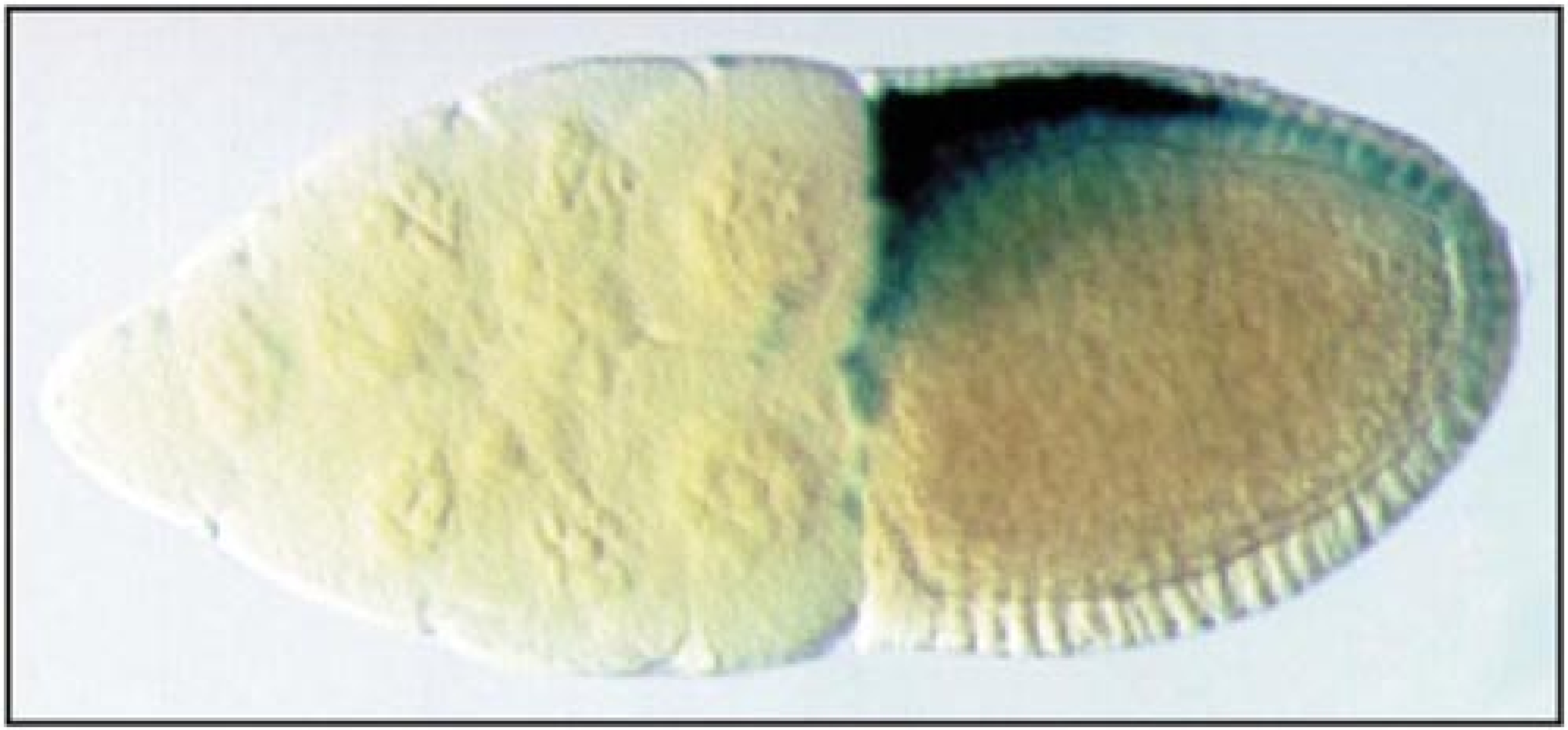
Egg Development



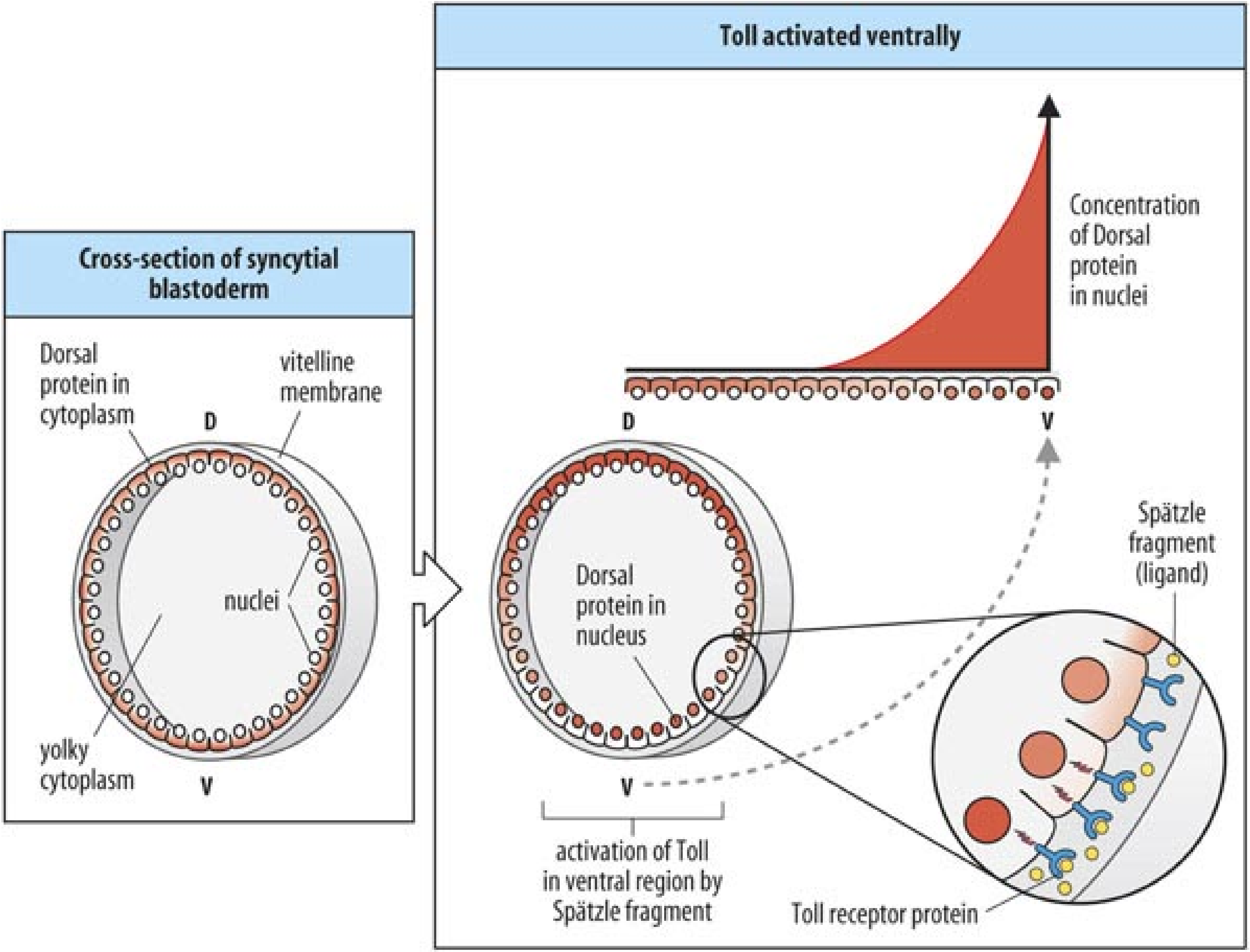
Growing egg chambers setup oocyte polarity



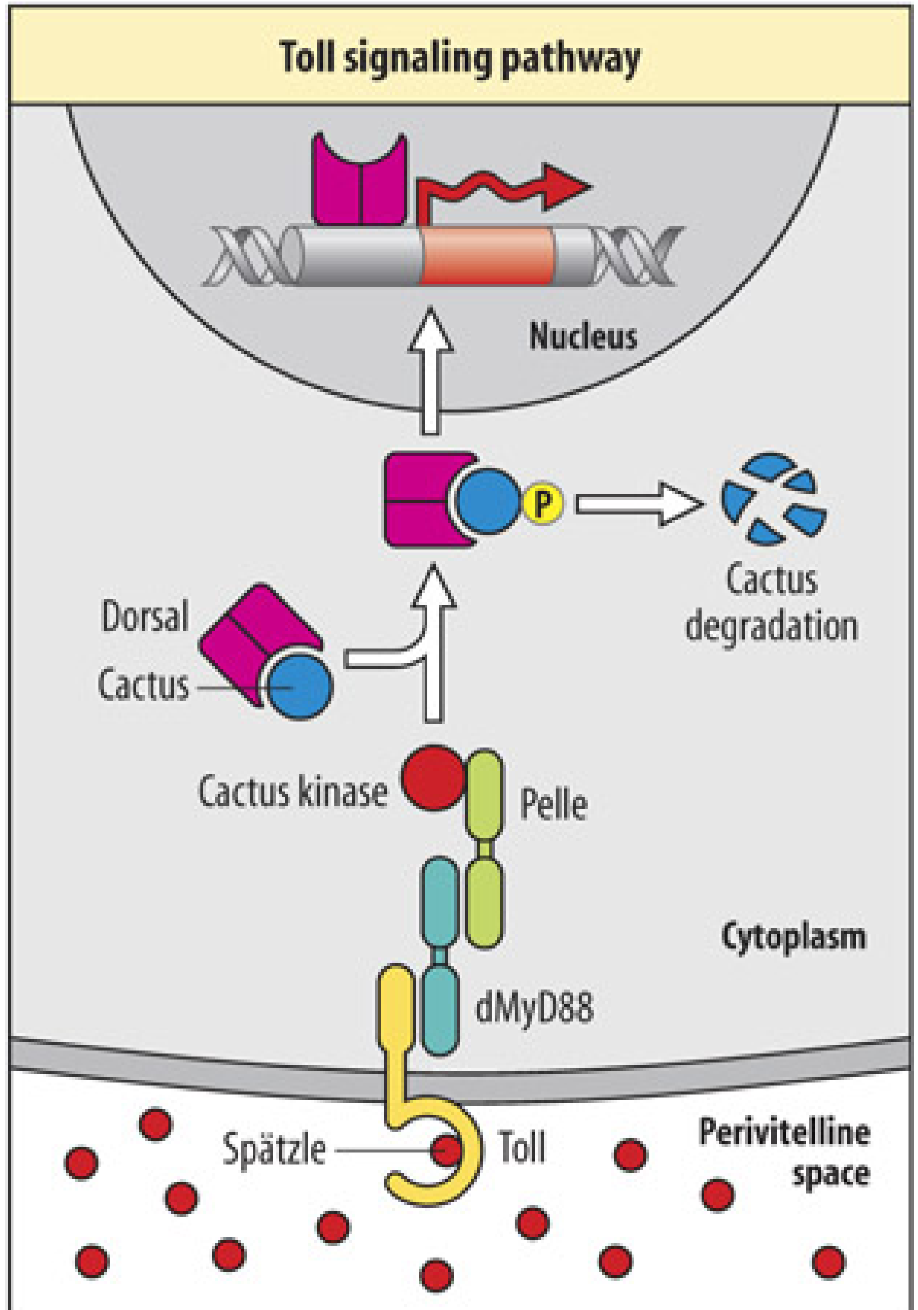
Follicle Cell Expression



Localization of *Dorsal*



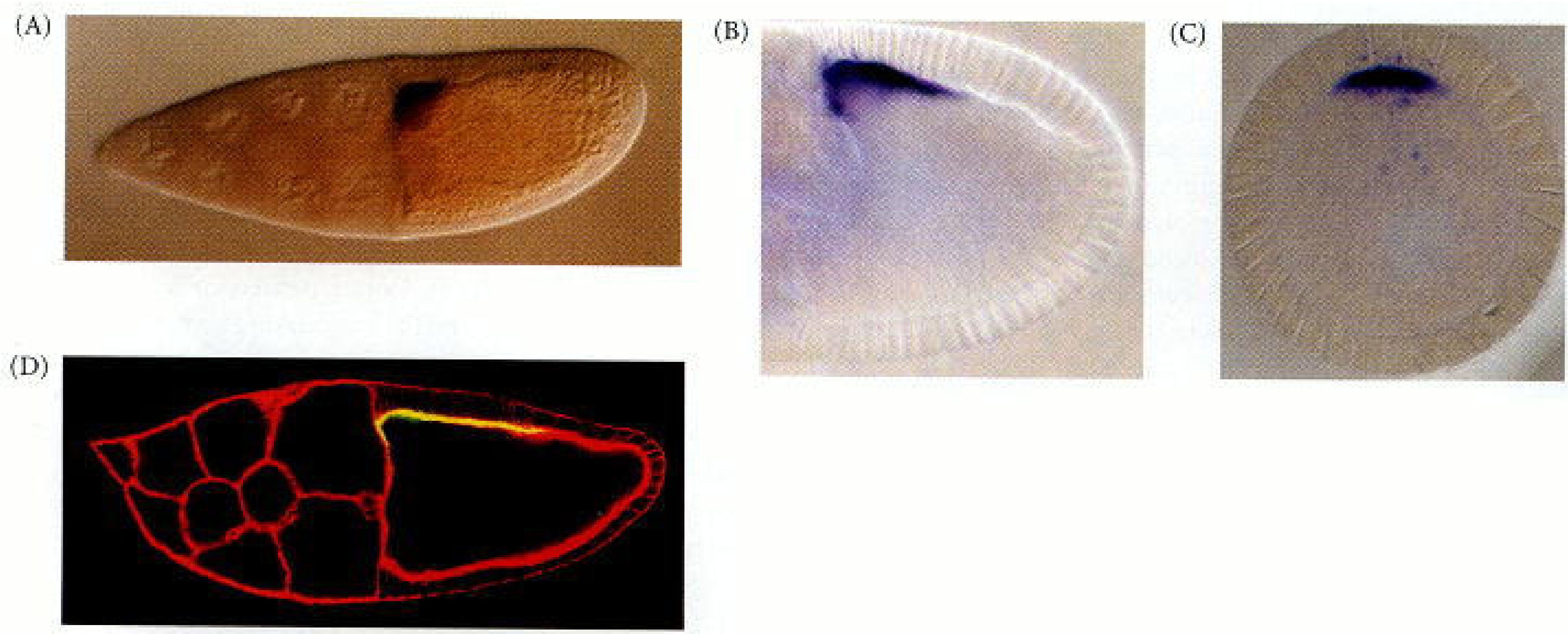
Toll Signaling Pathway



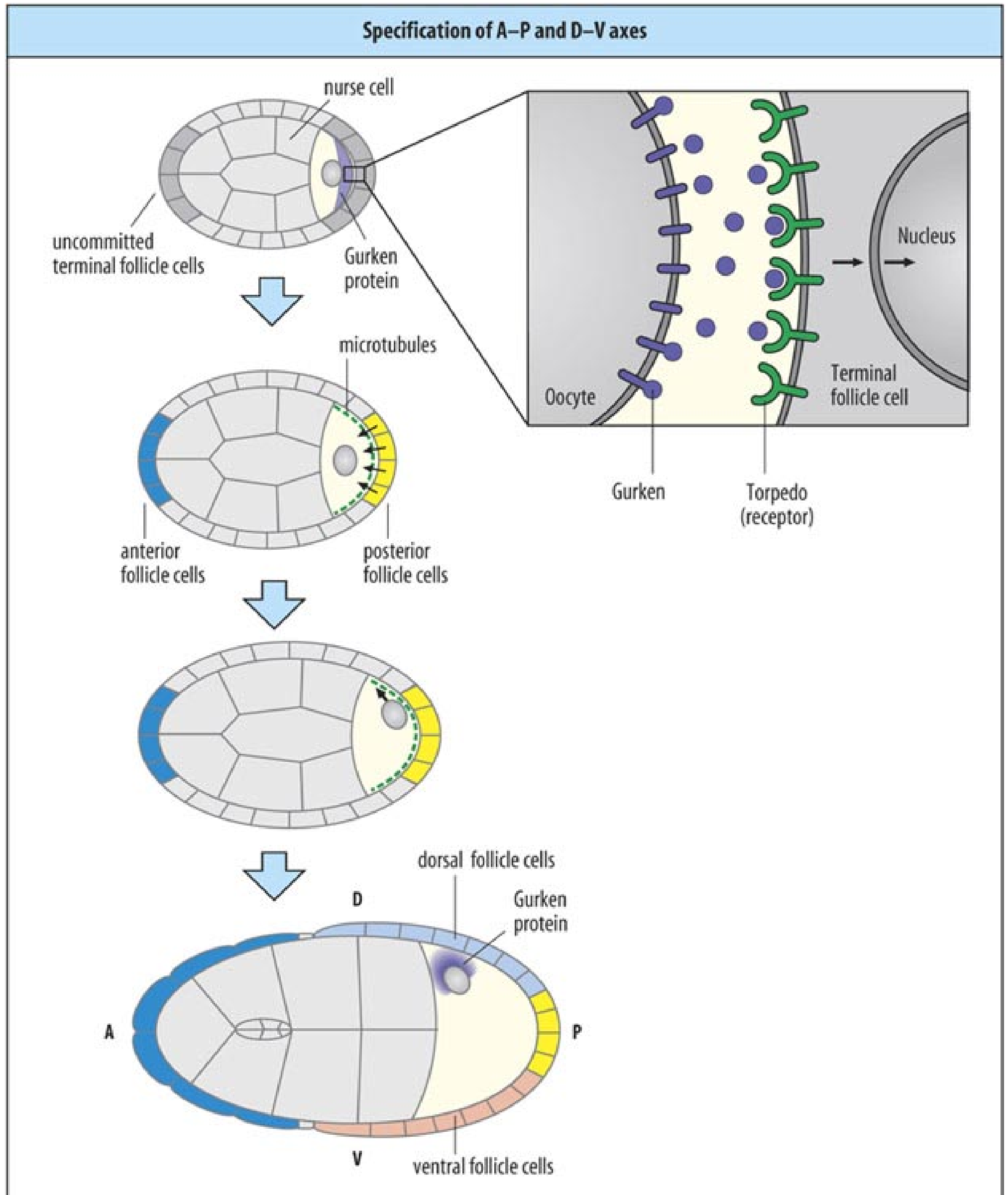
Gurken Localized to Dorsal Surface

- Localized during oogensis.
- Inhibits *Pipe* protein
- *Pipe* protein required for *Dorsal* function
  - *Pipe* active on ventral side (part of complex cascade)
  - Allows *Dorsal* protein to ventralize the opposite side of the cell.
  - Develops *Dorsal* gradient

