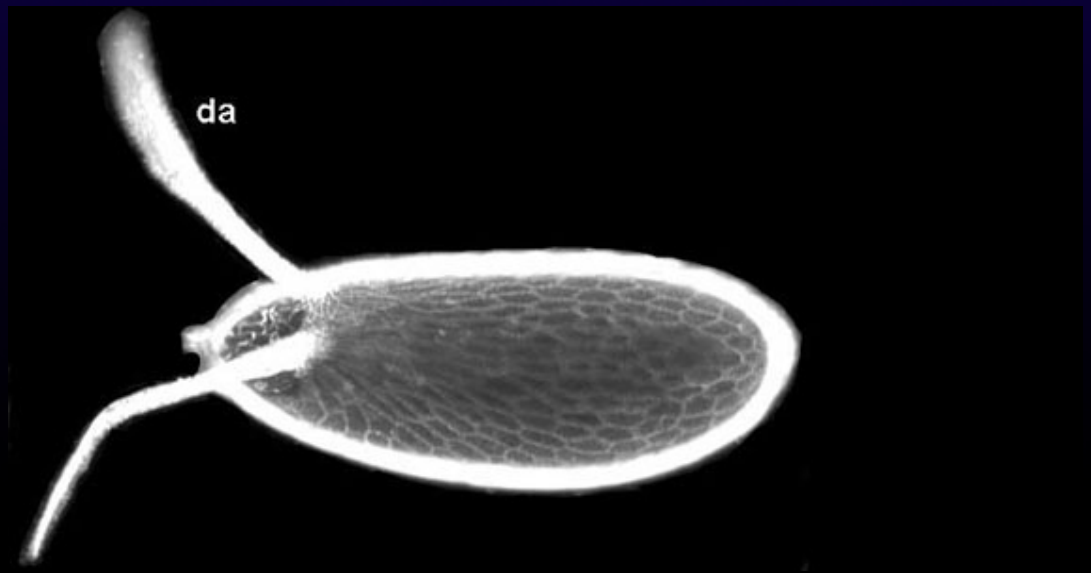
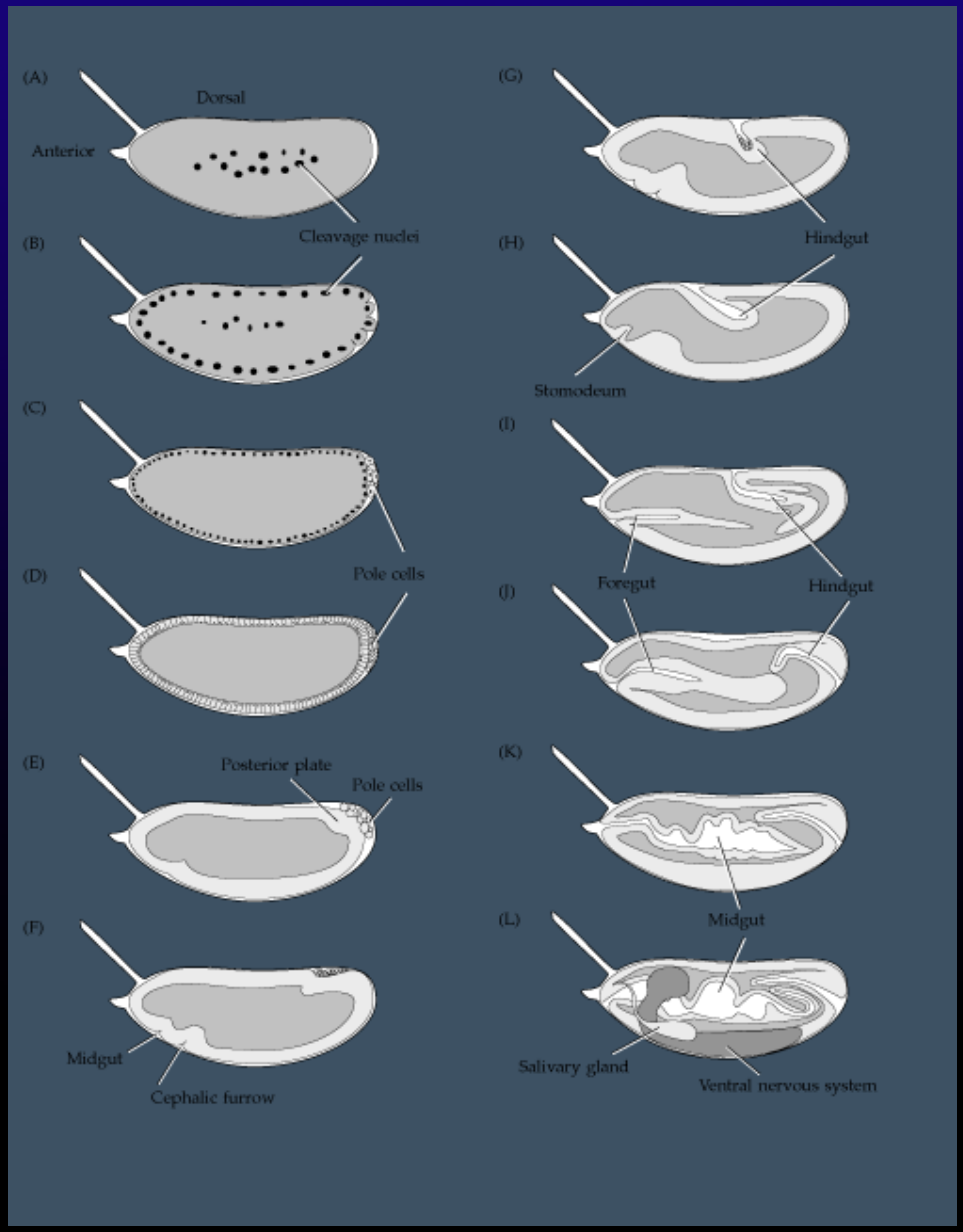
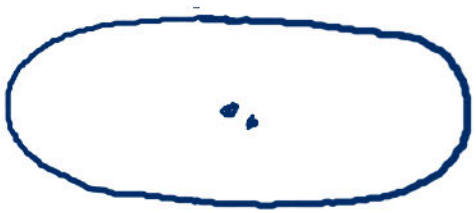
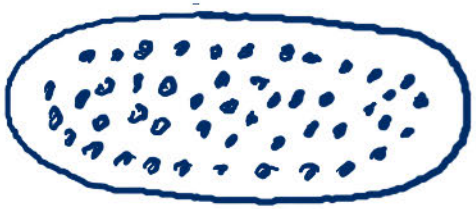
MCB 141
Developmental Biology

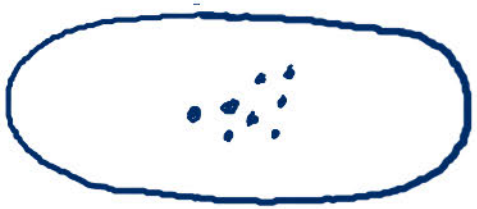
Jan. 29, 2015

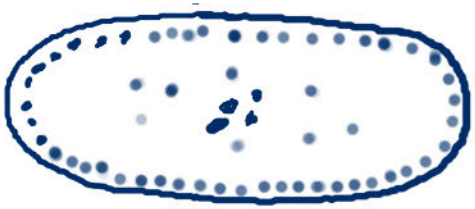

da

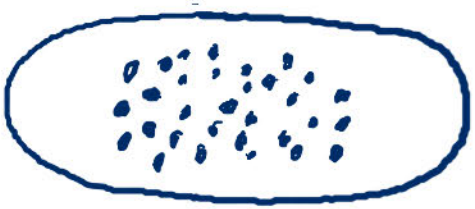

(A)
Dorsal
Anterior
Cleavage nuclei
(B)
(C)
Pole cells
(D)
(E)
Posterior plate
Pole cells
(F)
Midgut
Cephalic furrow
(G)
Hindgut
(H)
Stomodeum
(I)
Foregut
Hindgut
(J)
(K)
Midgut
(L)
Salivary gland
Ventral nervous system