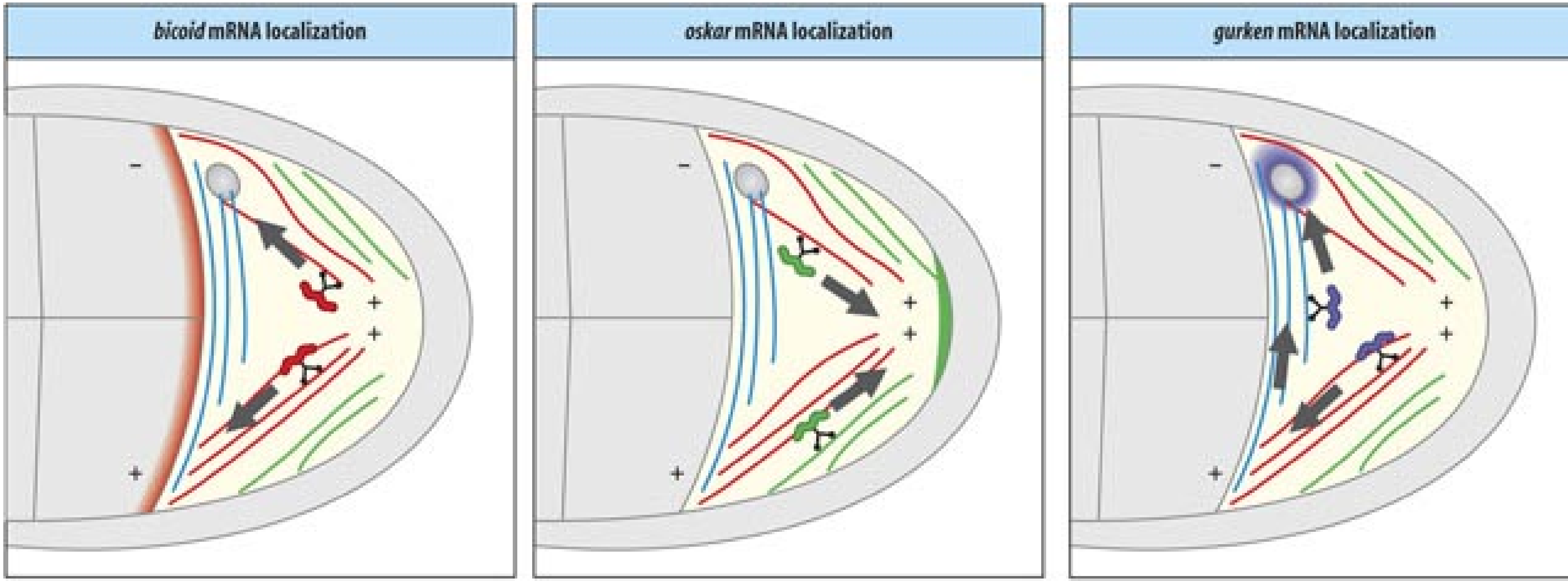
Localization of *Gurken*

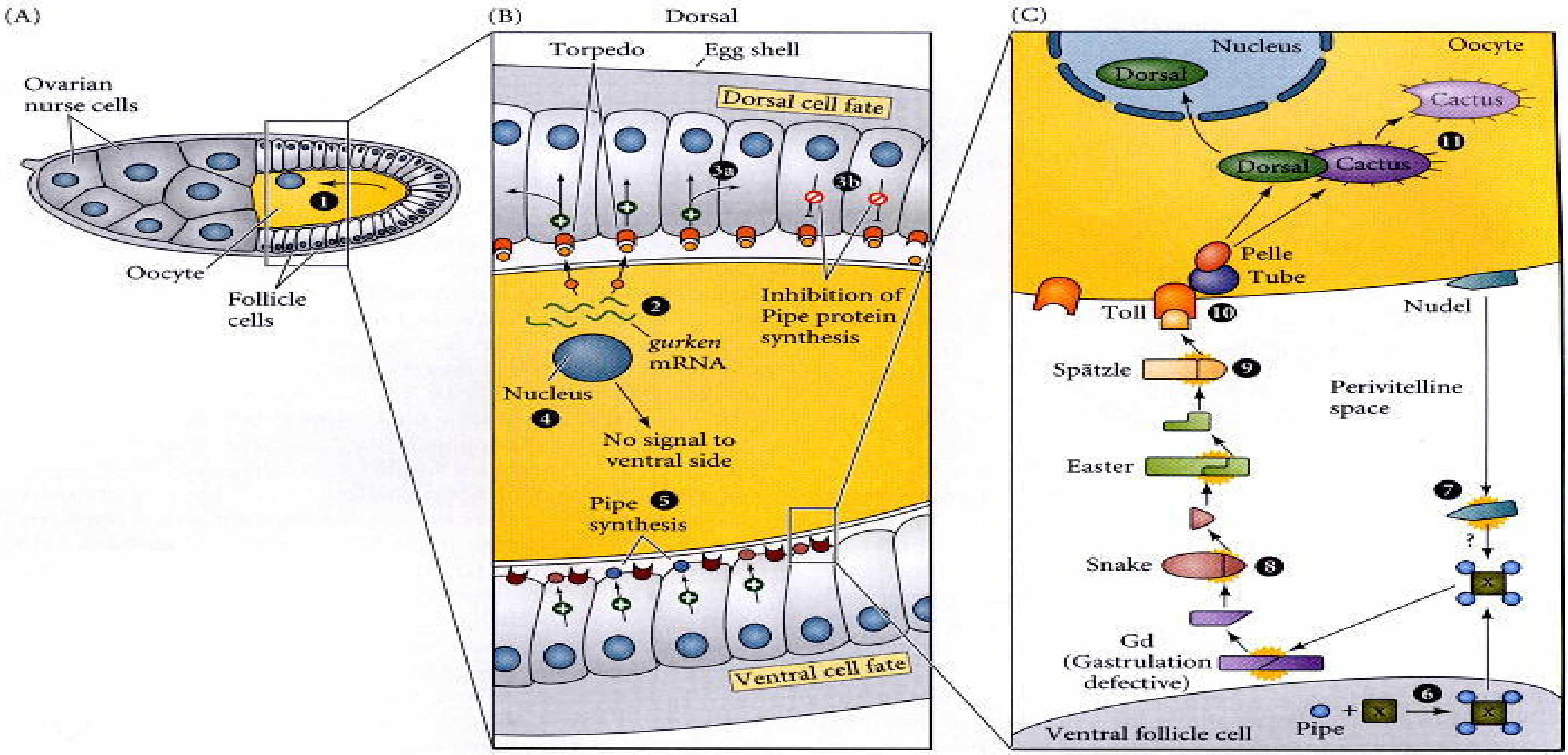


Localization of *gurken*



Localization of *bicoid*, *oskar* and *gurken*

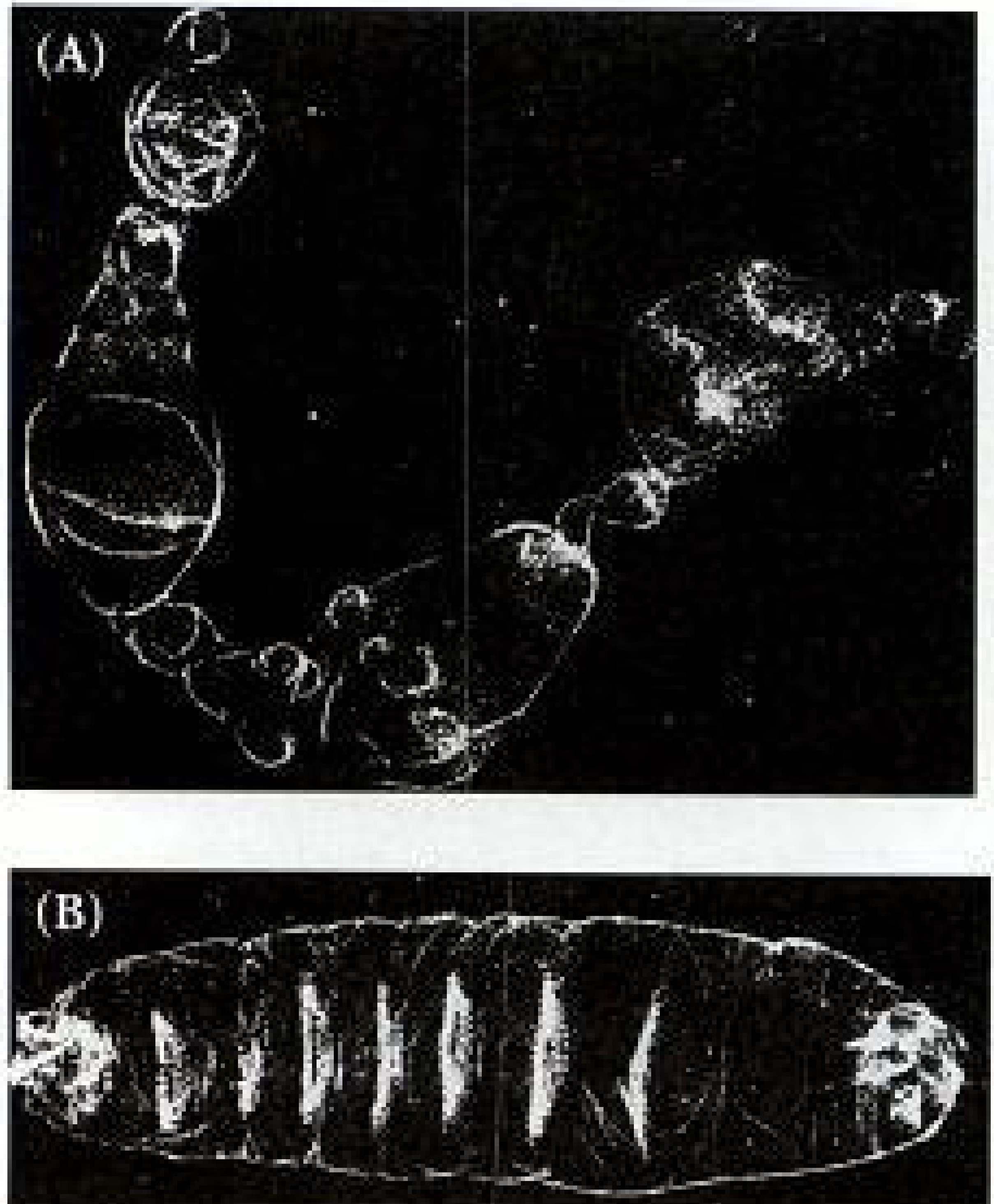




- ❶ Oocyte nucleus travels to anterior dorsal side of oocyte. It synthesizes *gurken* mRNA which remains between the nucleus and the follicle cells.
- ❷ *gurken* messages are translated. The Gurken protein is received by Torpedo proteins during mid-oogenesis.
- 3a Torpedo signal causes follicle cells to differentiate to a dorsal morphology.
- 3b Synthesis of Pipe protein is inhibited in dorsal follicle cells.
- ❹ Gurken protein does not diffuse to ventral side.
- ❺ Ventral follicle cells synthesize Pipe proteins.
- ❻ In ventral follicle cells, Pipe completes the modification of unknown factor (x).
- ❼ Nudel and factor (x) interact to split the Gastrulation-deficient (Gd) protein.
- ❽ The activated Gd protein splits the Snake protein, and the activated Snake protein cleaves the Easter protein.
- ❾ The activated Easter protein splits Spätzle; activated Spätzle binds to Toll receptor protein.
- ❿ Toll activation activates Tube and Pelle, which phosphorylate the Cactus protein. Cactus is degraded, releasing it from Dorsal.
- ⓫ Dorsal protein enters the nucleus and ventralizes the cell.

Snake Gene Mutation

Snake required for *Dorsal* localization during oogenesis

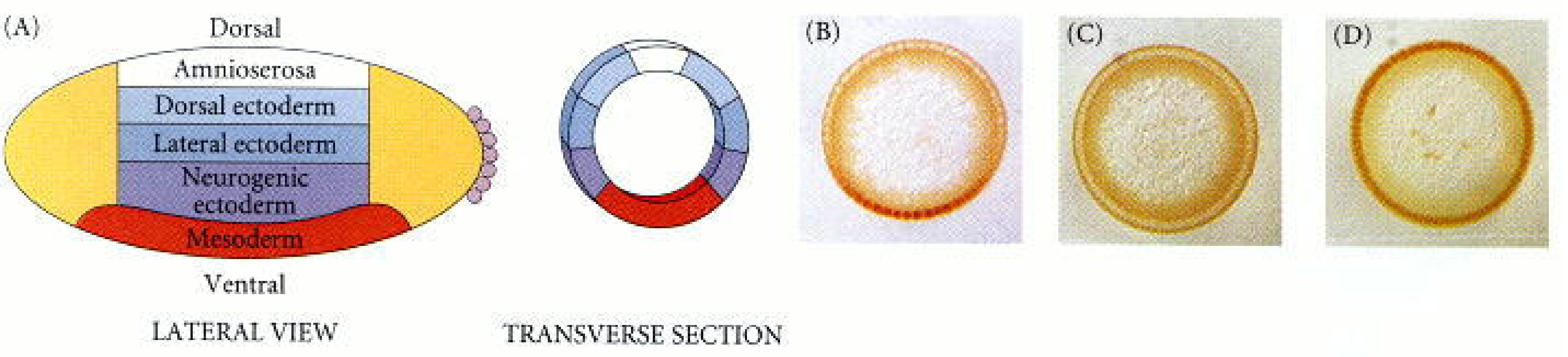


How can a protein gradient specify different regions of the embryo?

Dorsal protein is a transcription factor.

Activates or suppresses transcription of genes.

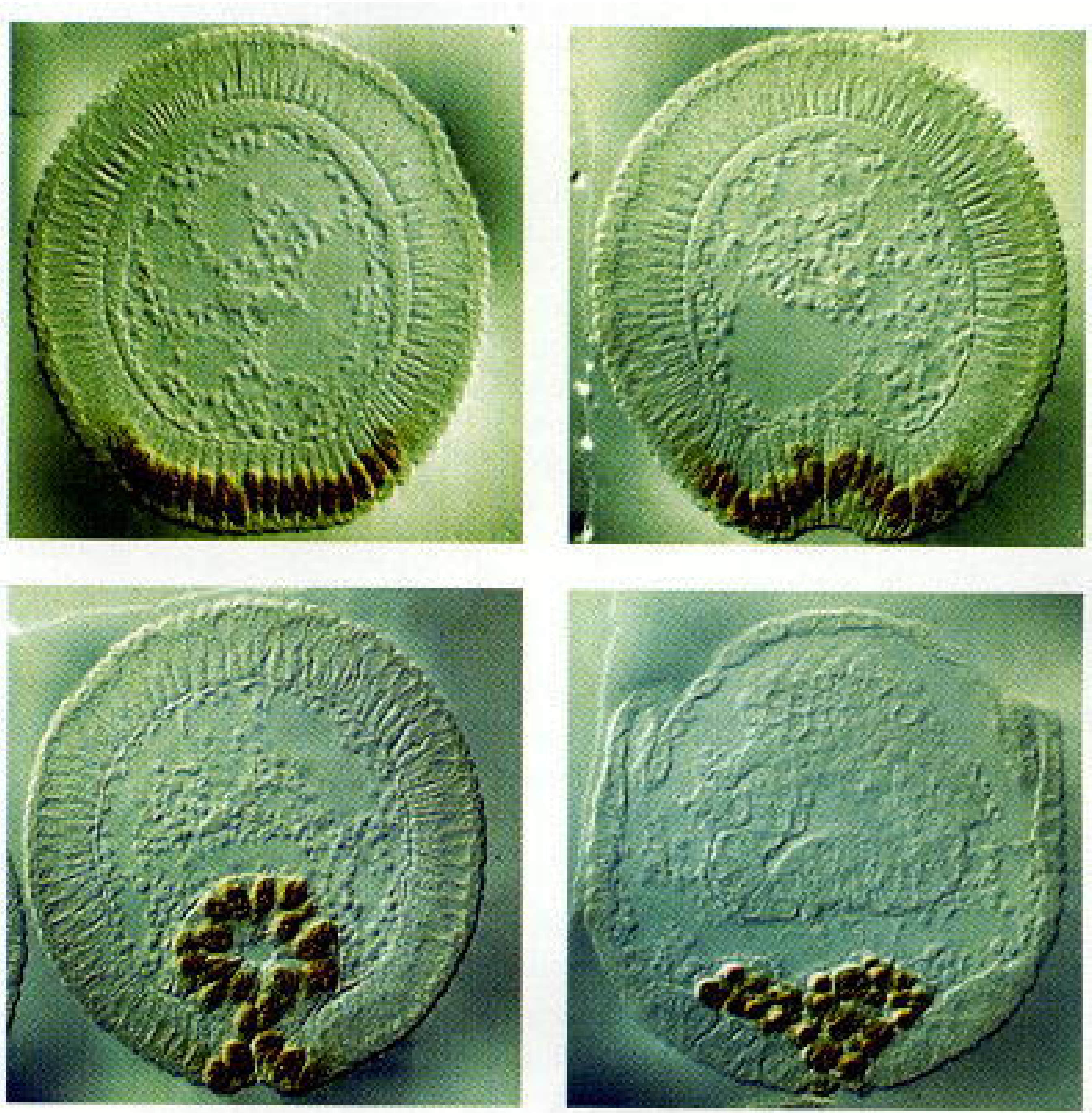
- Genes have different sensitivities to *Dorsal* protein



Example of Dorsal Gradient Function

- *Twist* protein required to specify mesoderm
    - High concentration of *Dorsal* is required to turn on *Twist* gene
    - *Twist* is only turned on in cells along ventral mid-line

Localization of Twist Protein During Gastrulation



Summary

Antero-posterior

mRNAs: *bicoid* forms anterior to posterior gradient; *hunchback* uniform; *nanos* and *caudal* uniform

↓

Anterior to posterior gradient of Bicoid protein formed. *hunchback* mRNA translation suppressed in posterior region by Nanos. *caudal* mRNA translation repressed by Bicoid

Dorso-ventral

Spätzle protein activates Toll receptor on ventral side

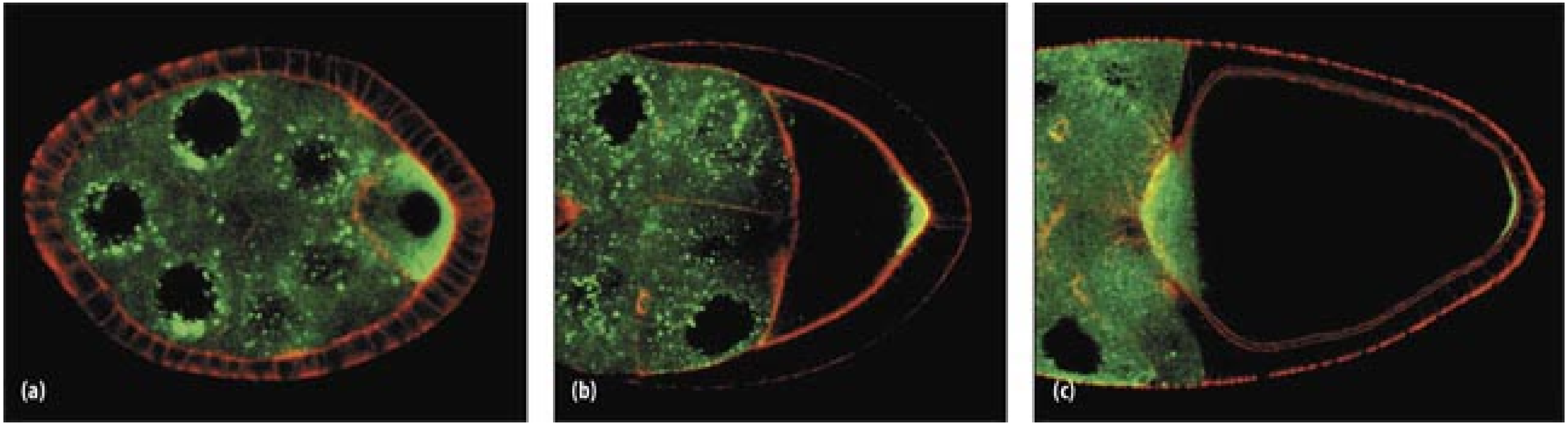
↓

Dorsal protein enters ventral nuclei, giving ventral to dorsal gradient

Termini: Torso receptor activated by Trunk at ends of egg

Staufen required for *bicoid* and *oskar* mRNA localization

Staufen protein binds to *bicoid* and *oskar* mRNA



Polarization of *Drosophila* oocyte

Antero-posterior

oocyte localized at posterior end of follicle by cadherin

⇩

oocyte Gurken protein induces posterior follicle cells via Torpedo

⇩

posterior signal from follicle cells reorganizes oocyte cytoskeleton ⇨

⇩

bicoid mRNA localized in anterior, *oskar* and other mRNAs in posterior

Dorso-ventral

nucleus moves dorsally

⇩

oocyte Gurken induces dorsal follicle cells

⇩

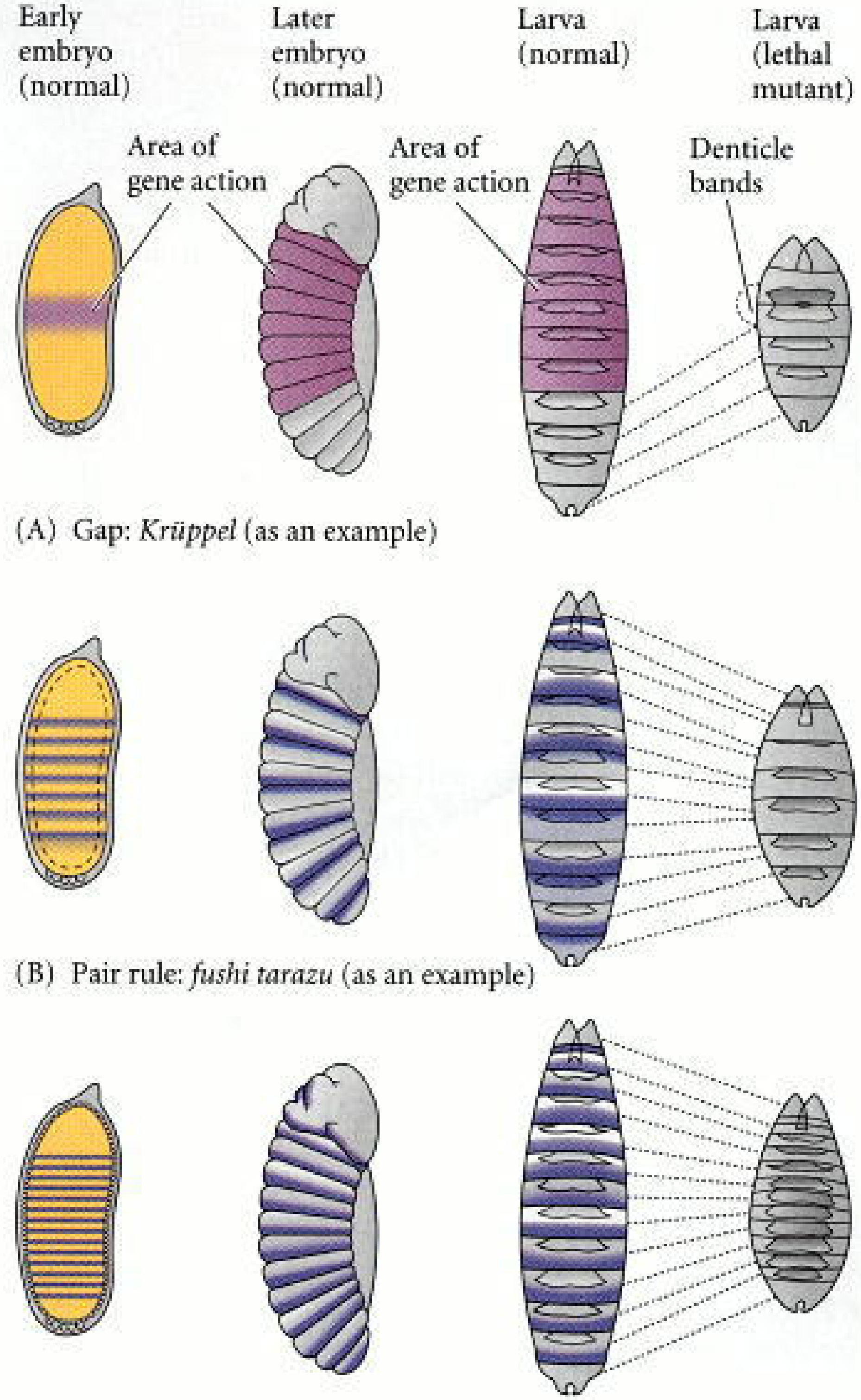
ventral follicle cells deposit ventral proteins in oocyte viteline envelope

Termini: follicle cells at both ends of the egg deposit ligand for the Torso protein in the vitelline envelope

Segmentation Genes

Classified according to mutant phenotype

- **Gap** – several contiguous segments missing.
- **Pair-rule** – part of every other segment missing.
- **Segment polarity** – defects (deletions, duplications, polarity reversals) in every segment.



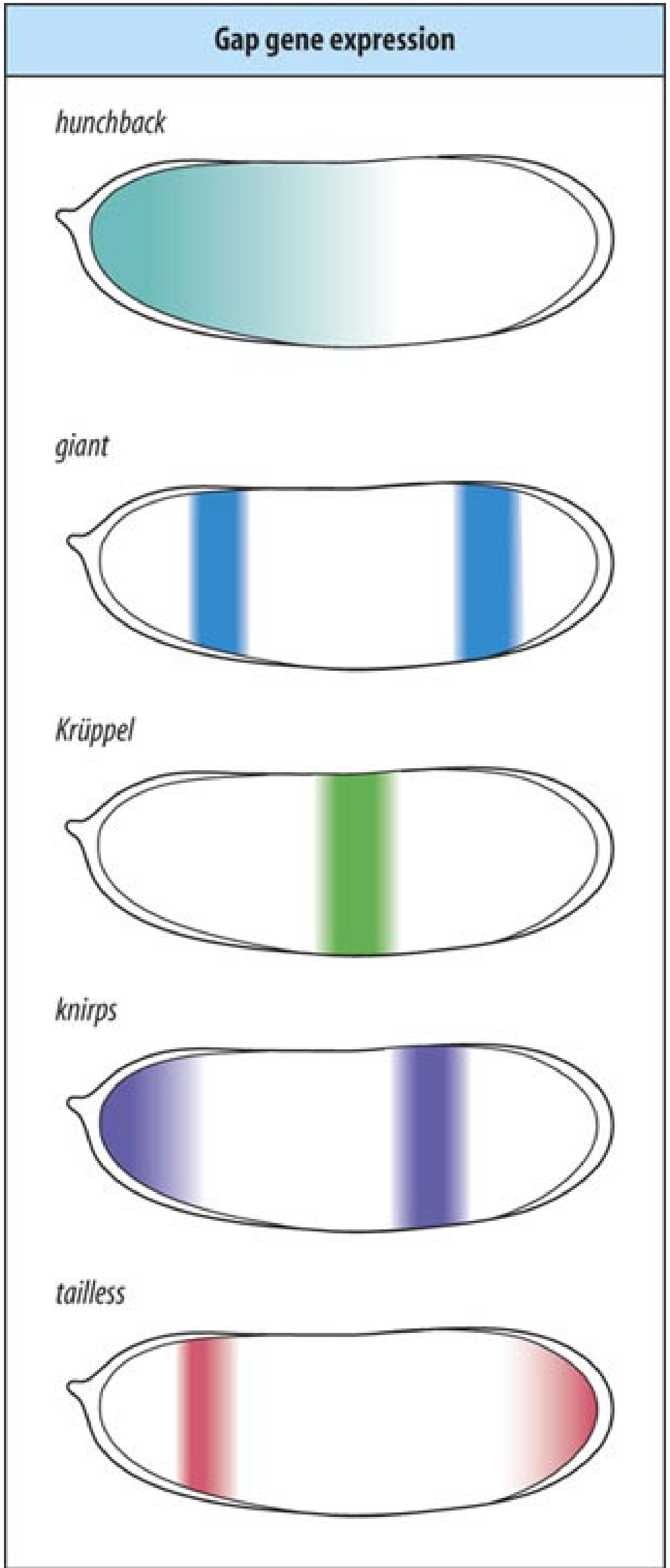
(A) Gap: *Krüppel* (as an example)

(B) Pair rule: *fushi tarazu* (as an example)

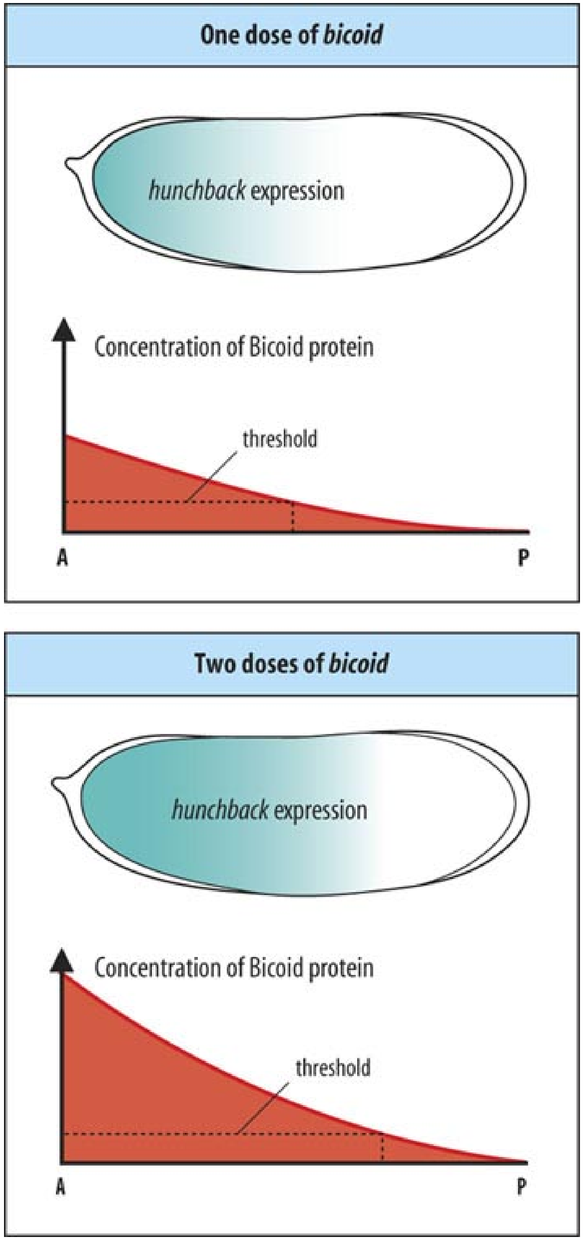
(C) Segment polarity: *engrailed* (as an example)

Gap Genes

Activated or repressed by maternal effect genes.



bicoid controls *hunchback* expression

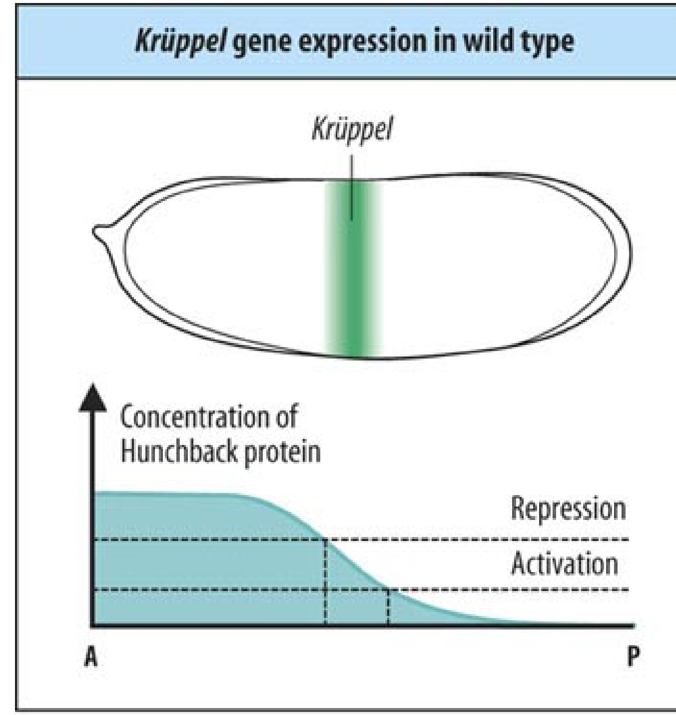


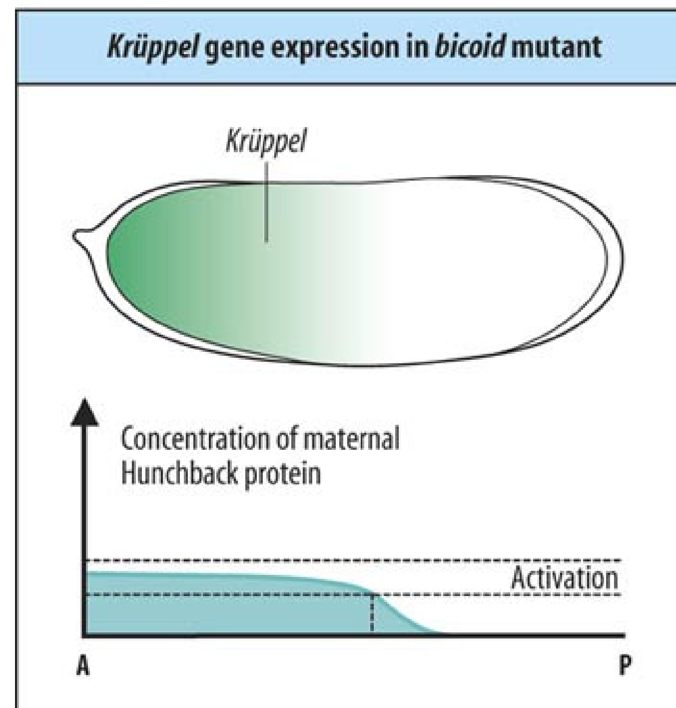
Gap gene expression is complex and not completely known.