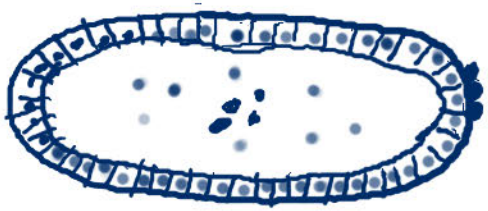


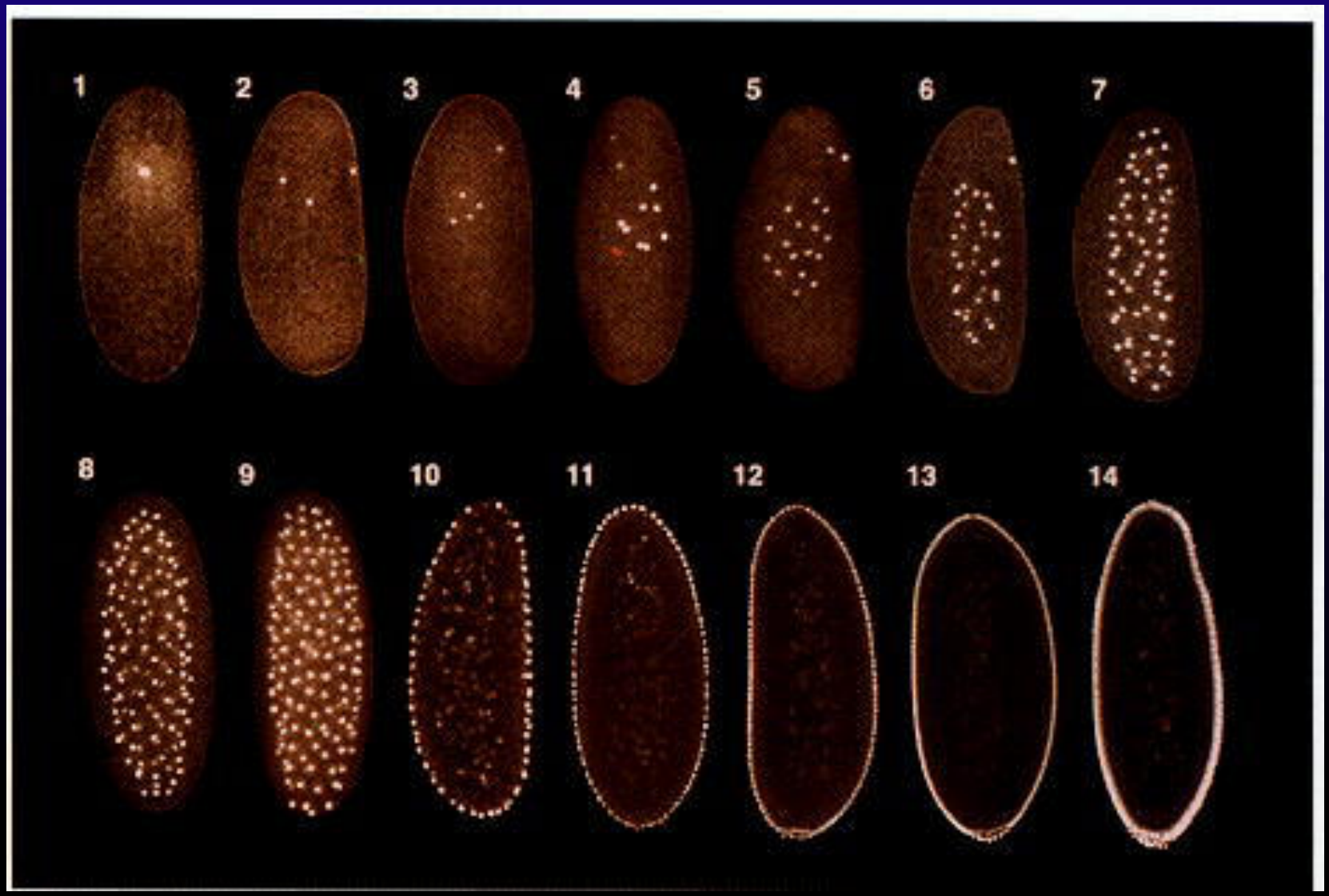


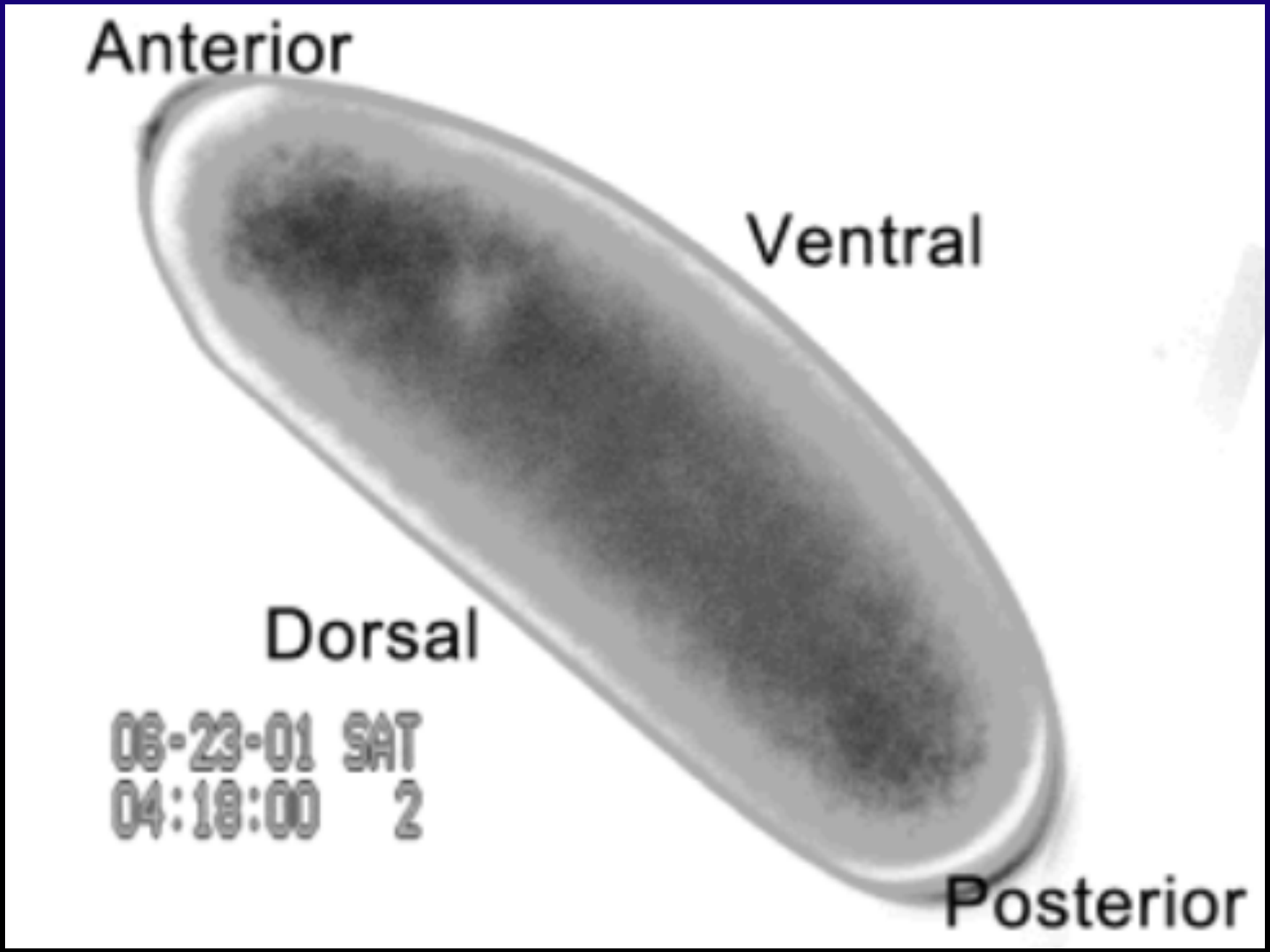


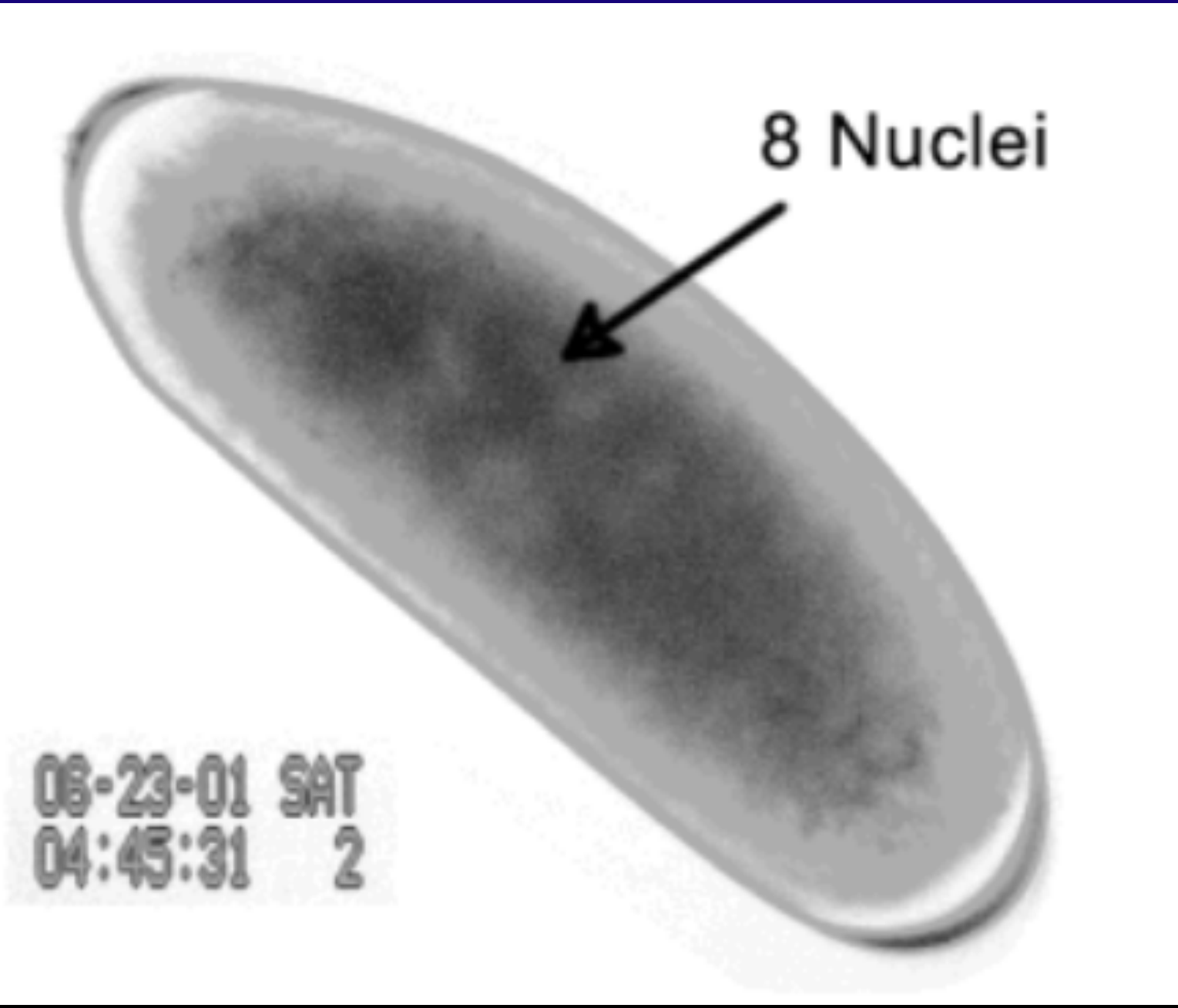


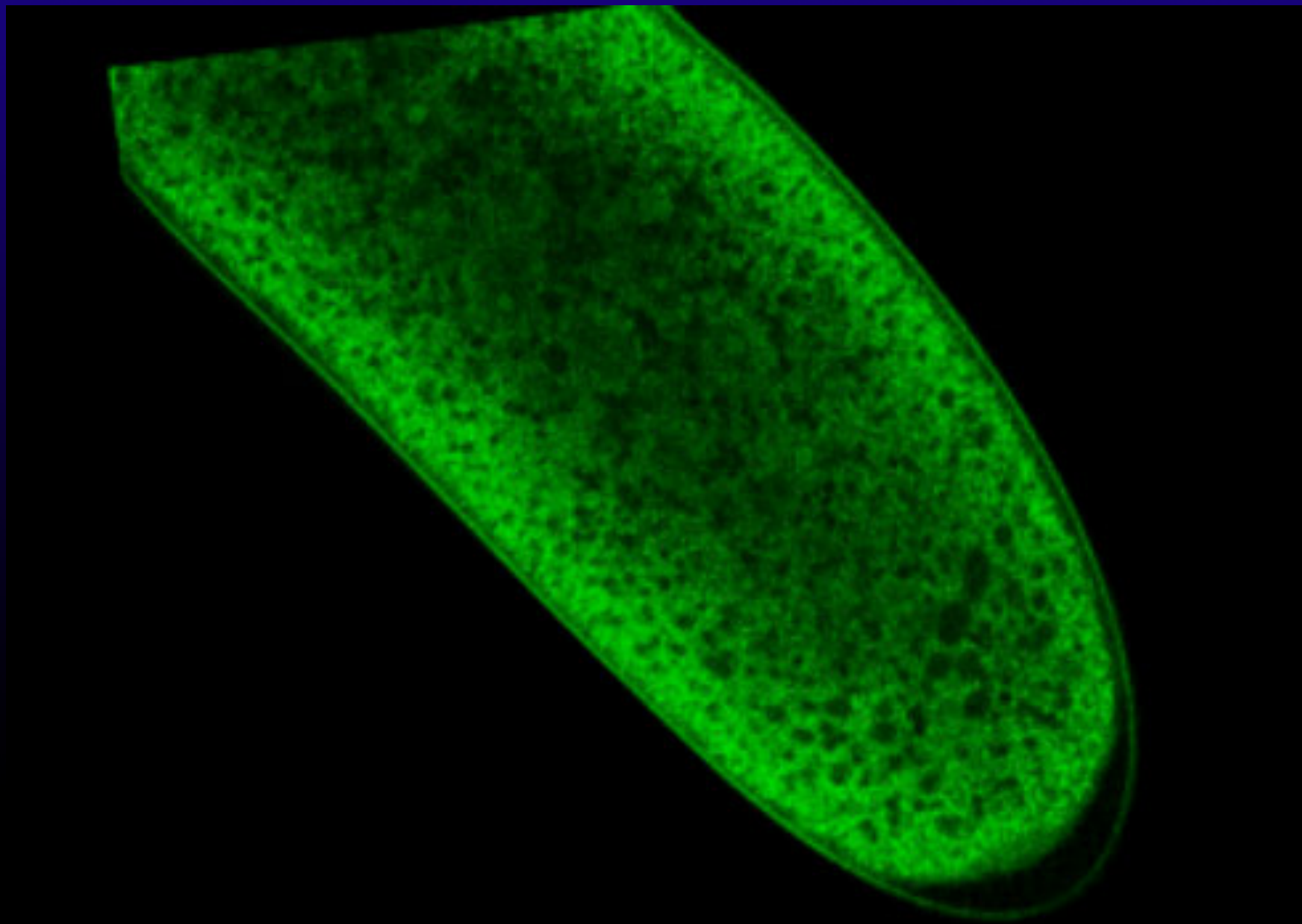


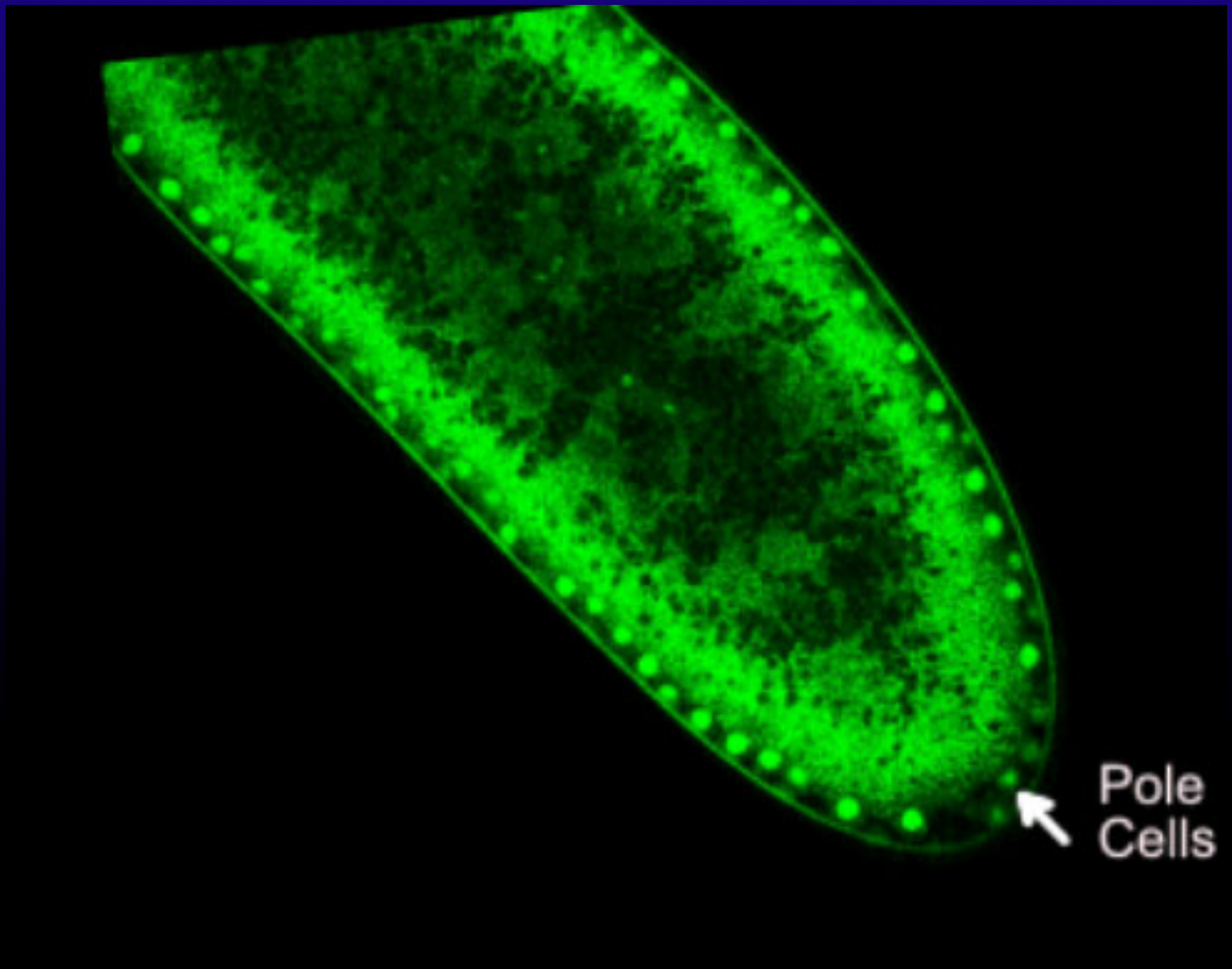