What do we know?

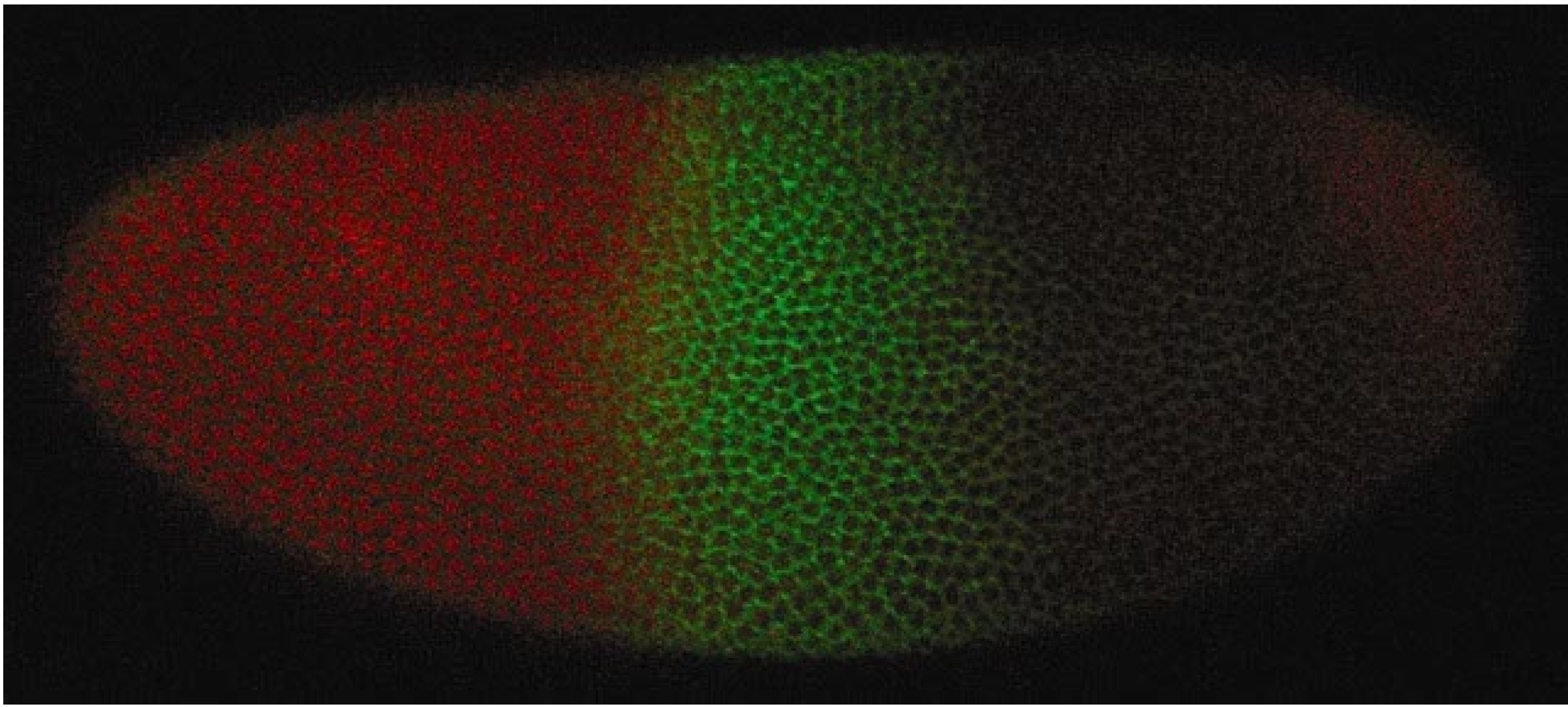
Kruppel expression is controlled by Hunchback protein

- High levels of Hunchback inhibits Kruppel
- Medium levels of Hunchback activates Kruppel





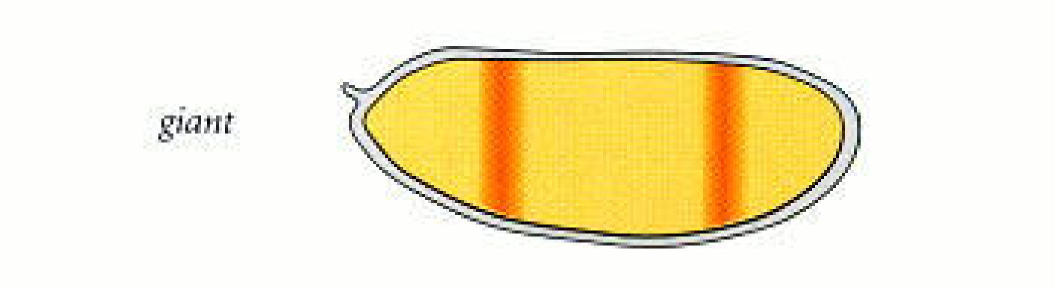
Red=Hunchback
Green=Kruppel



giant

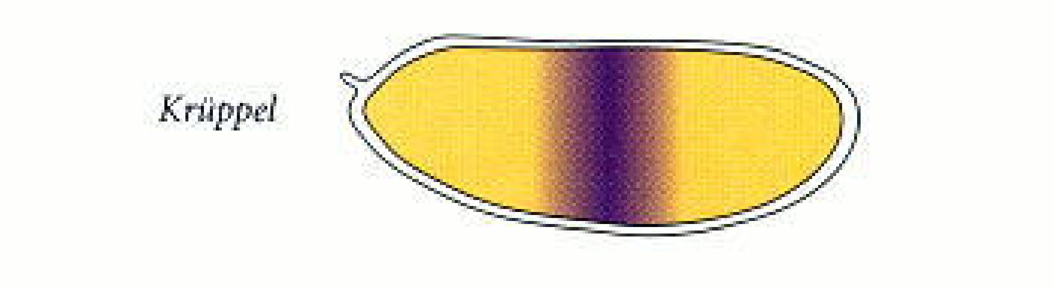
High levels of hunchback and bicoid induce expression of giant in anterior end but repress expression of giant at posterior end.

Caudal induces giant at posterior end.



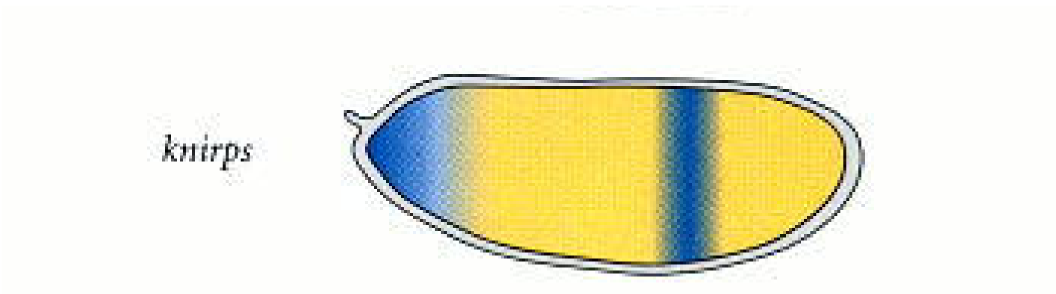
kruppel

Kruppel expressed where there are low levels of hunchback and caudal.

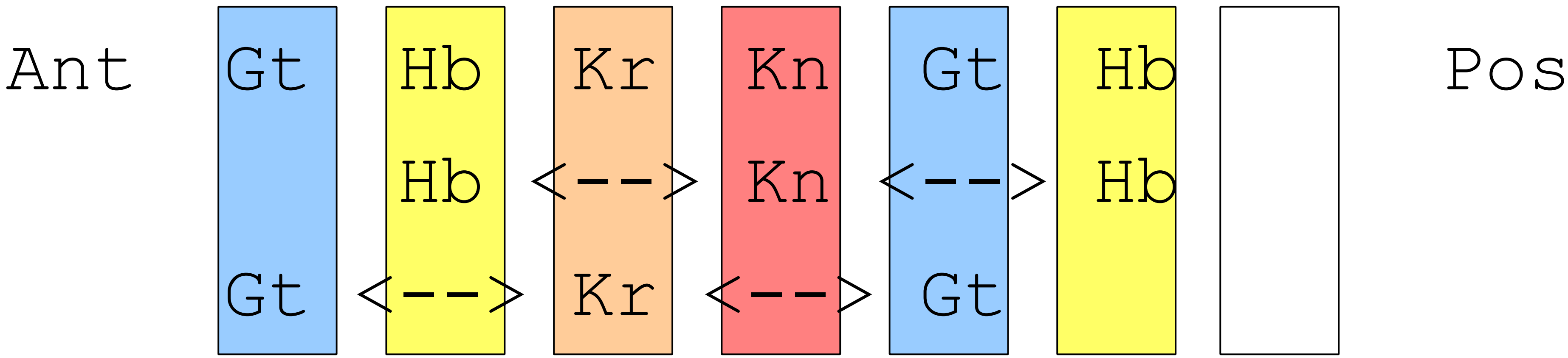


knirps

Hunchback inhibits knirps at anterior end and caudal activates knirps at posterior end.



Gap gene pattern

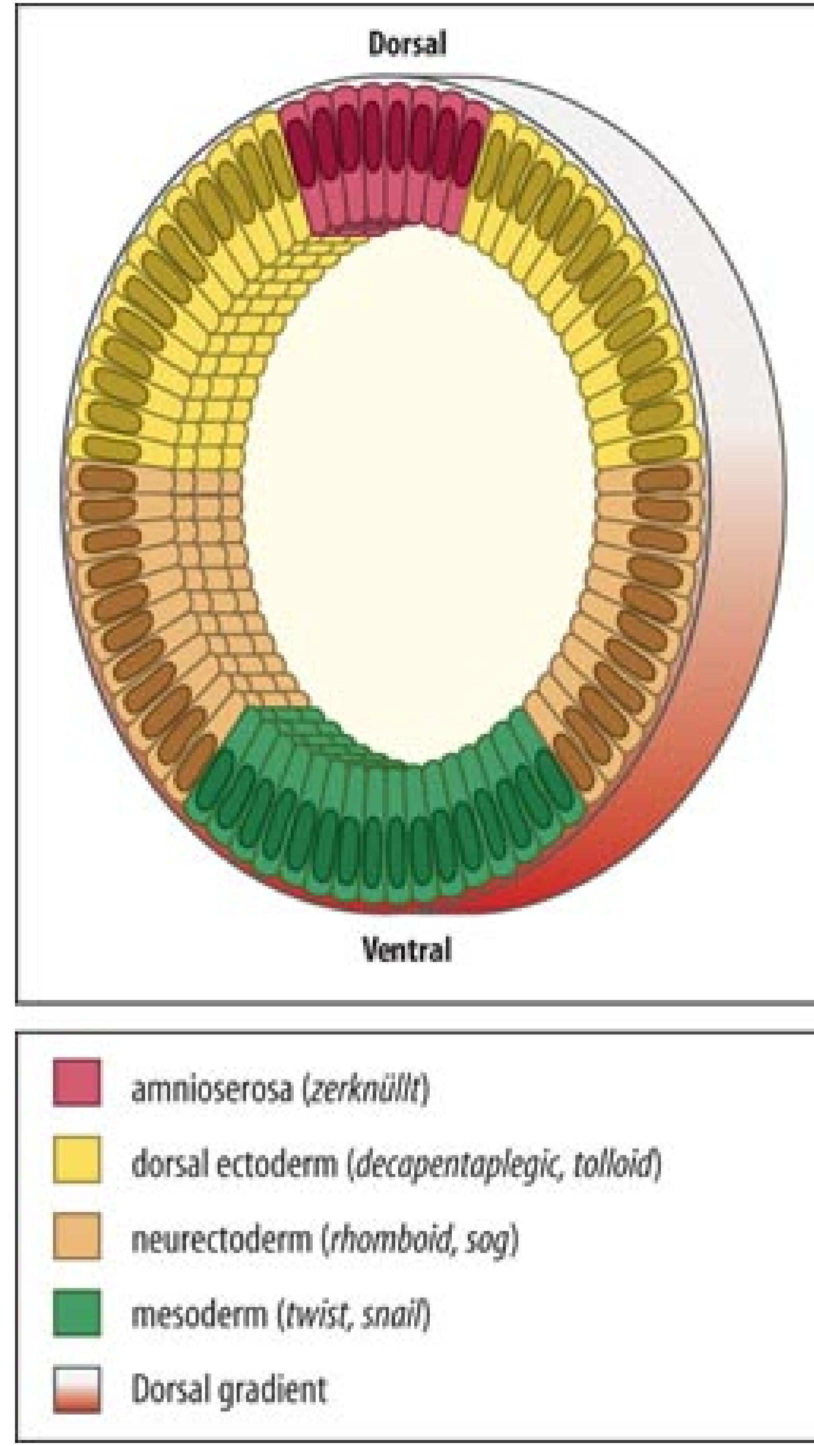


Domains overlap

Nonadjacent domains mutually repress

- Gt/Kr mutual repression
- Hb/Kn mutual repression

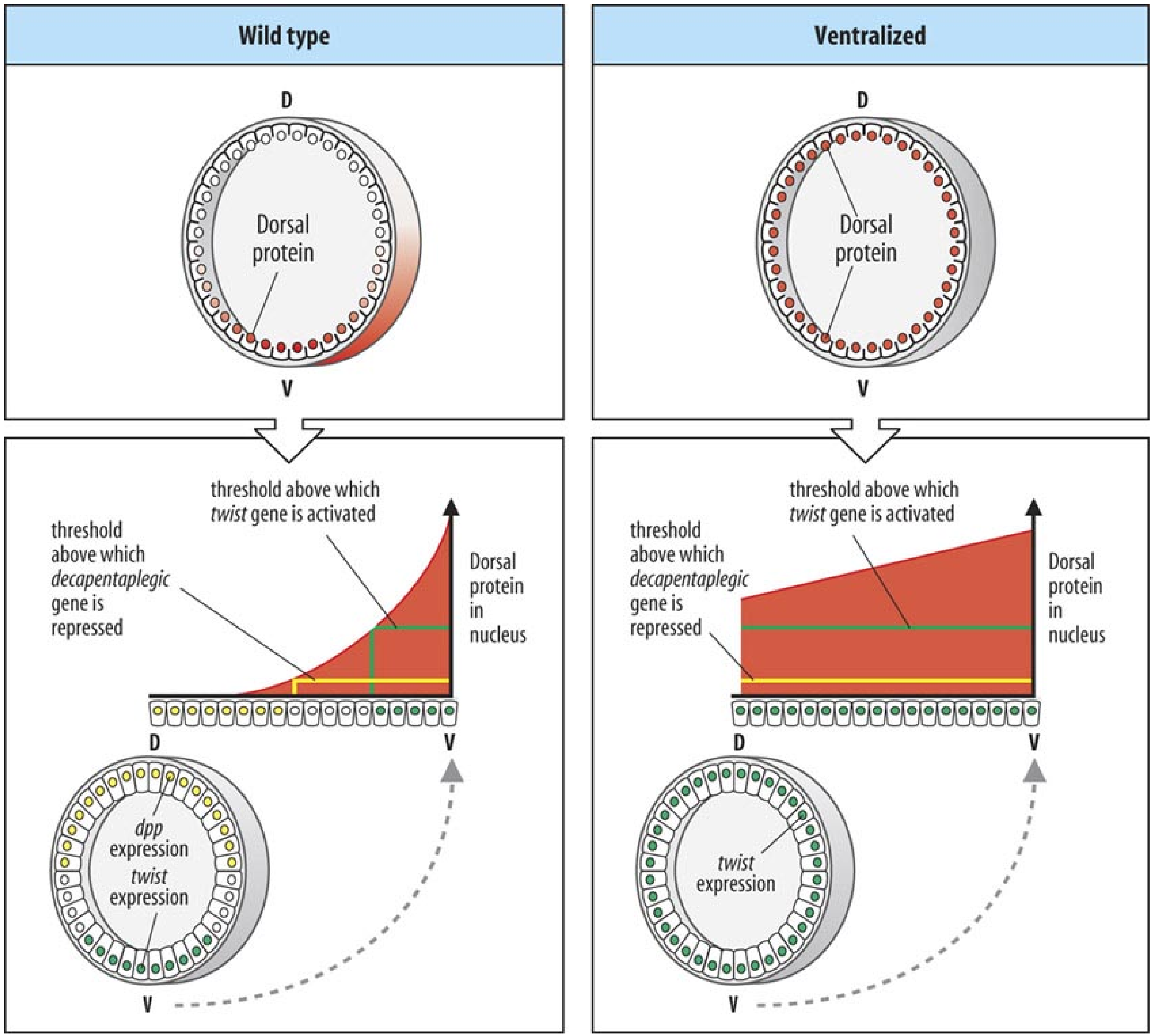
Dorsal divides up the dorsal/ventral axis