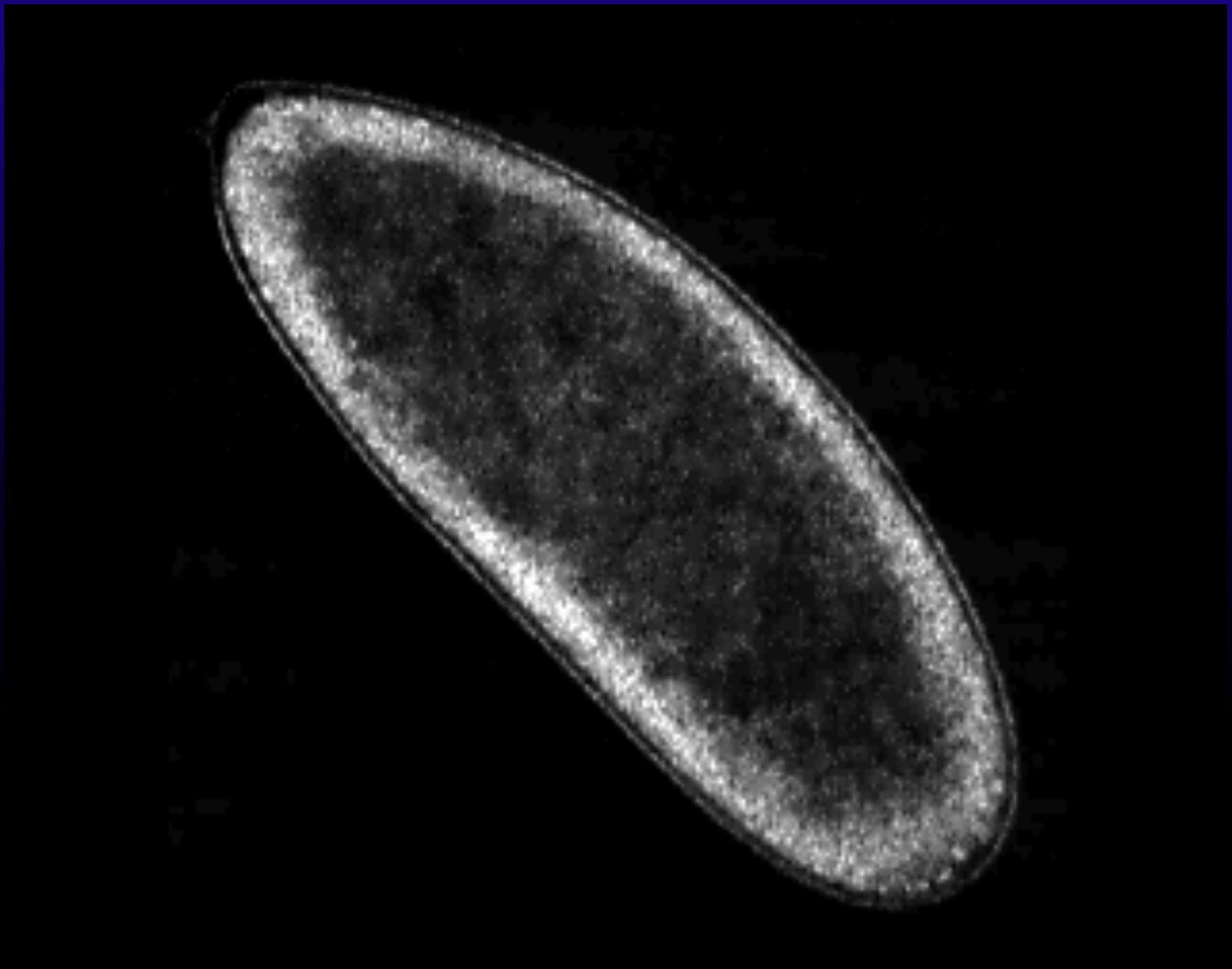

1
2
3
4
5
6
7
8
9
10
11
12
13
14

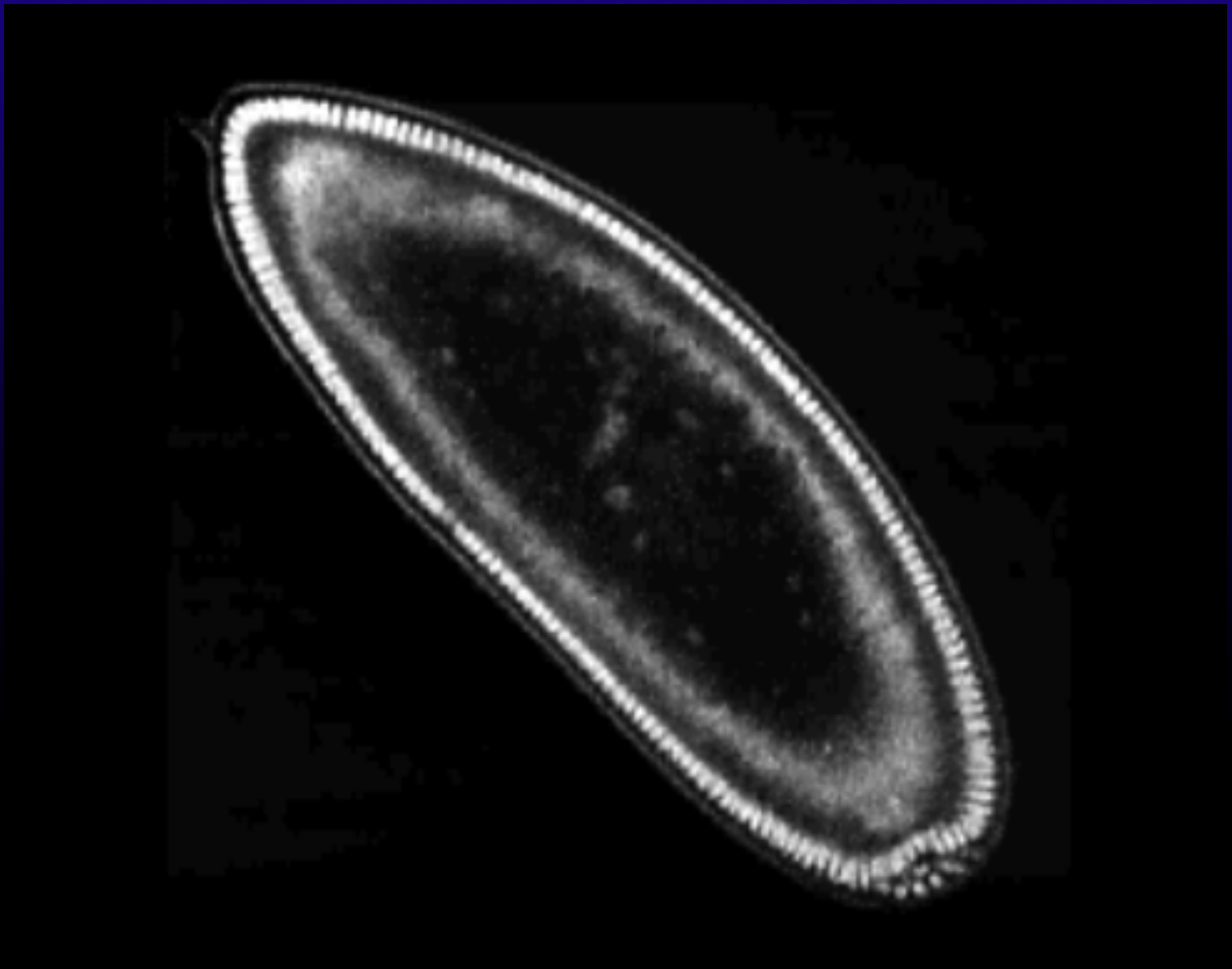

Anterior
Ventral
Dorsal
06-23-01 SAT
04:18:00 2
Posterior

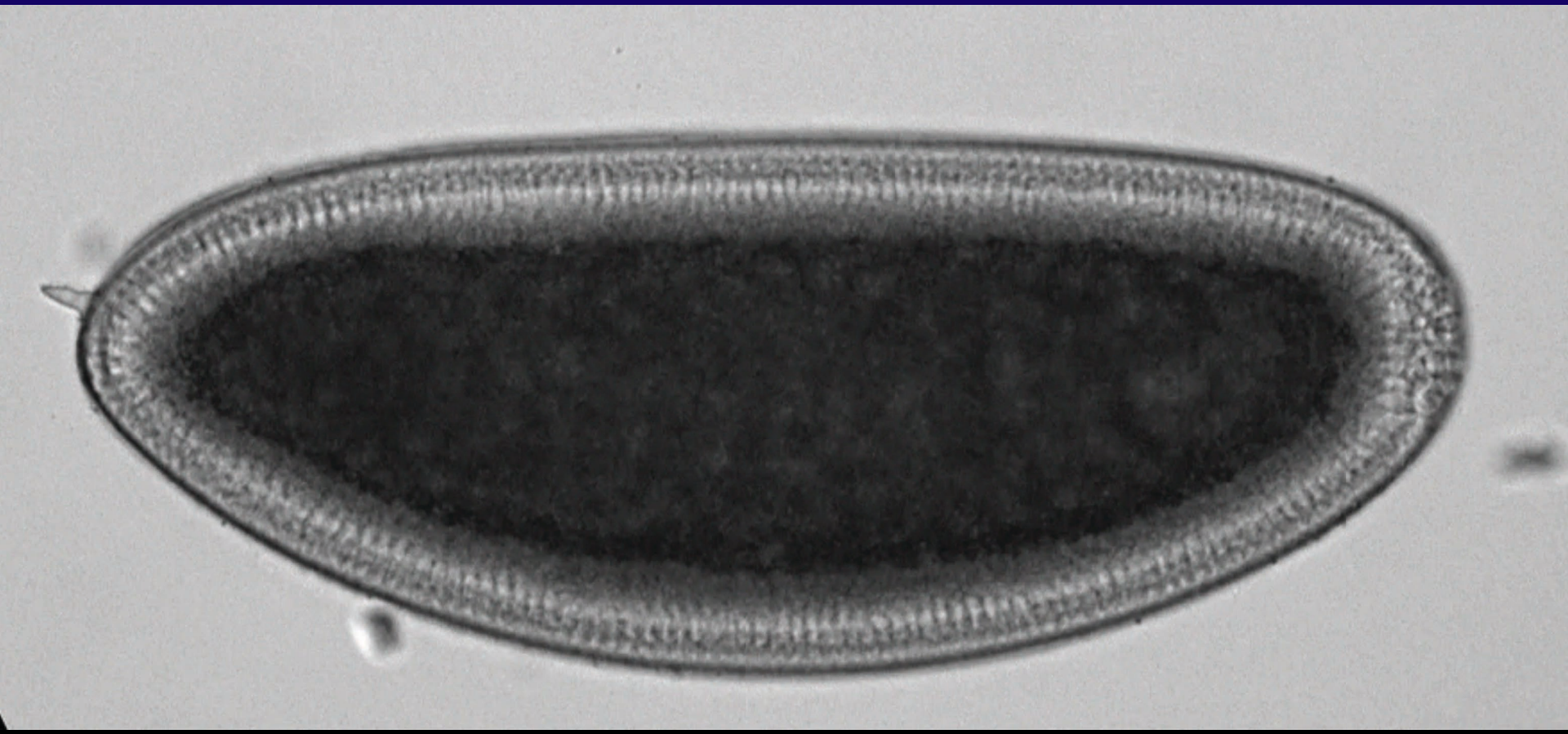

8 Nuclei
06-23-01 SAT
04:45:31 2

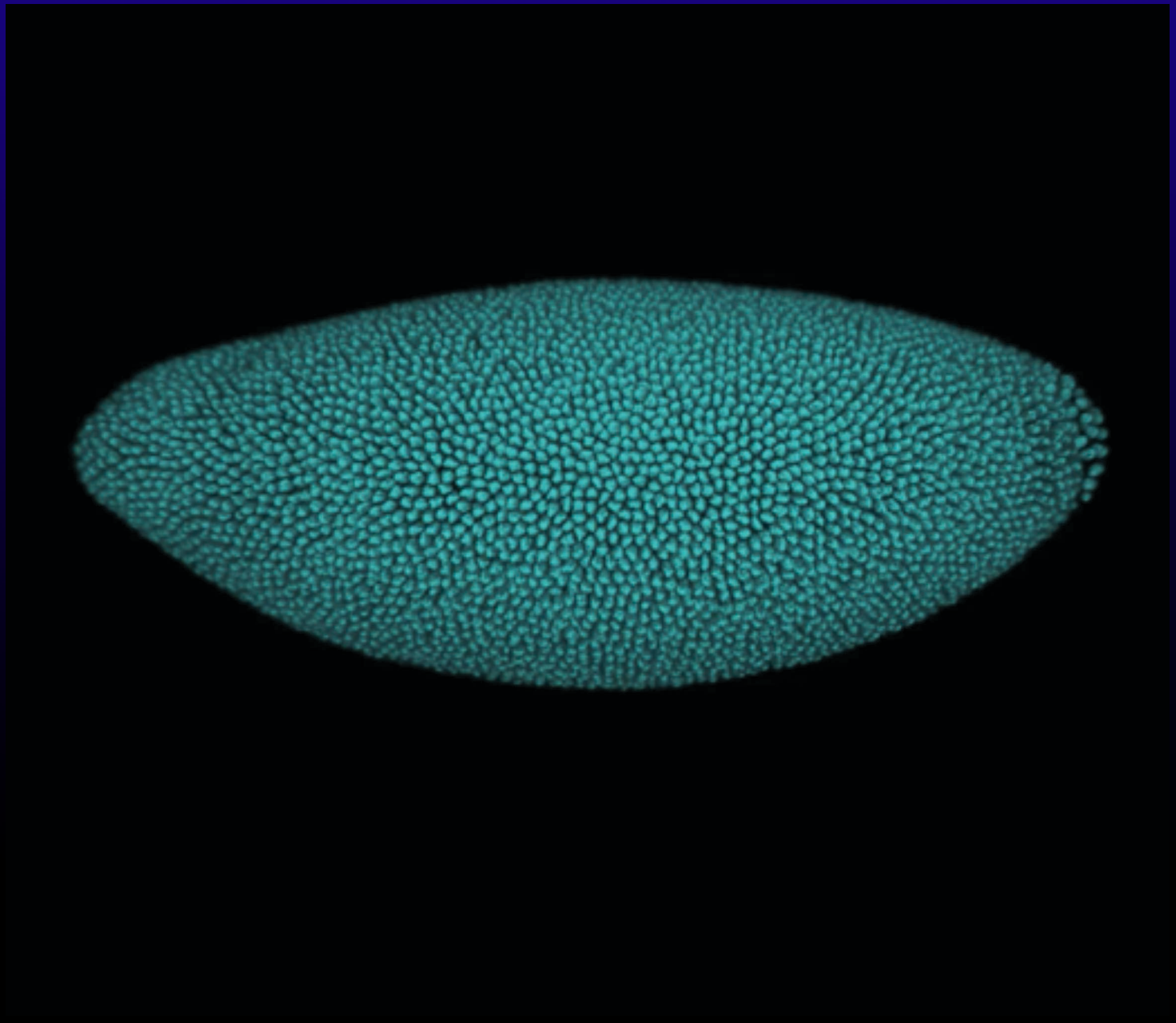


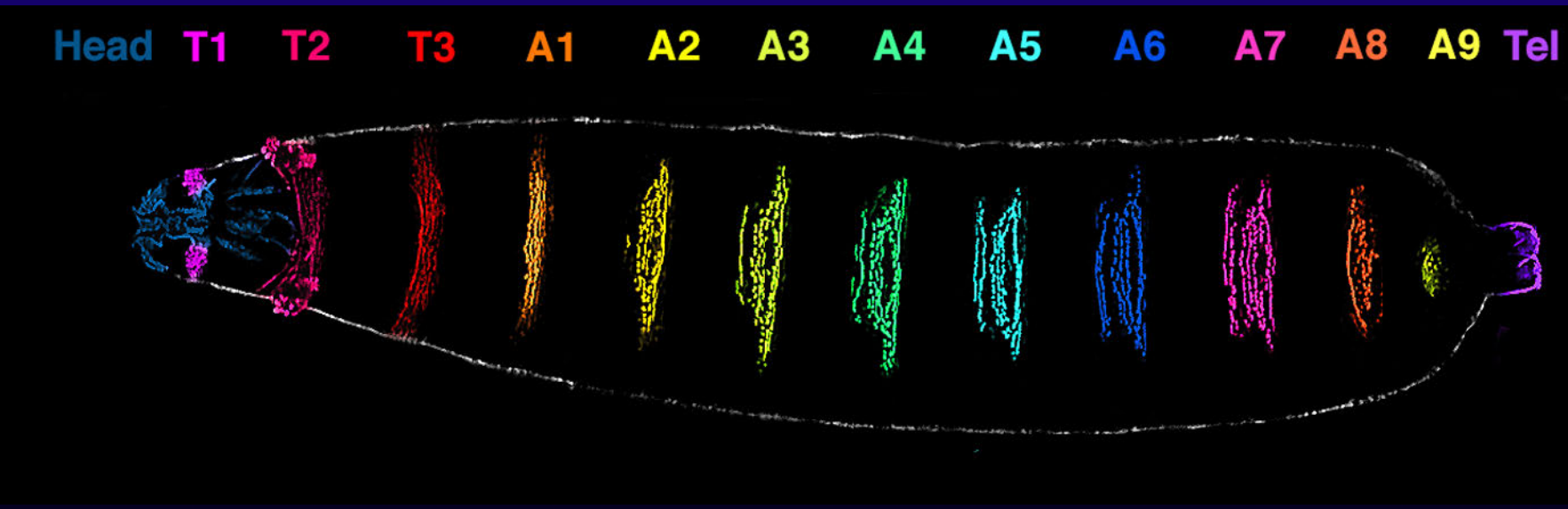

Pole
Cells







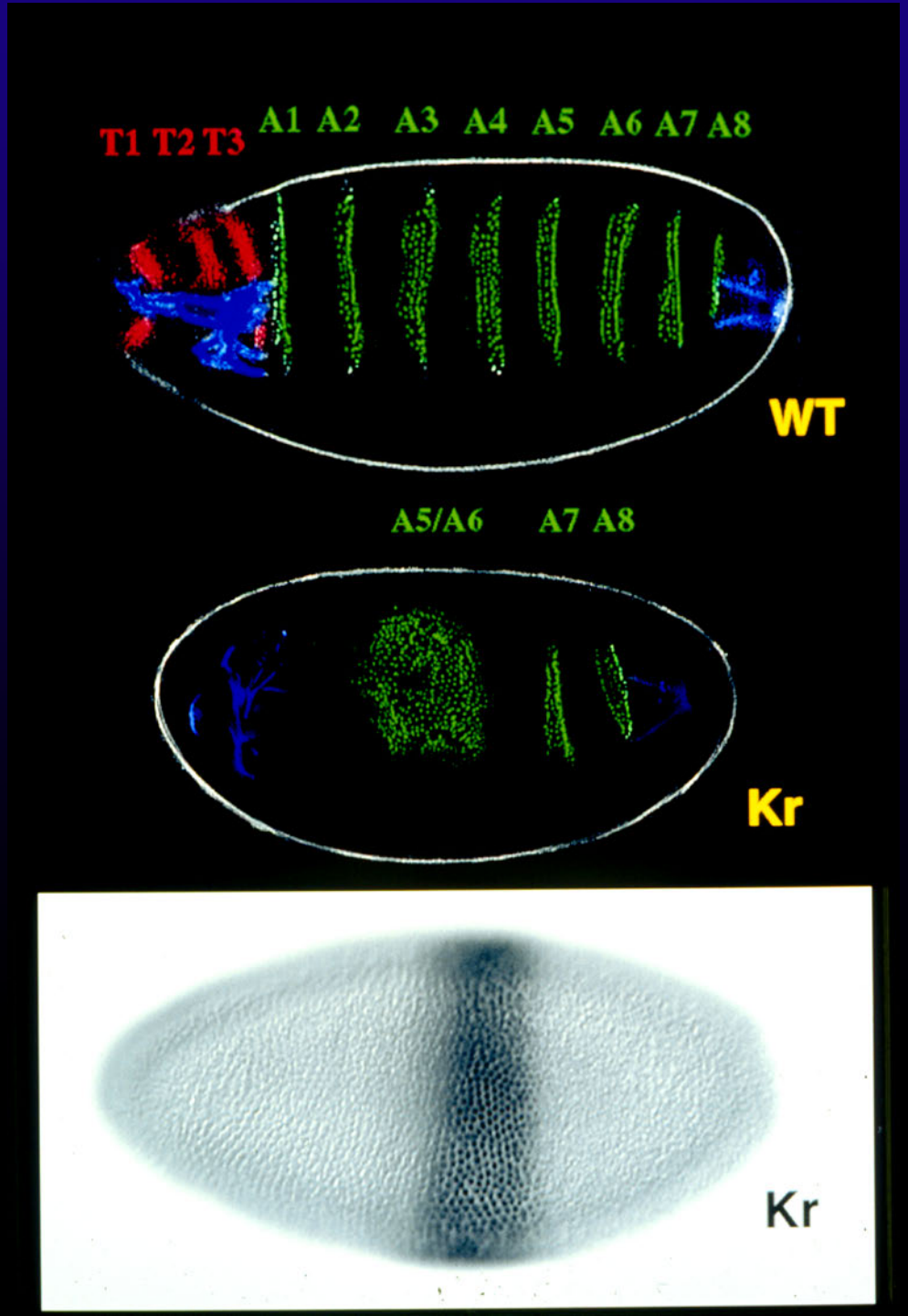



Head
T1
T2
T3
A1
A2
A3
A4
A5
A6
A7
A8
A9
Tel

Gap mutants

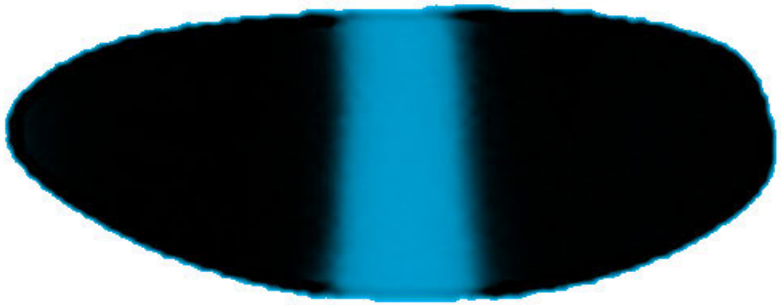