Dorsal Gradient Function

- *Decapentaplegic* (dpp) required to specify dorsal ectoderm
  - *Dorsal* protein suppresses *dpp* gene
  - *dpp* only expressed where Dorsal protein is low – on dorsal side away from ventral midline

Dorsal controls both *dpp* and *twist*

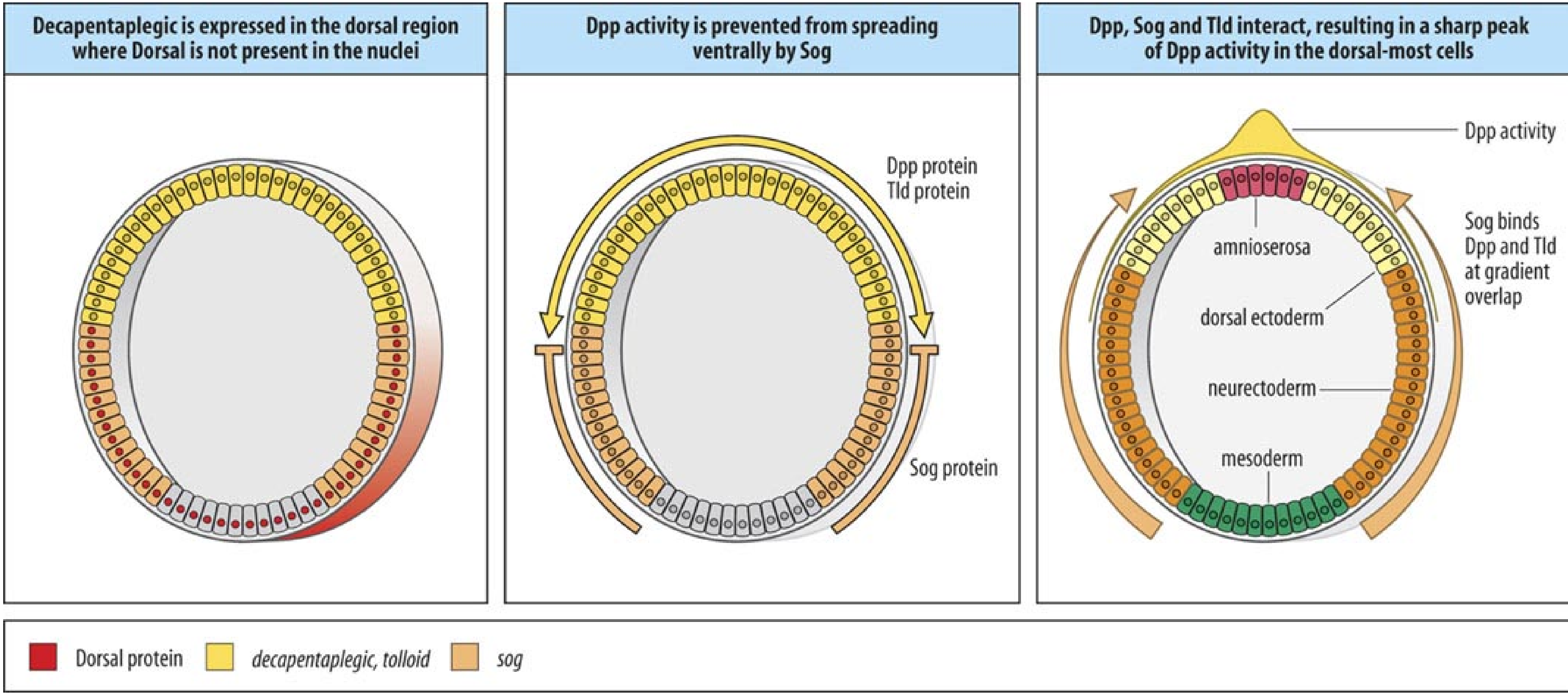


Dorsal Gradient Function

Sog required to specify the neuroectoderm

- Transcription of *Sog* is activated by an intermediate amount of Dorsal protein
- Sog can only have an effect when it is not express with *twist*
- *Sog* is expressed in a narrow band above *twist* expression and below *dpp* expression

Amniosera determined by interaction of *Sog*, *tolliod*, *Dorsal* and *dpp*.



Summary

Antero-posterior

Bicoid protein gradient switches *hunchback* on at high concentration

↓

Hunchback activates and represses gap genes like *Krüppel*, *knirps*, *giant*

↓

gap-gene products and gap genes interact to sharpen expression boundaries

↓

axis is divided into unique domains containing different combinations of transcription factors

Dorso-ventral

ventro-dorsal gradient of intranuclear Dorsal protein forms

↓

ventral activation of *twist*, *snail*, and repression of *decapentaplegic*

↓

Decapentaplegic expressed dorsally

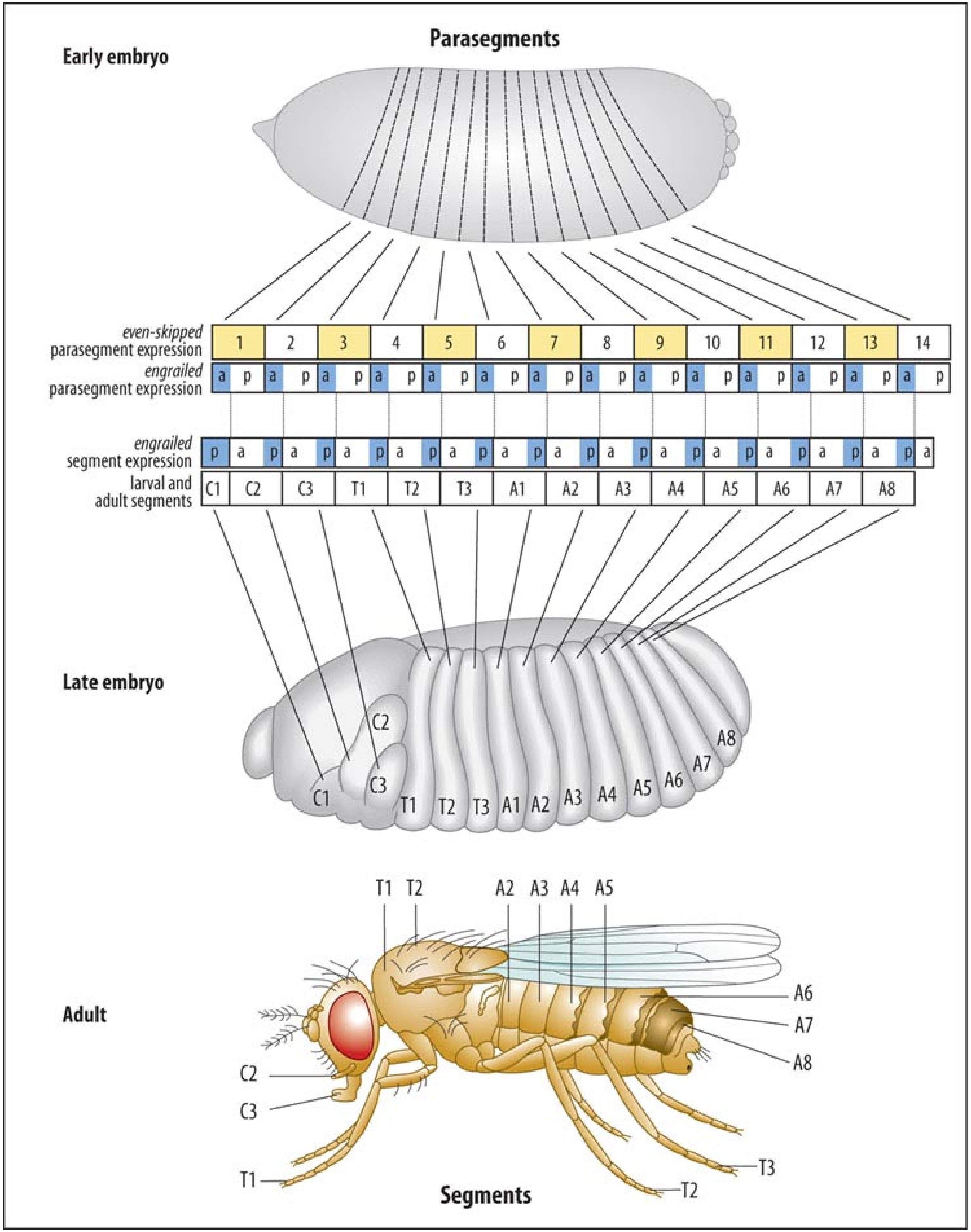
↓

gradient of Decapentaplegic activity patterns dorsal region

↓

dorso-ventral axis divided into prospective mesoderm, neurectoderm, epidermis, amnioserosa

Pair-rule genes



Primary Pair-rule genes

Gene promoters of primary pair-rule genes have different enhancers required for expression in different segments in the embryo.

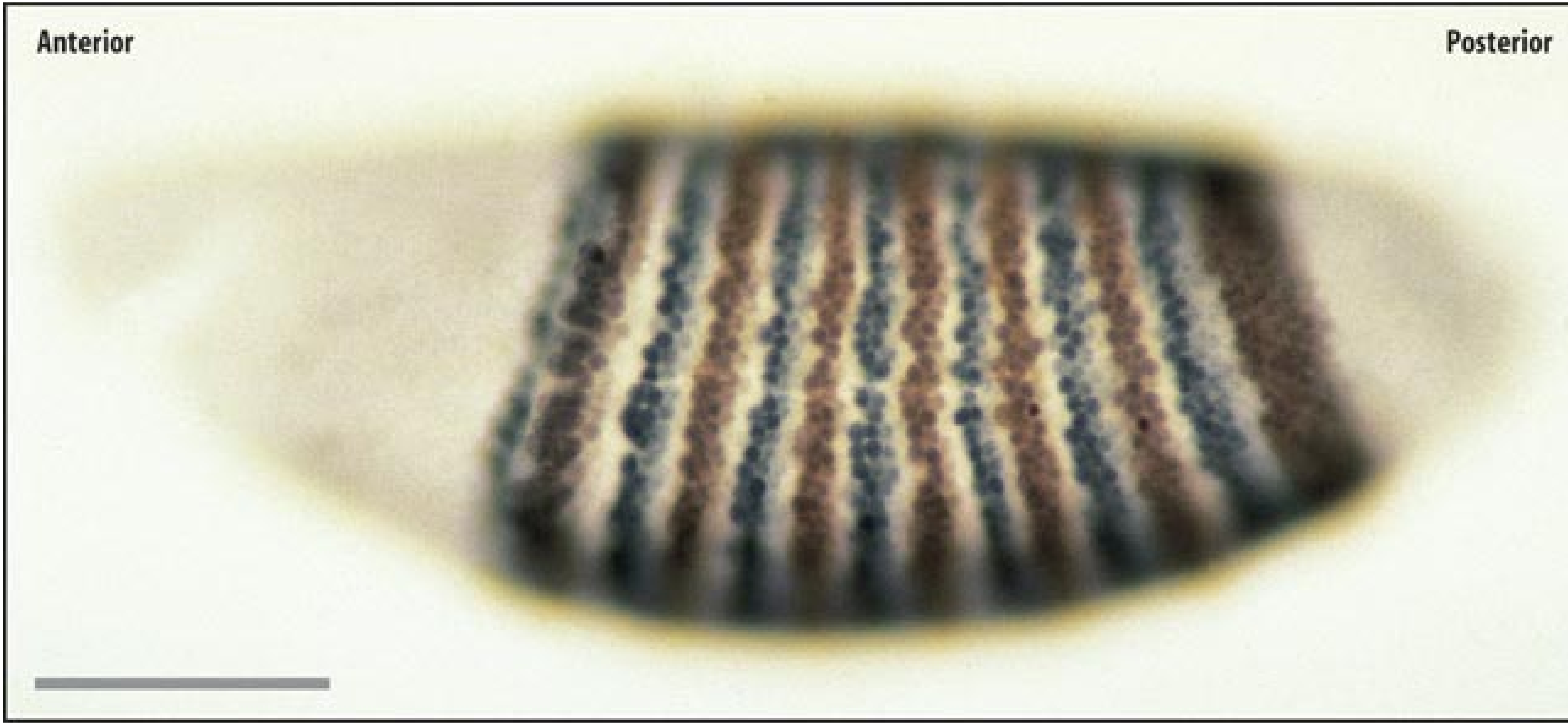
Each enhancer is subject to activation by a different gap gene.

Example even-skipped gene

Stripe 2 enhancer

- Low bicoid/hunchback activate
- Giant/kruppel repressed
- Acts like an on/off switch for gene