large contiguous deletions

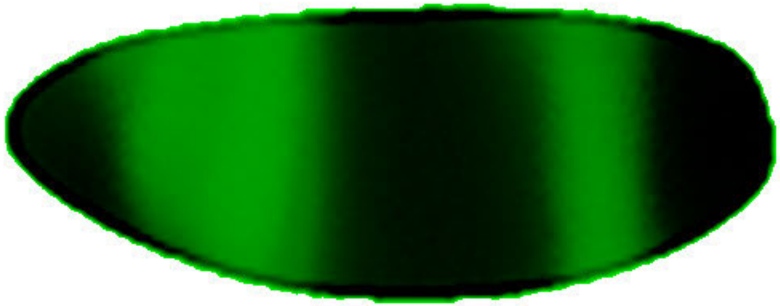
Krüppel
giant
knirps
hunchback



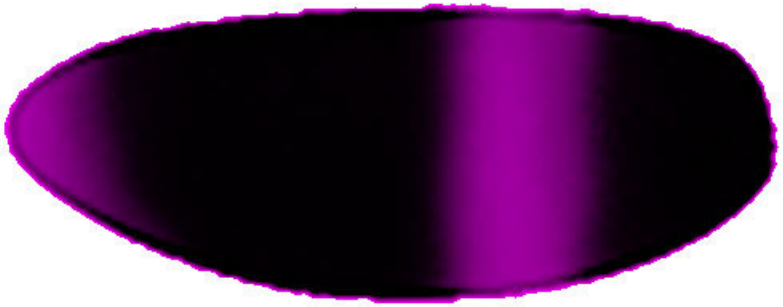
Hb

Kr





Gt

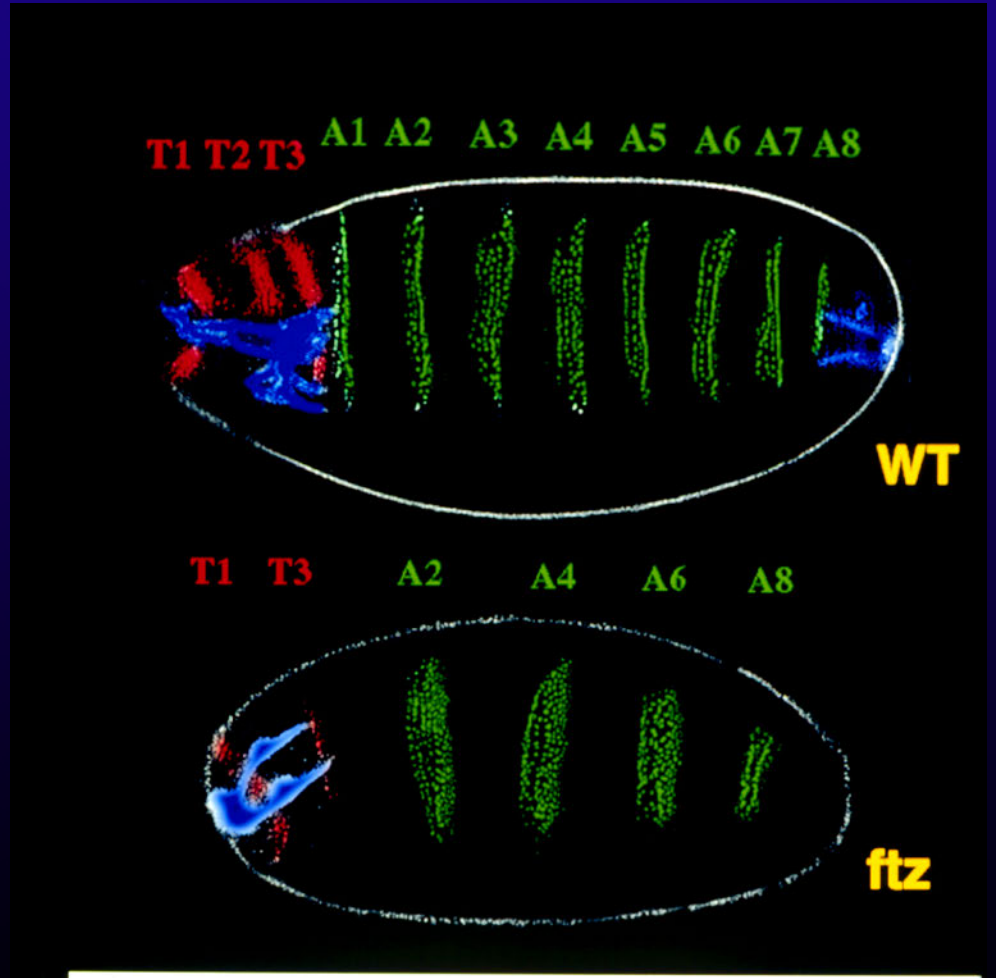


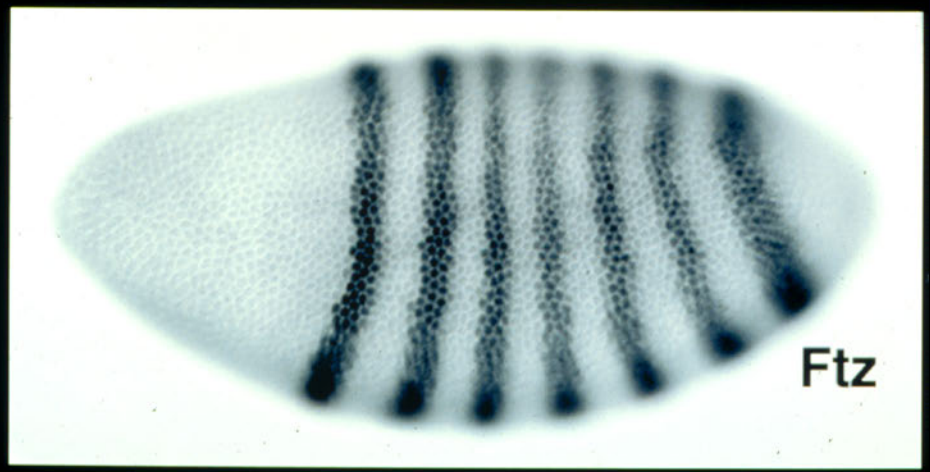