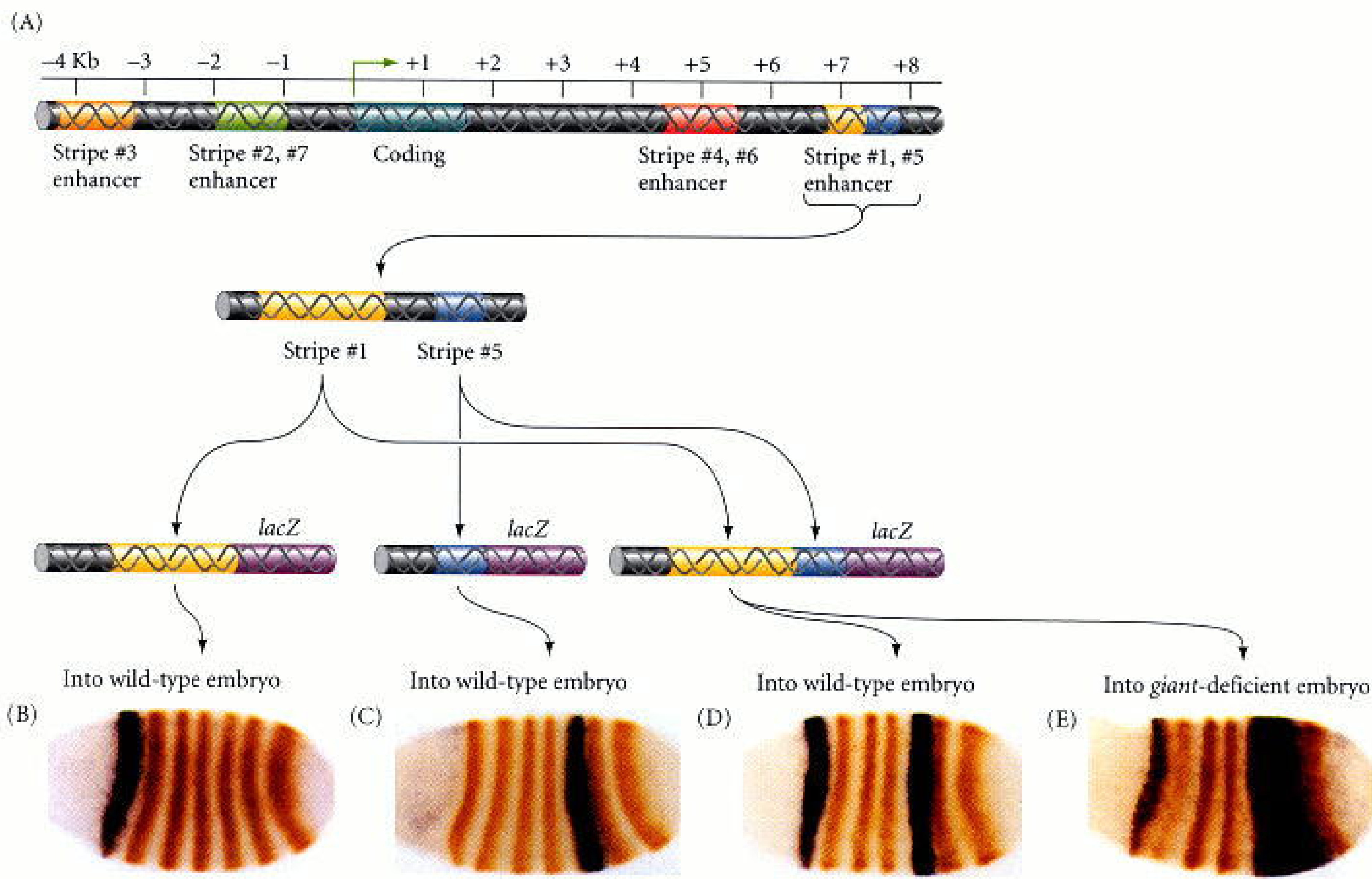
Pair-rule gene expression



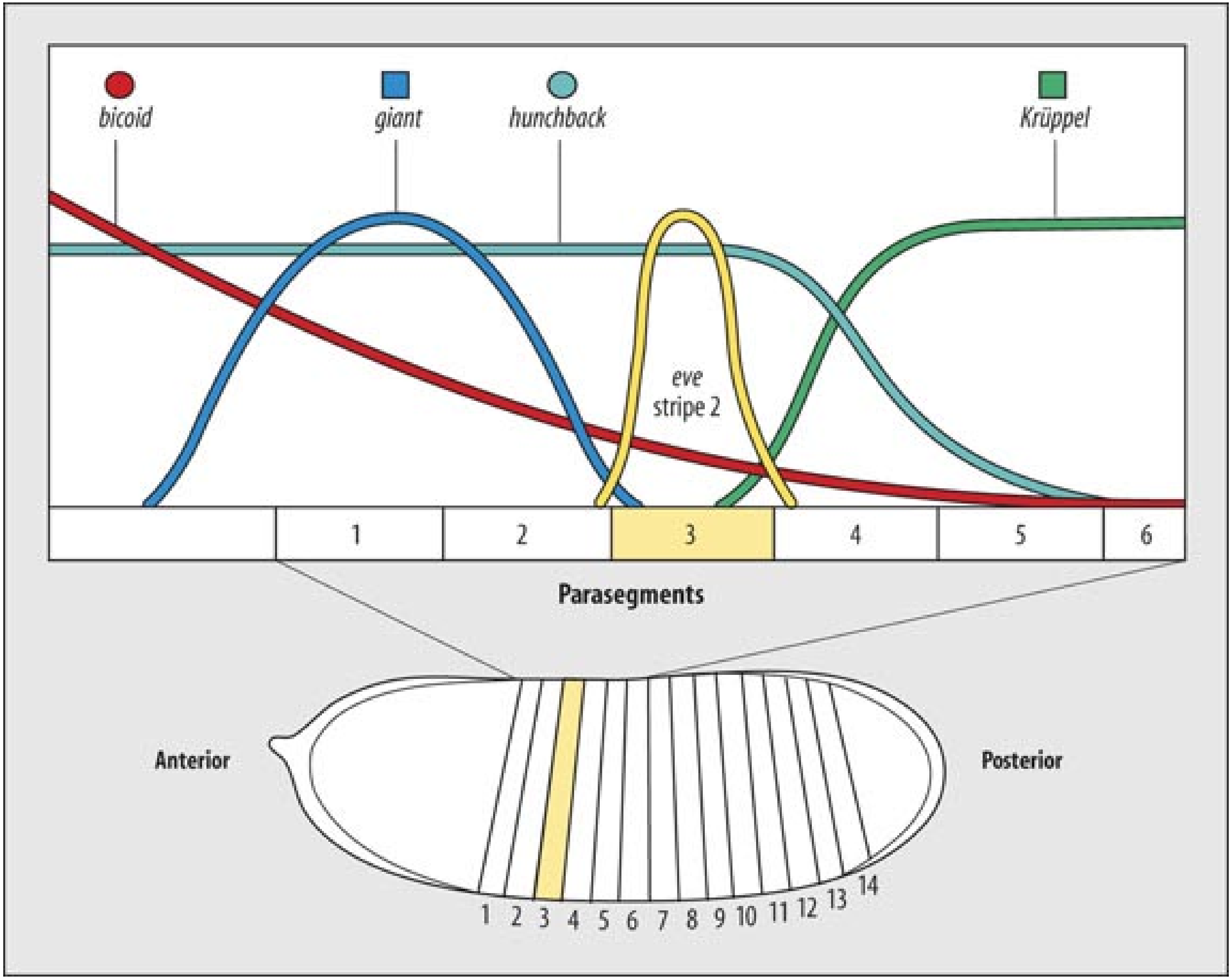
Blue=*even-skipped* Brown=*fushi tarazu*

Immediately prior to cellularization

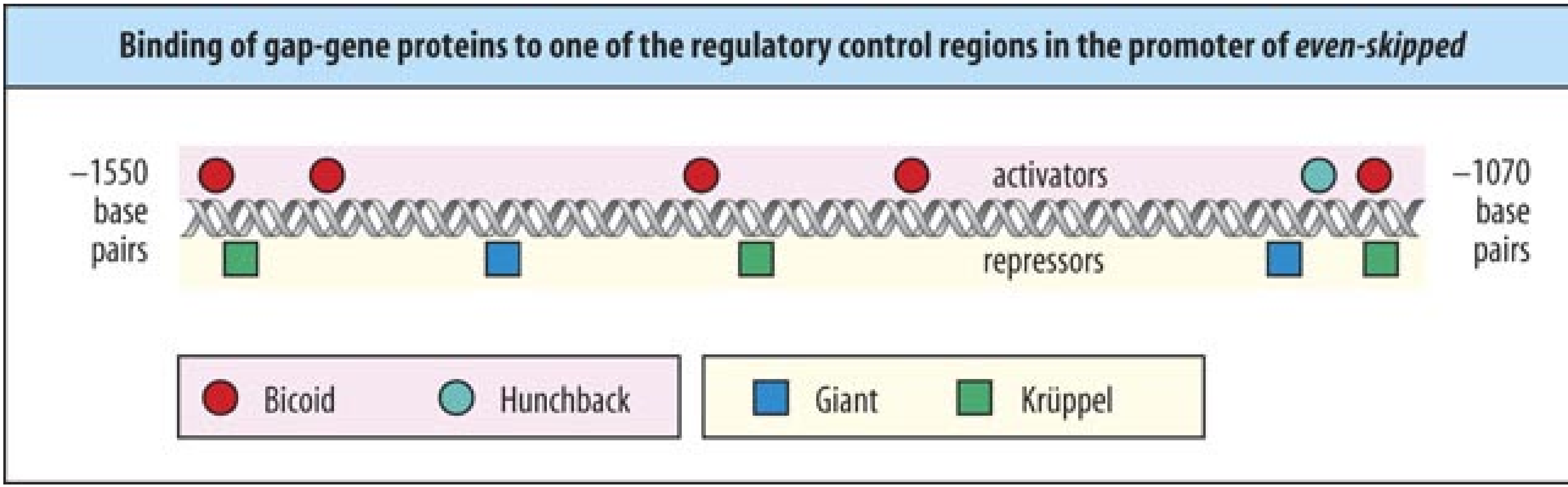
Protein localization using antibodies


(A)
–4 Kb
–3
–2
–1
+1
+2
+3
+4
+5
+6
+7
+8
Stripe #3 enhancer
Stripe #2, #7 enhancer
Coding
Stripe #4, #6 enhancer
Stripe #1, #5 enhancer
Stripe #1
Stripe #5
lacZ
lacZ
lacZ
Into wild-type embryo
Into wild-type embryo
Into wild-type embryo
Into giant-deficient embryo
(B)
(C)
(D)
(E)

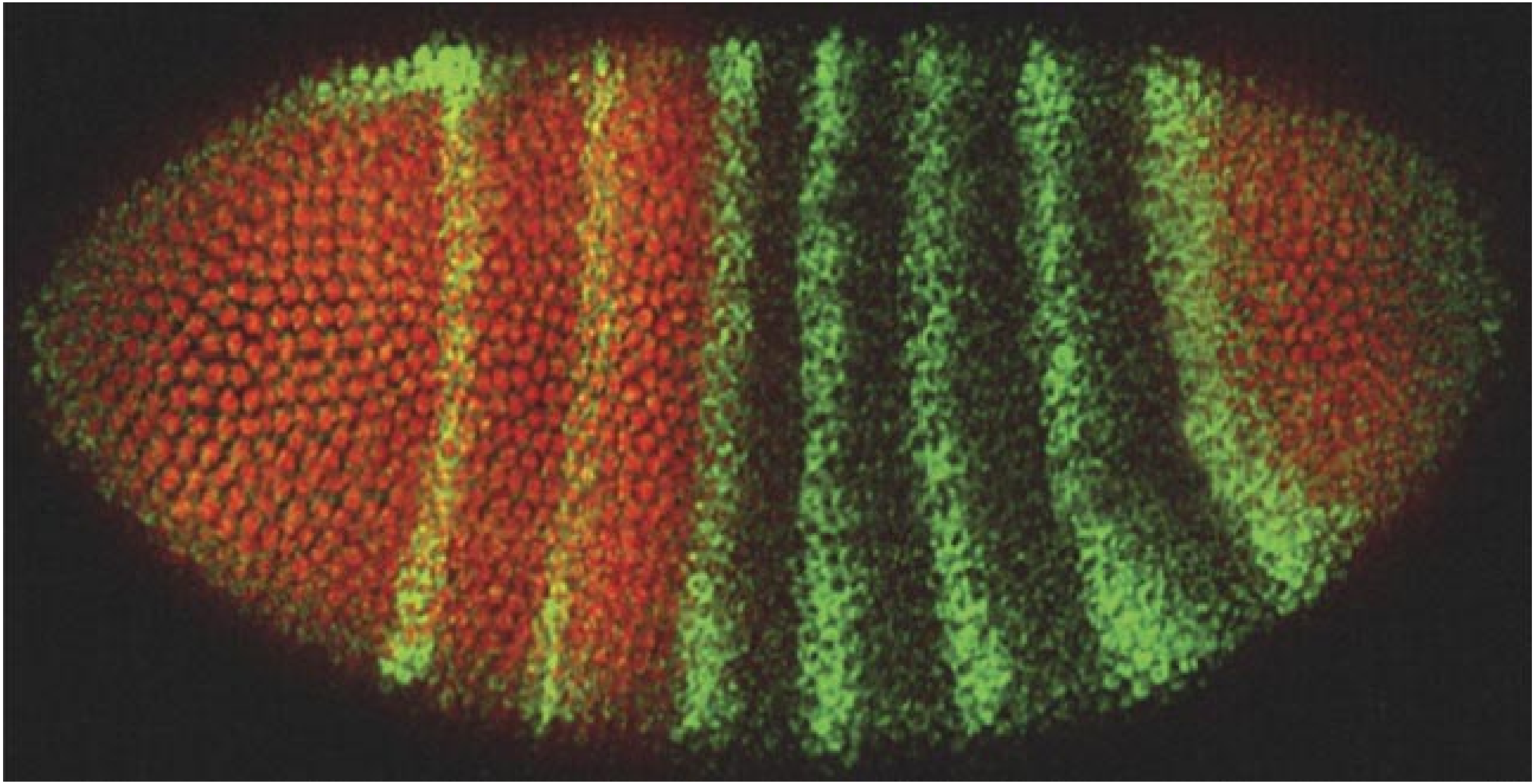
Gap genes specifiy second *even-skipped* stripe



Stripe 2 Enhancer of *even-skipped*



Red=*hunchback*
Green=*eve-skipped*



Secondary Pair-Rule Genes

Expressed in context of primary pair-rule genes

As in primary pair-rule gene they self enhance expression

Result – each of the eight pair-rule genes is expressed in a seven stripe pattern

However, the stripes are not in the same location

Result – each row of cells expresses a different combination of pair-rule genes

ftz gene expression

Fushi tarazu gene (ftz) secondary pair-rule gene

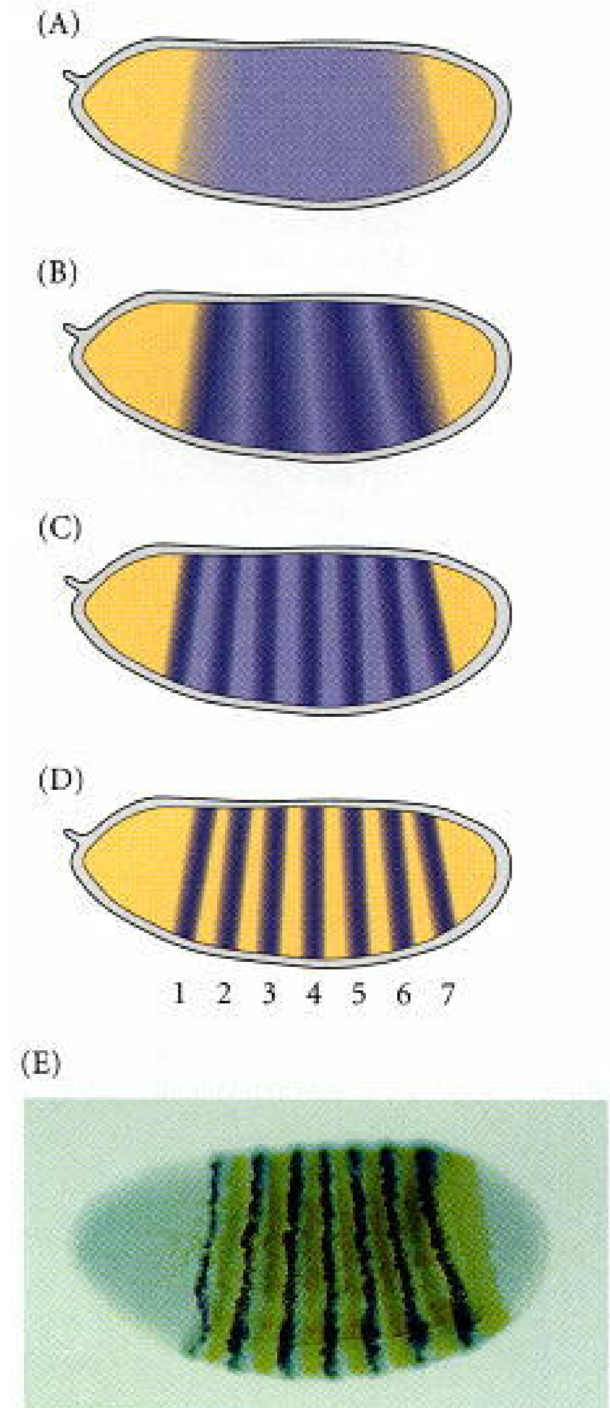
- Expressed in context of primary pair-rule genes

A-D *ftz* gene expression

- Localizes to 7 bands

E *ftz* and *eve* gene expression

- *ftz* gene is expressed where *eve* is not (*ftz* green, *eve* blue)



Pair-rule gene summary

production of local combinations of gap-gene transcription factors

↓

activation of each pair-rule gene in seven transverse stripes along the antero-posterior axis

↓

pair-rule gene expression defines 14 parasegments, each pair-rule gene
being expressed in alternate parasegments

Segment Polarity (segmentation) Genes

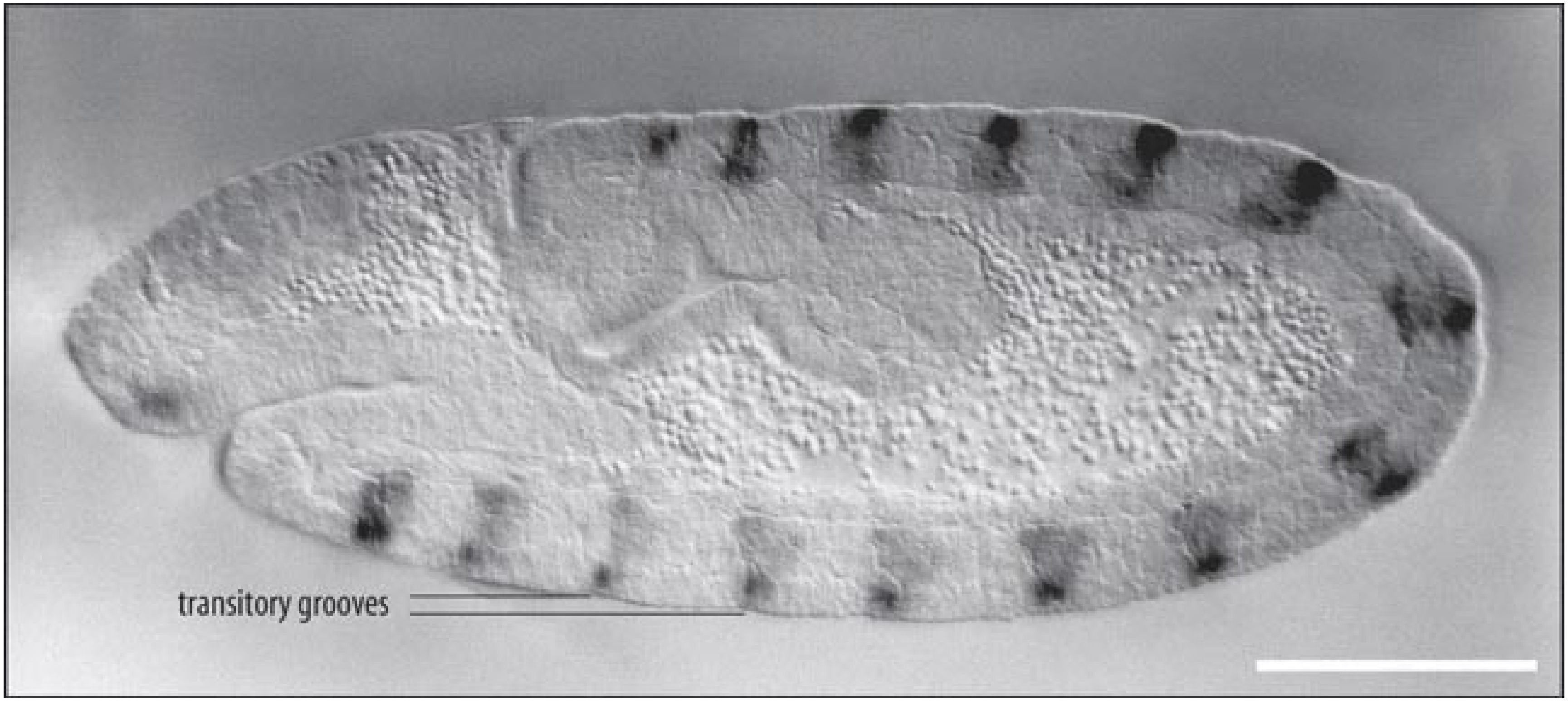
Cell-cell interactions – syncytium no longer present