Kni

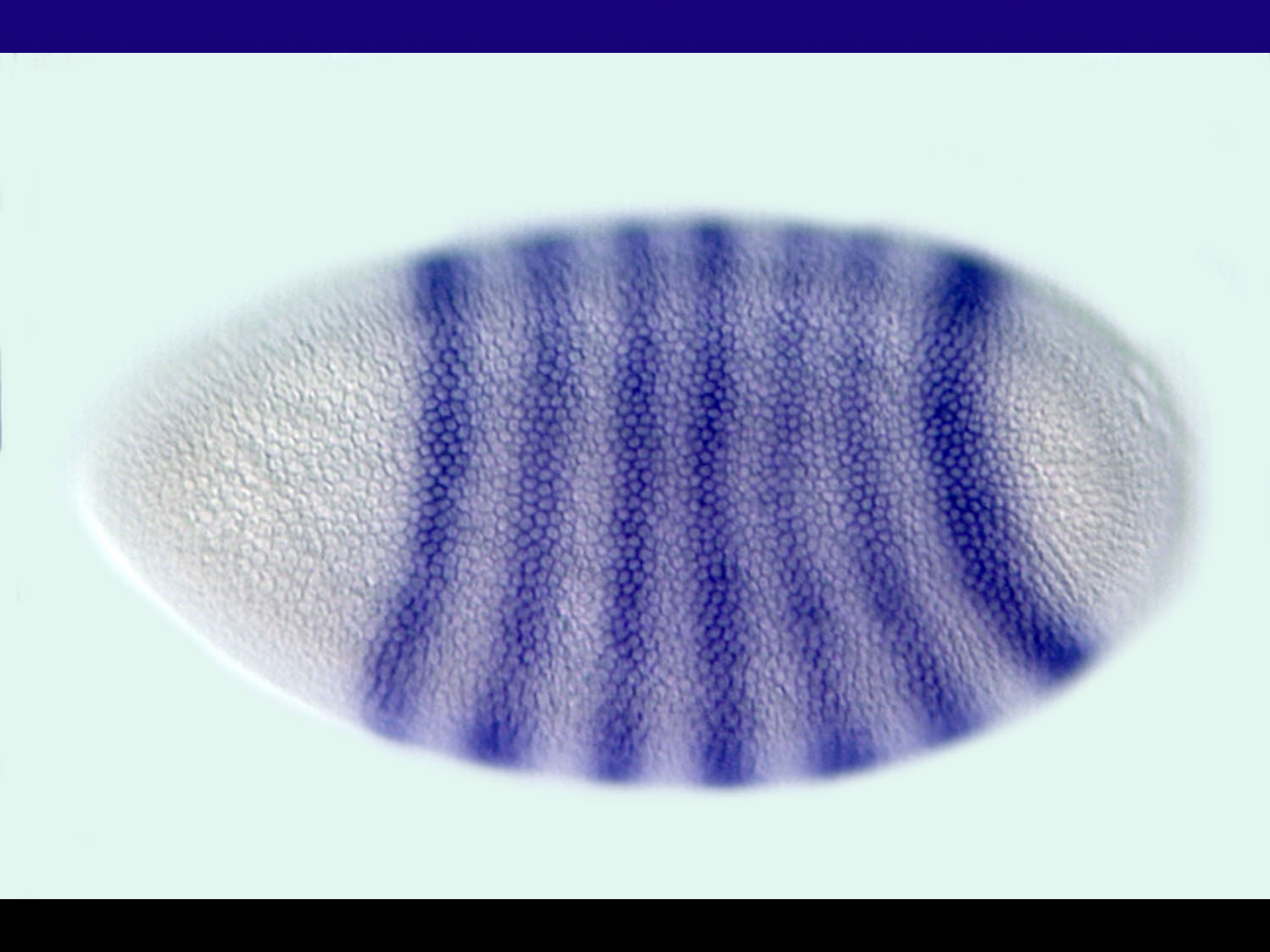
Pair-rule

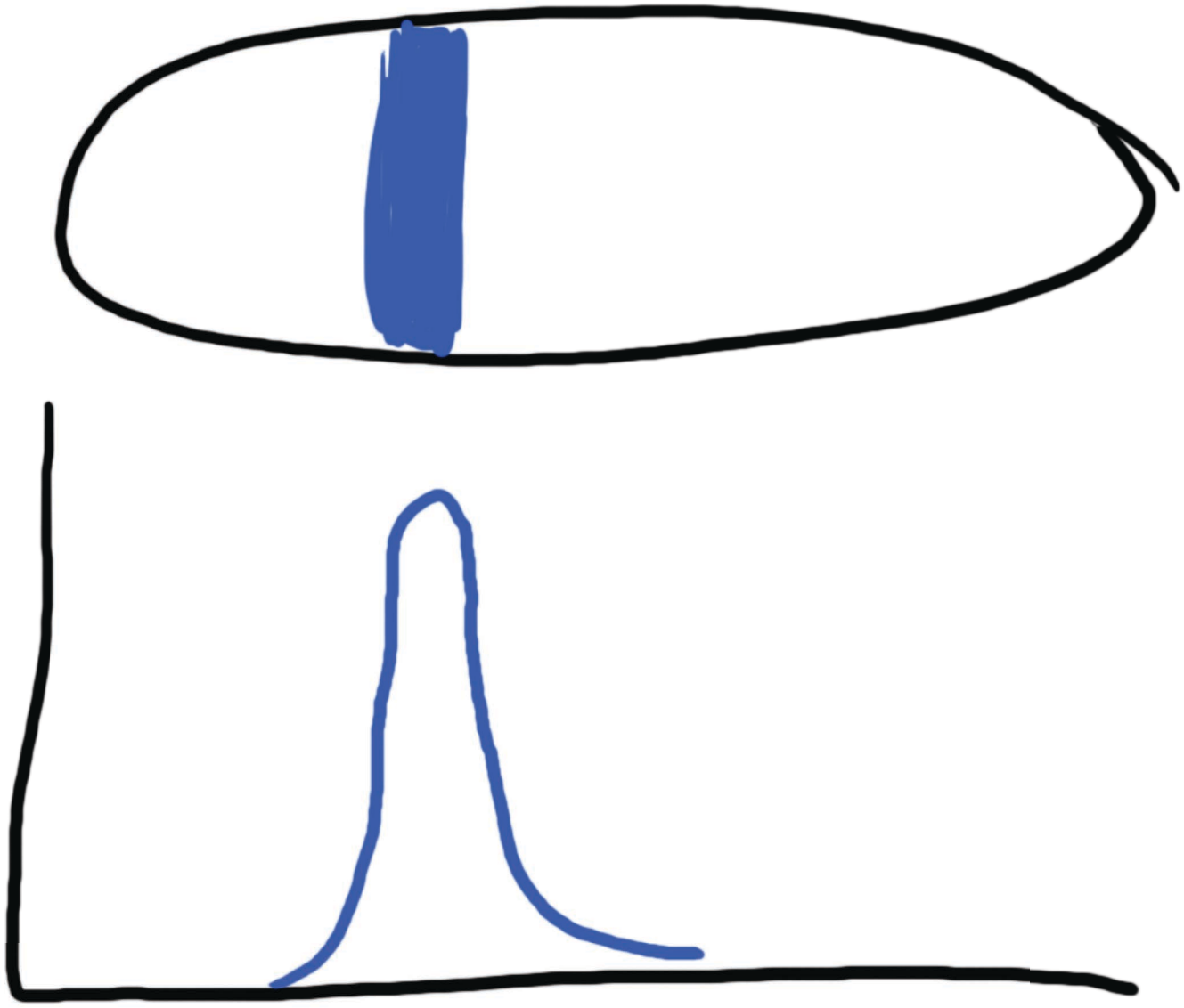
Two segment periodicity

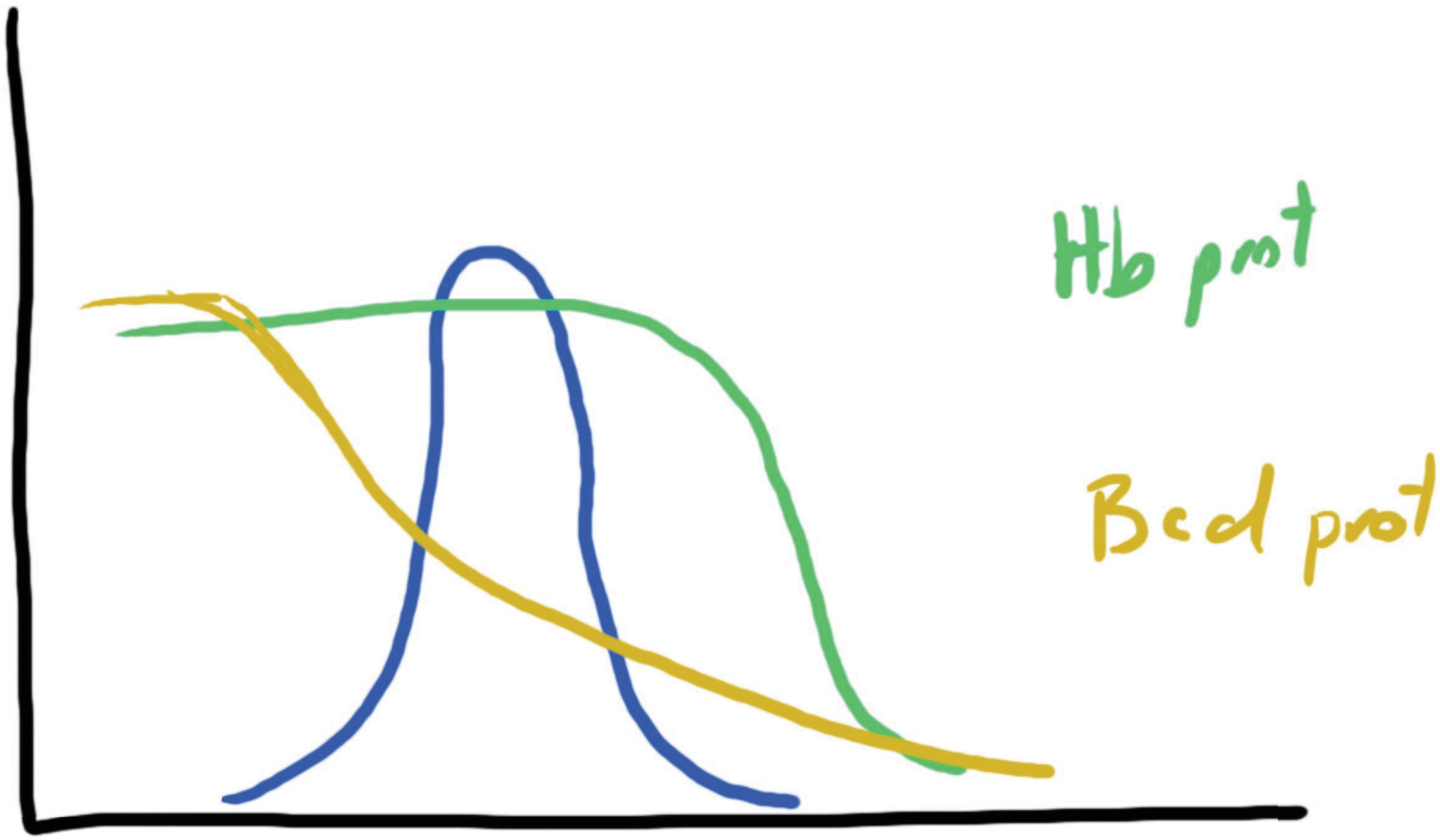
fushi tarazu
even-skipped
paired
odd-skipped
runt
hairy
odd-paired
sloppy paired

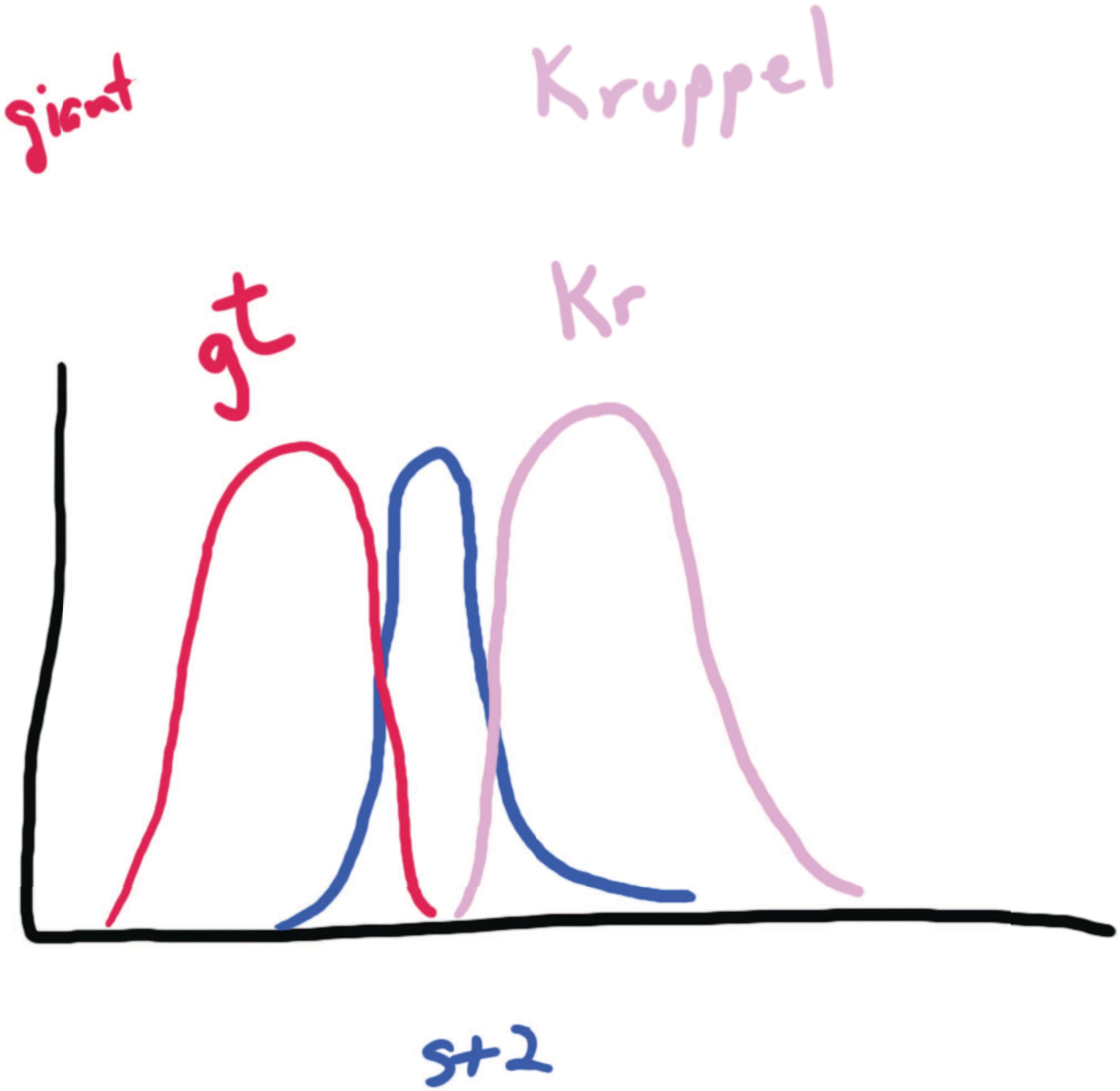


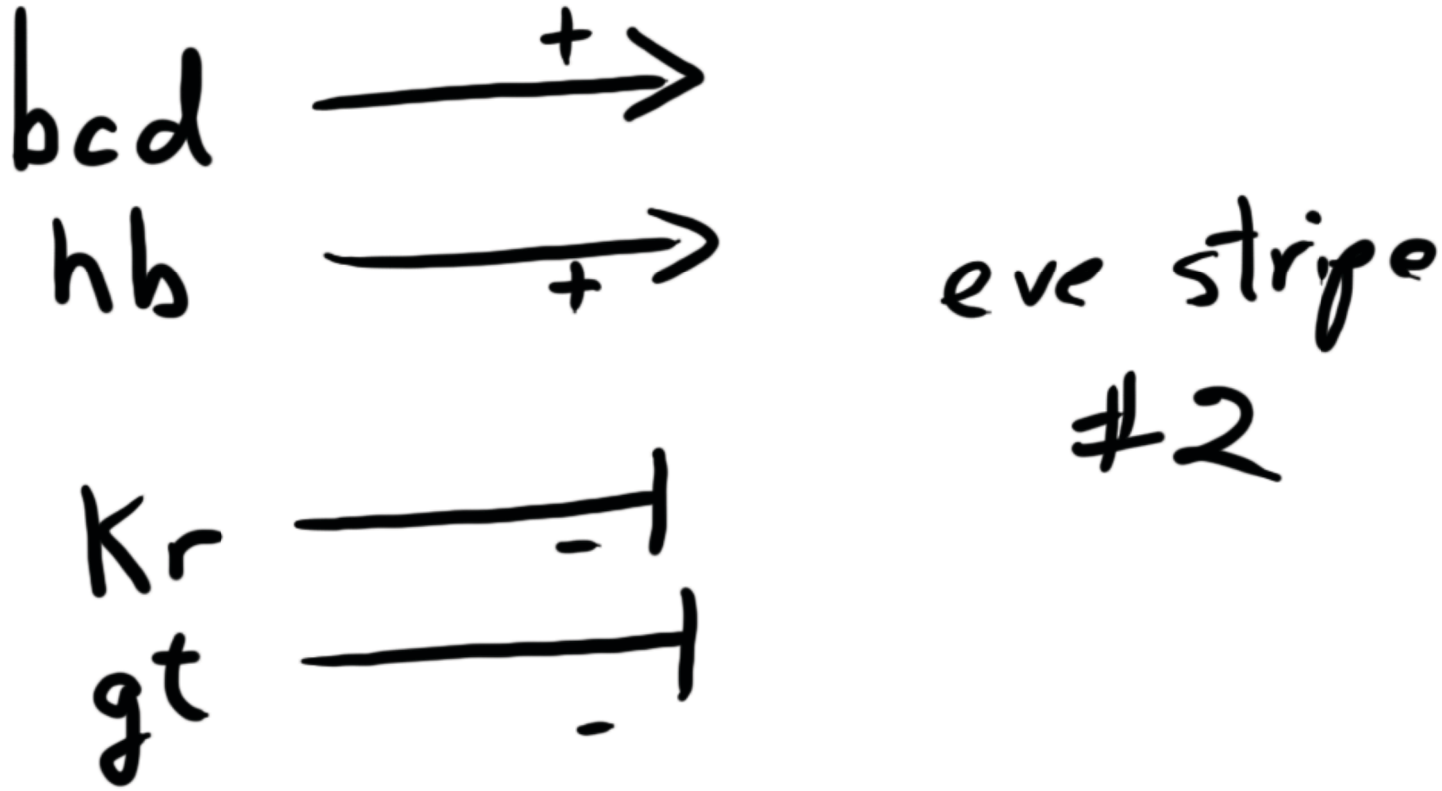


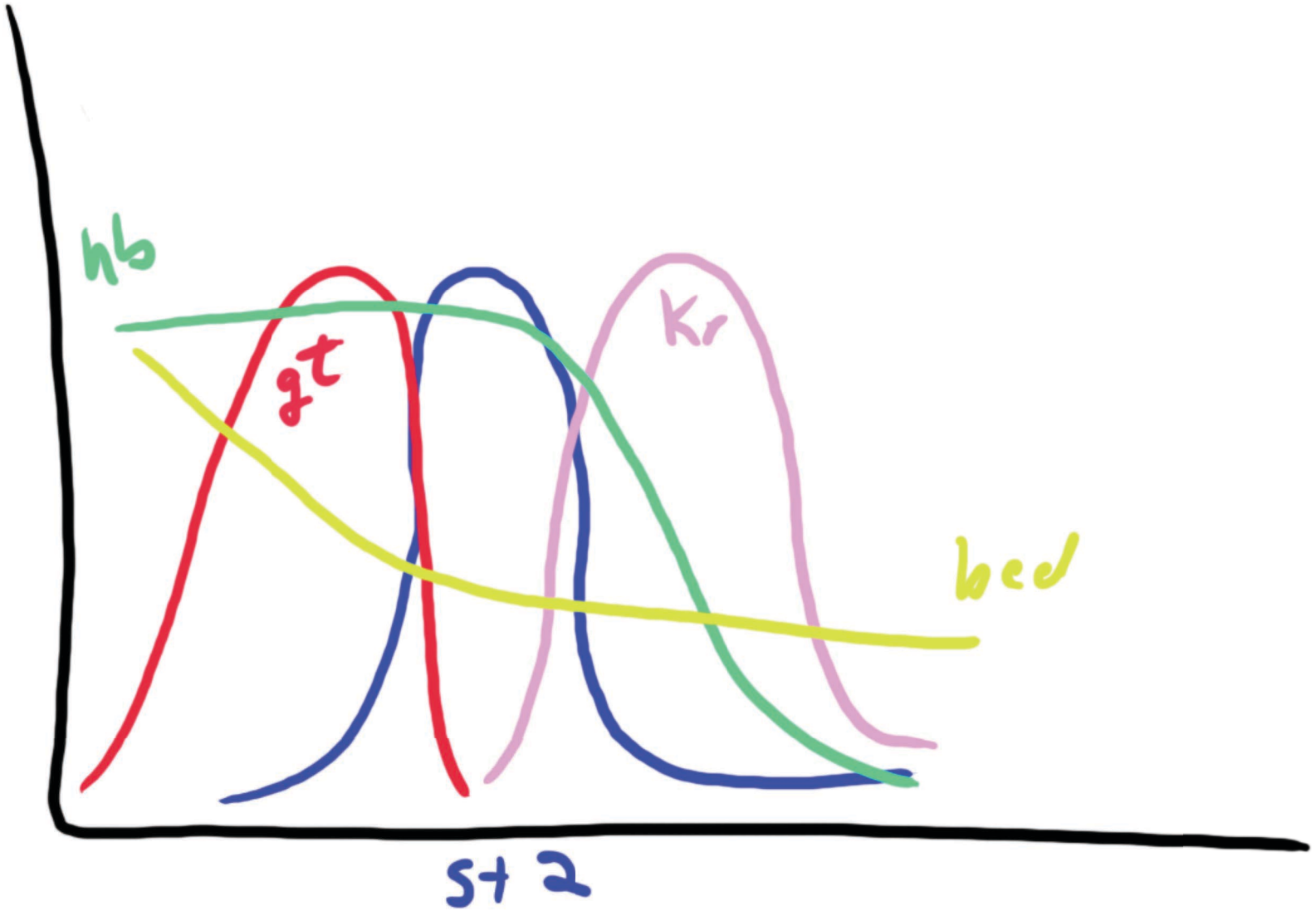


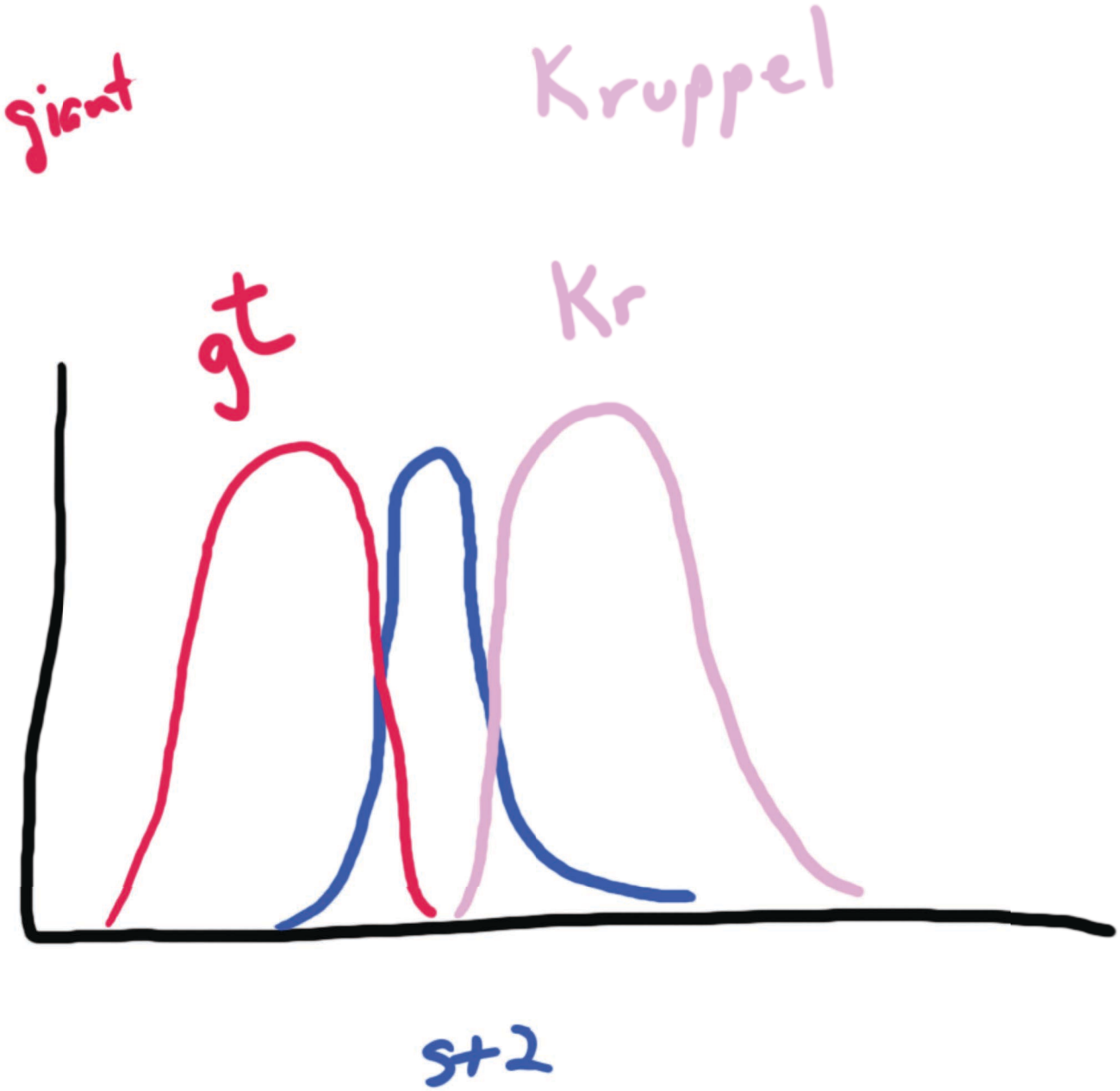