Convert parasegment (from pair-rule genes) to actual segments

Expression based on pair-rule expression

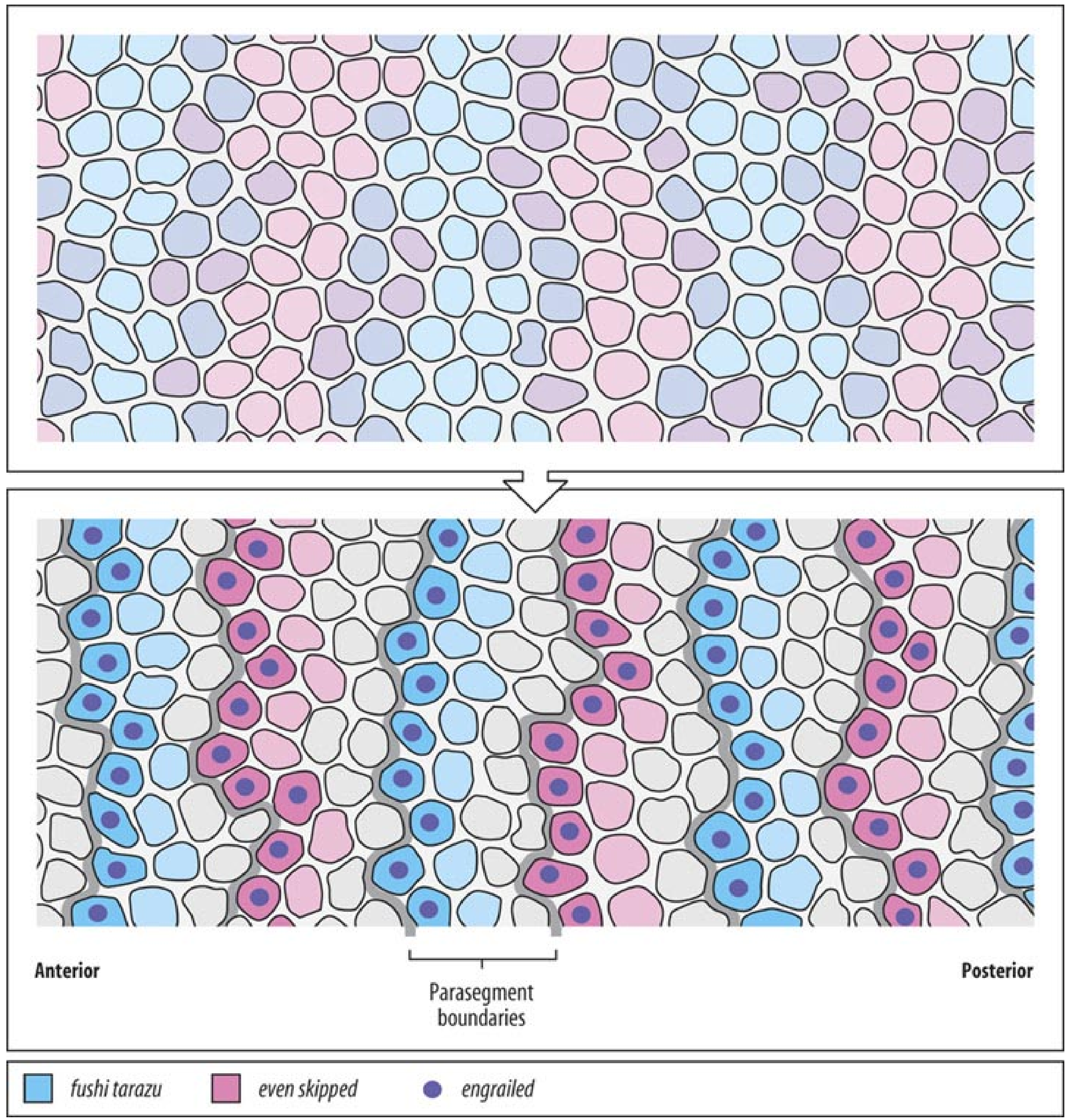
Expressed in part of every segment

engrailed Expression



Expressed in the anterior end of each parasegment after cellularization.
Turned on by pair-rule gene expression.

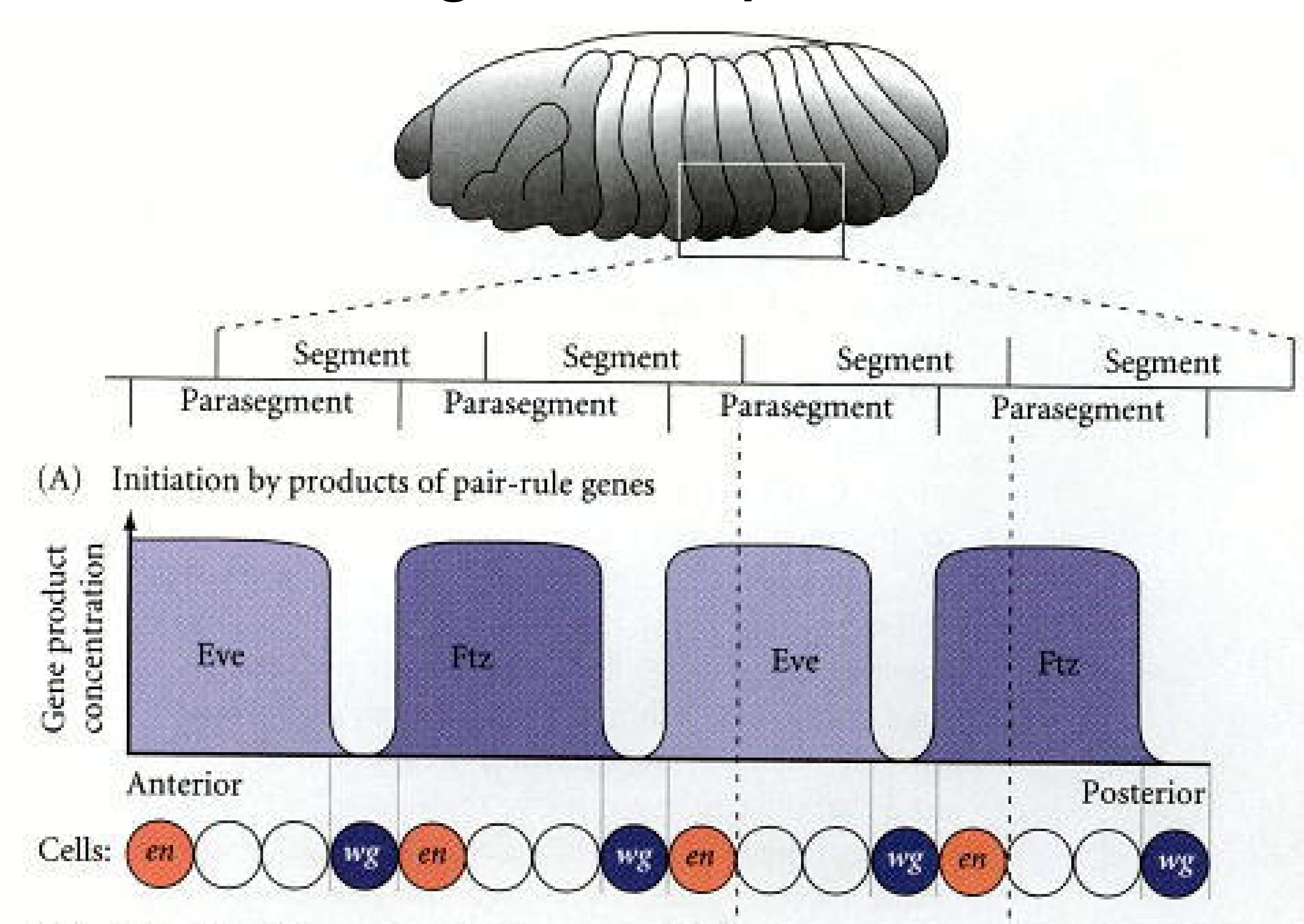
Ftz (blue), eve (pink), engrailed (dark nuclei)



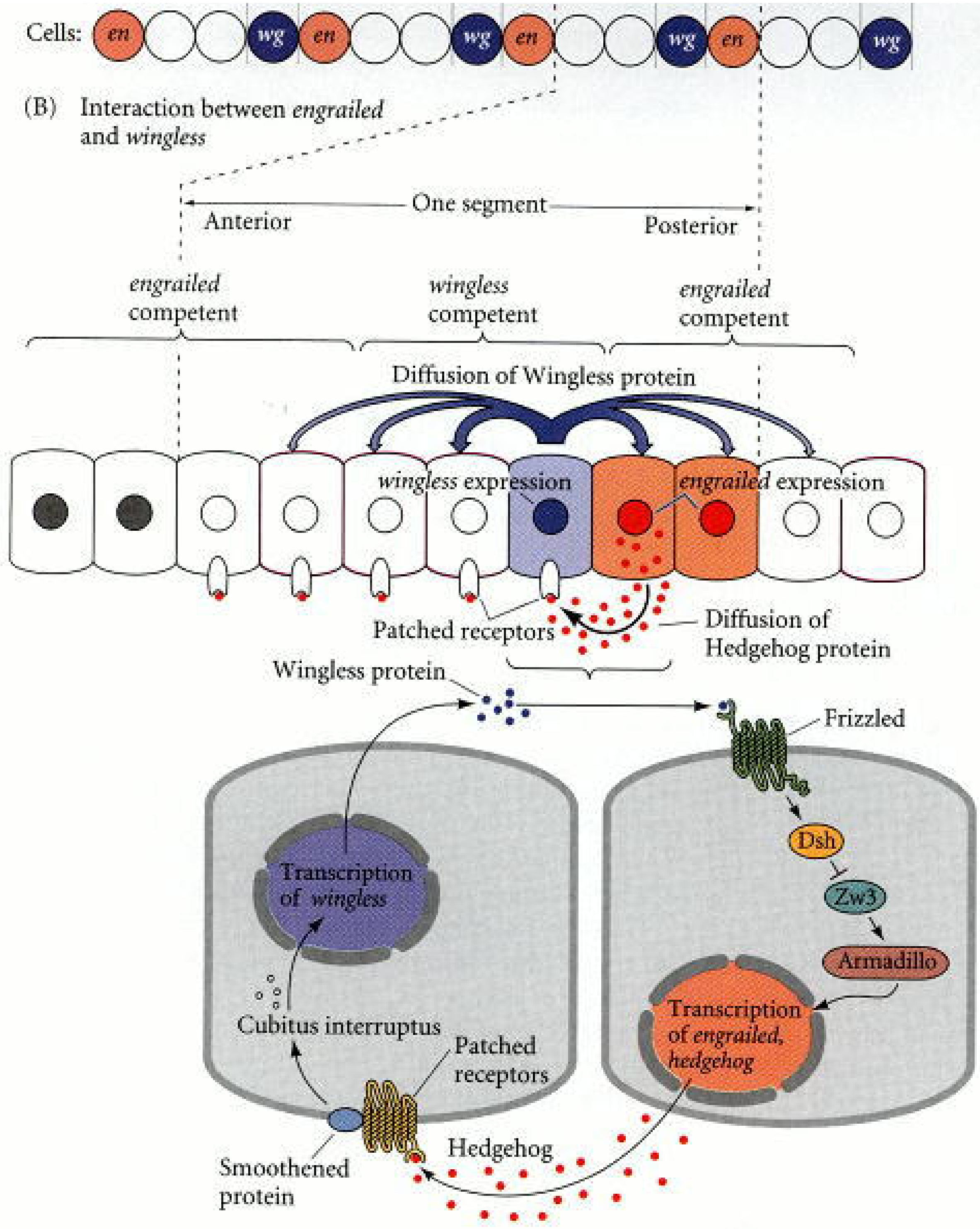
Hedgehog and Wingless

- Each is expressed in a single band in each parasegment
  - Engrailed activated hedgehog transcriptions and represses wingless transcription
  - Engrailed is activated in cells high in eve and ftz but low in other pair-rule proteins
  - Results in a 14 stripe pattern

Wingless Expression



Controlled by Wnt Cascade

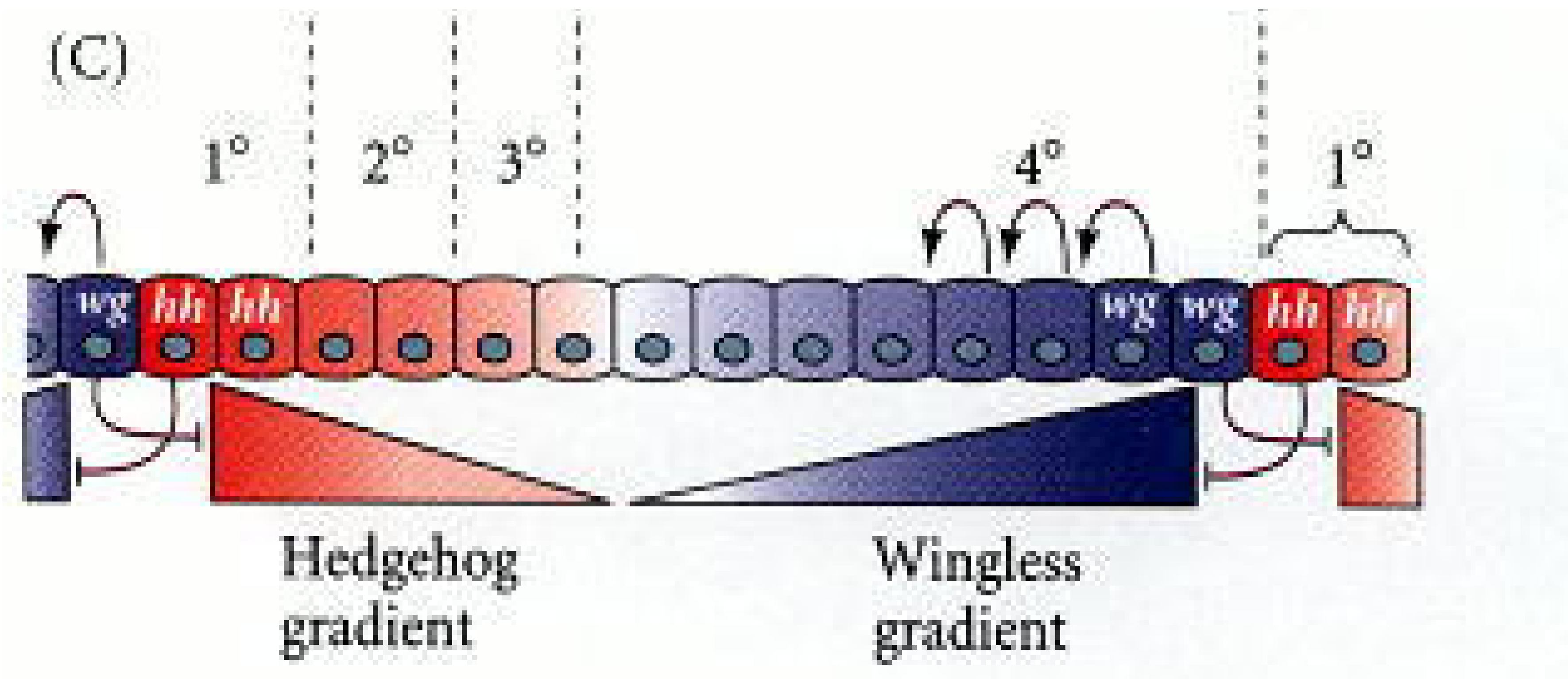


Hedgehog/wingless gradients

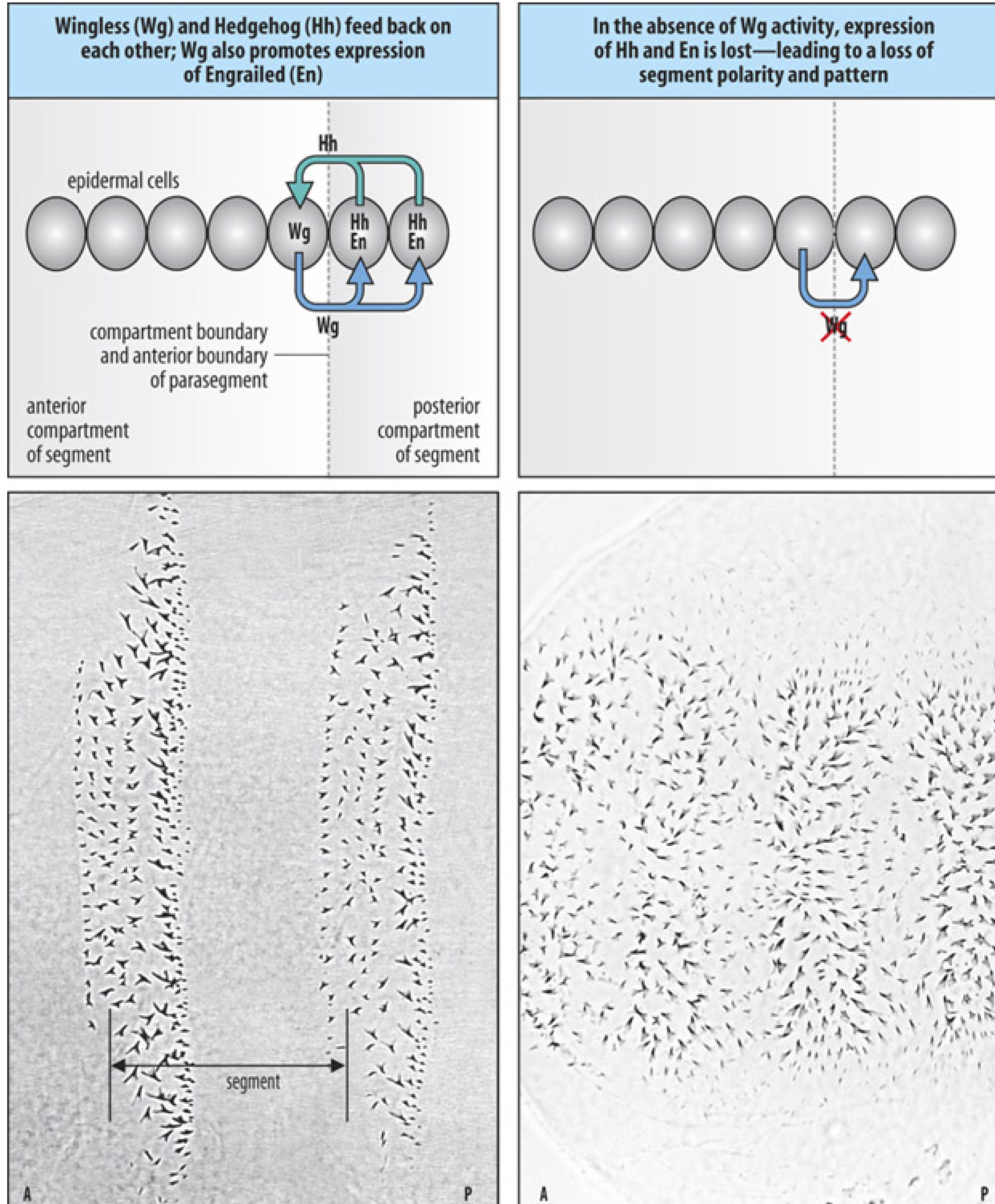
Stable boundary forms

Overlapping gradients can control other genes

Anterior/posterior of each segment established



Compartment Boundary

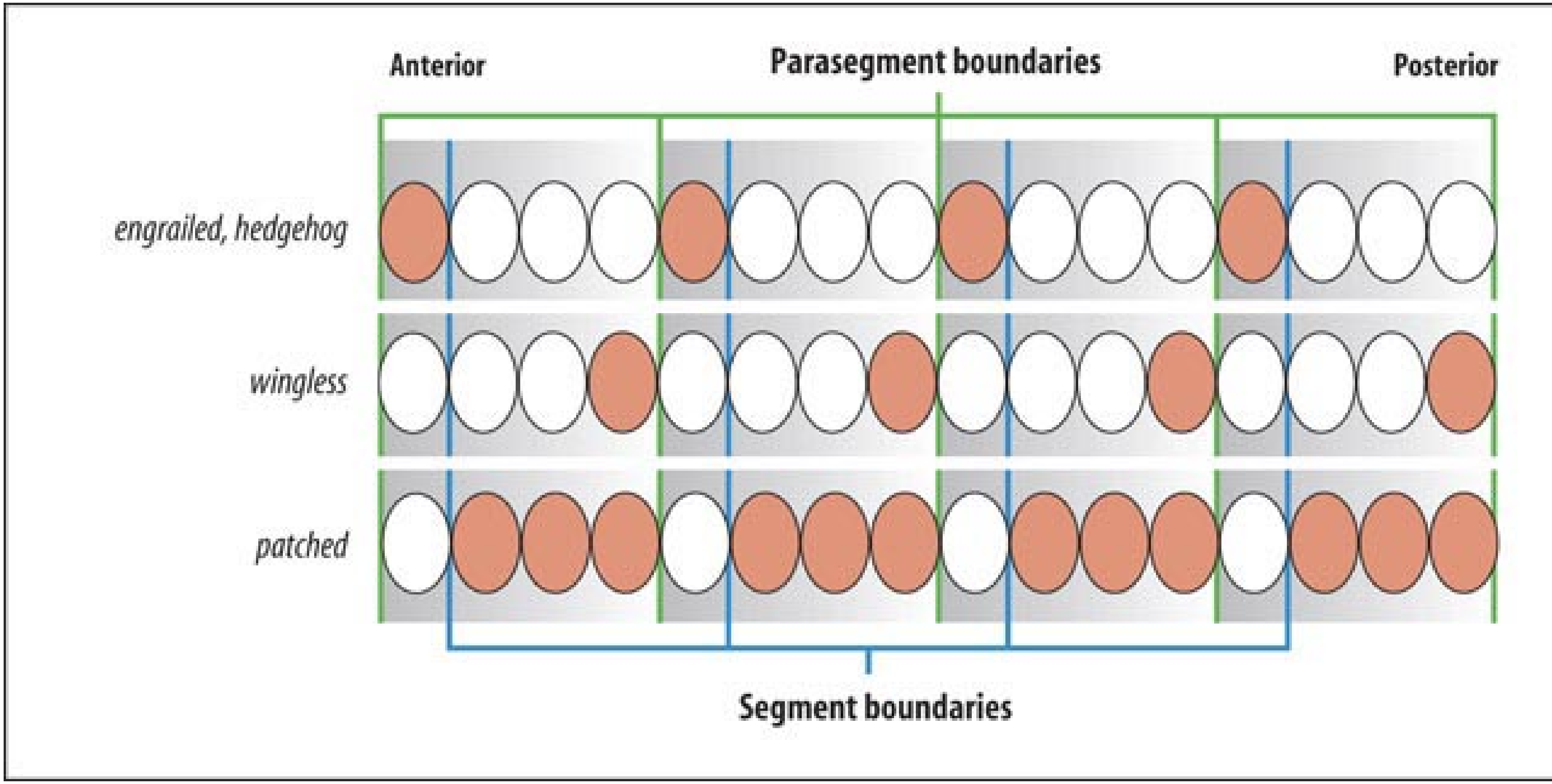


Strengthening of Pattern

Reciprocal activation

- Hedgehog diffuses to neighboring cells and activates wingless
- Wingless diffuses to neighboring cells and activates hedgehog

Formation of Segments



Homeotic Selector Genes

Establish segment identity

Activated once segment polarity is determined by segment polarity genes

Turned on by unique combination of gap and pair-rule genes

Antennapedia Complex

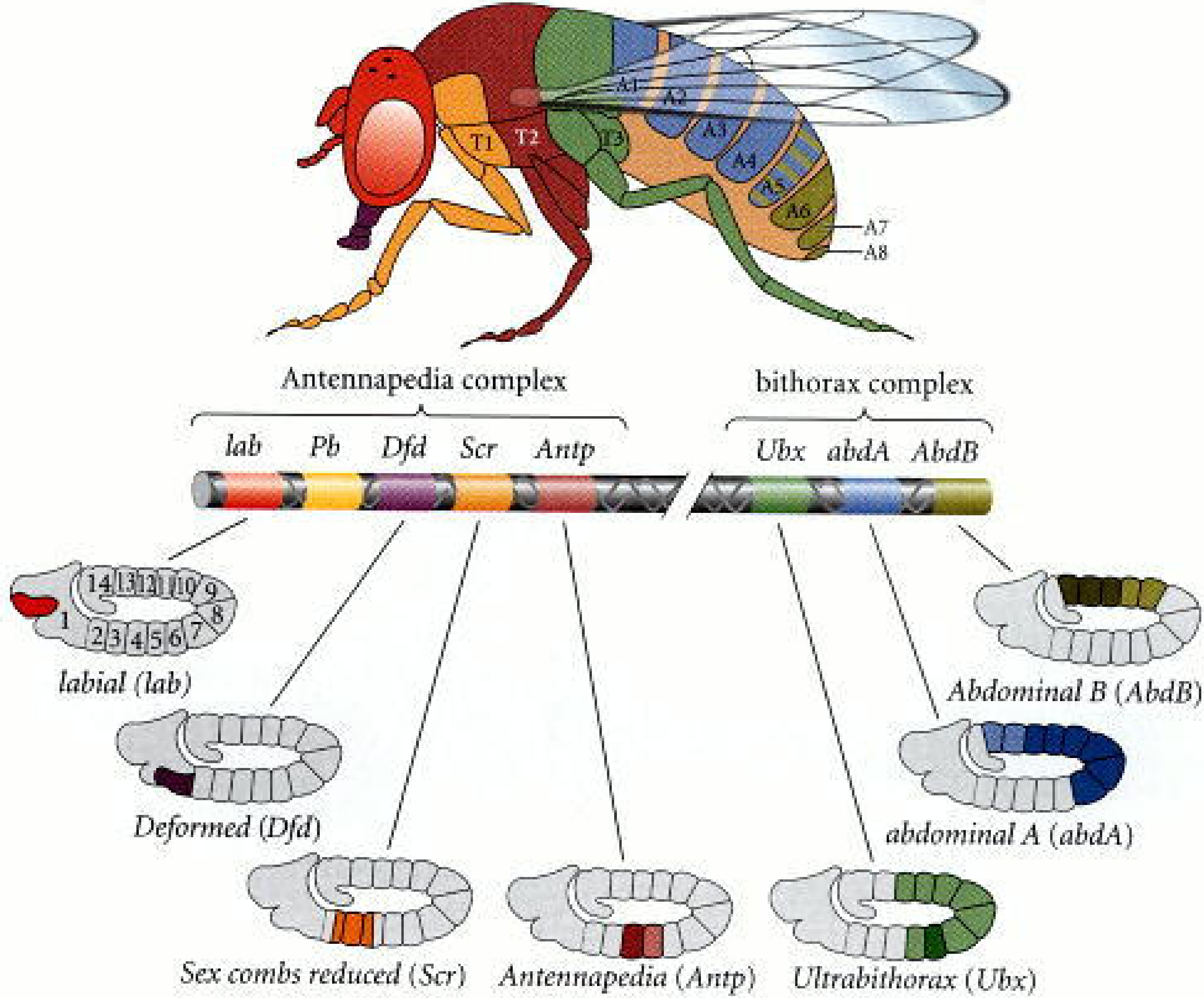
Contains 5 genes

- Labial (lab) – head
- Deformed (dfd) – head
- Antennapedia (Antp) – thorax
- Sex combs reduced (scr) – thorax
- Proboscipedia (pb) – adult head

Bithorax Complex

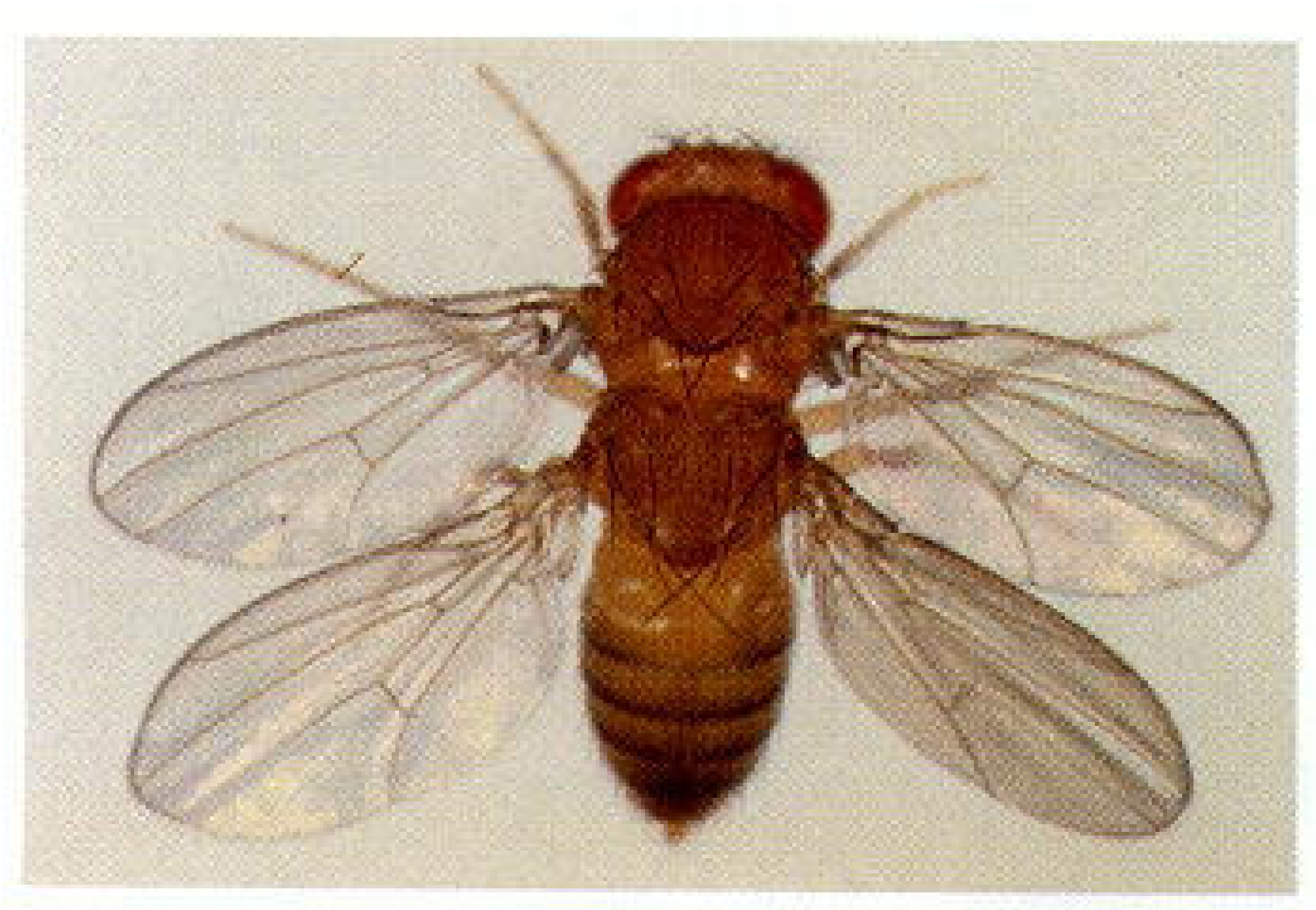
Contains three genes

- Ultrabithorax (Ubx) – third thoracic segment
- Abdominal A (abdA) – abdomen
- Abdominal B (abdB) – abdomen

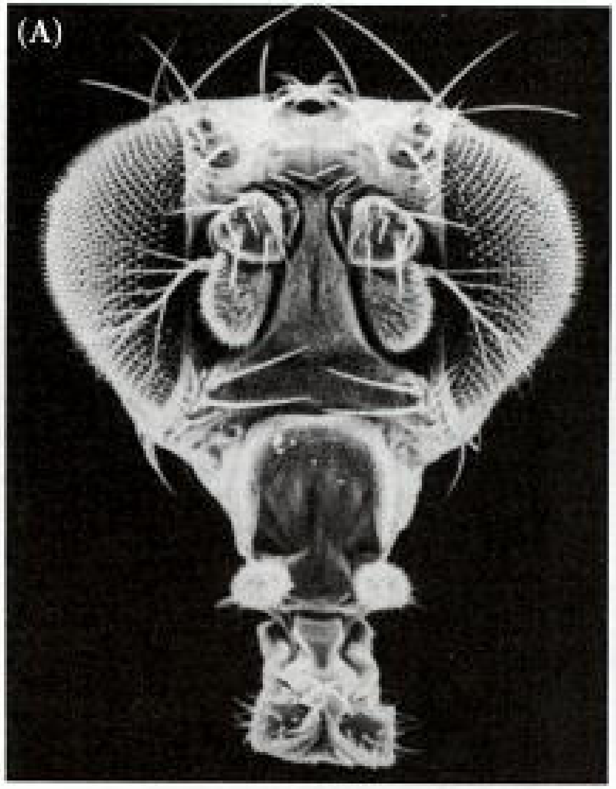


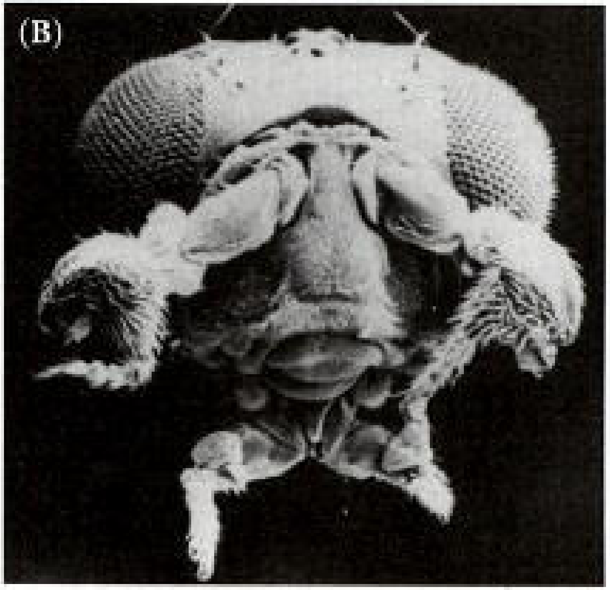
A1
A2
A3
A4
A5
A6
A7
A8
T1
T2
T3
Antennapedia complex
bithorax complex
lab
Pb
Dfd
Scr
Antp
Ubx
abdA
AbdB
14 13 12 11 10 9
1
2 3 4 5 6 7 8
labial (lab)
Deformed (Dfd)
Sex combs reduced (Scr)
Antennapedia (Antp)
Ultrabithorax (Ubx)
abdominal A (abdA)
Abdominal B (AbdB)

Ultrabithorax Mutant



Antennapedia Mutation





Gap and pair-rule genes specify homeotic genes

- Antp
  - Activated by hunchback
  - Expression is limited to T2 (mid-thoracic segment)
- abdA/AbdB
  - Repressed by hunchback and kruppel
  - Expression is limited to abdomen

Bithorax Characterized Parasegments