s+2


Hb prot
Bcd prot


giant
Kruppel
gt
Kr
s+2


bcd
+
hb
+
Kr
-
gt
-
eve stripe
#2


hb
gt
Kr
bed
St 2

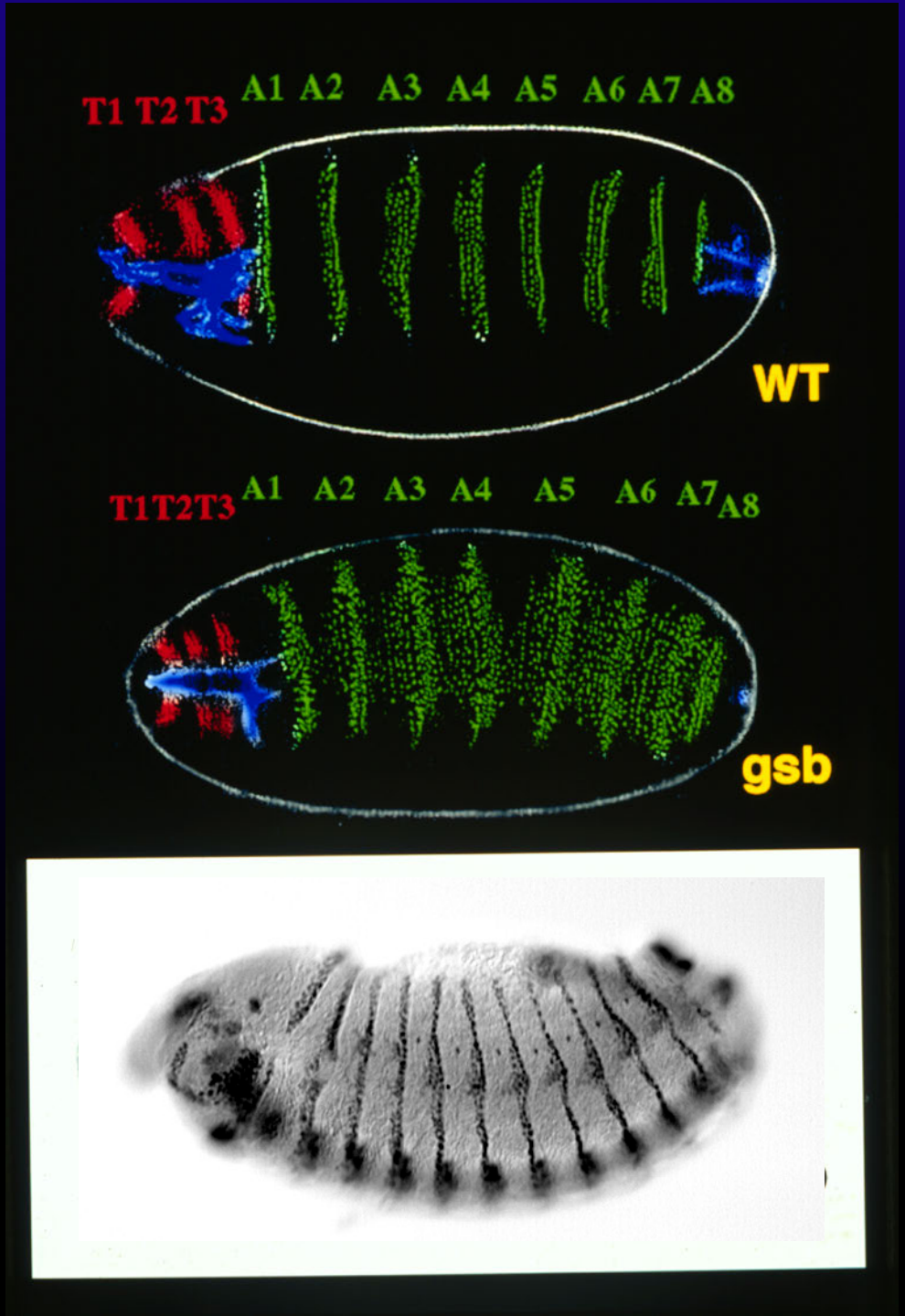

giant
Kruppel
gt
Kr
s+2

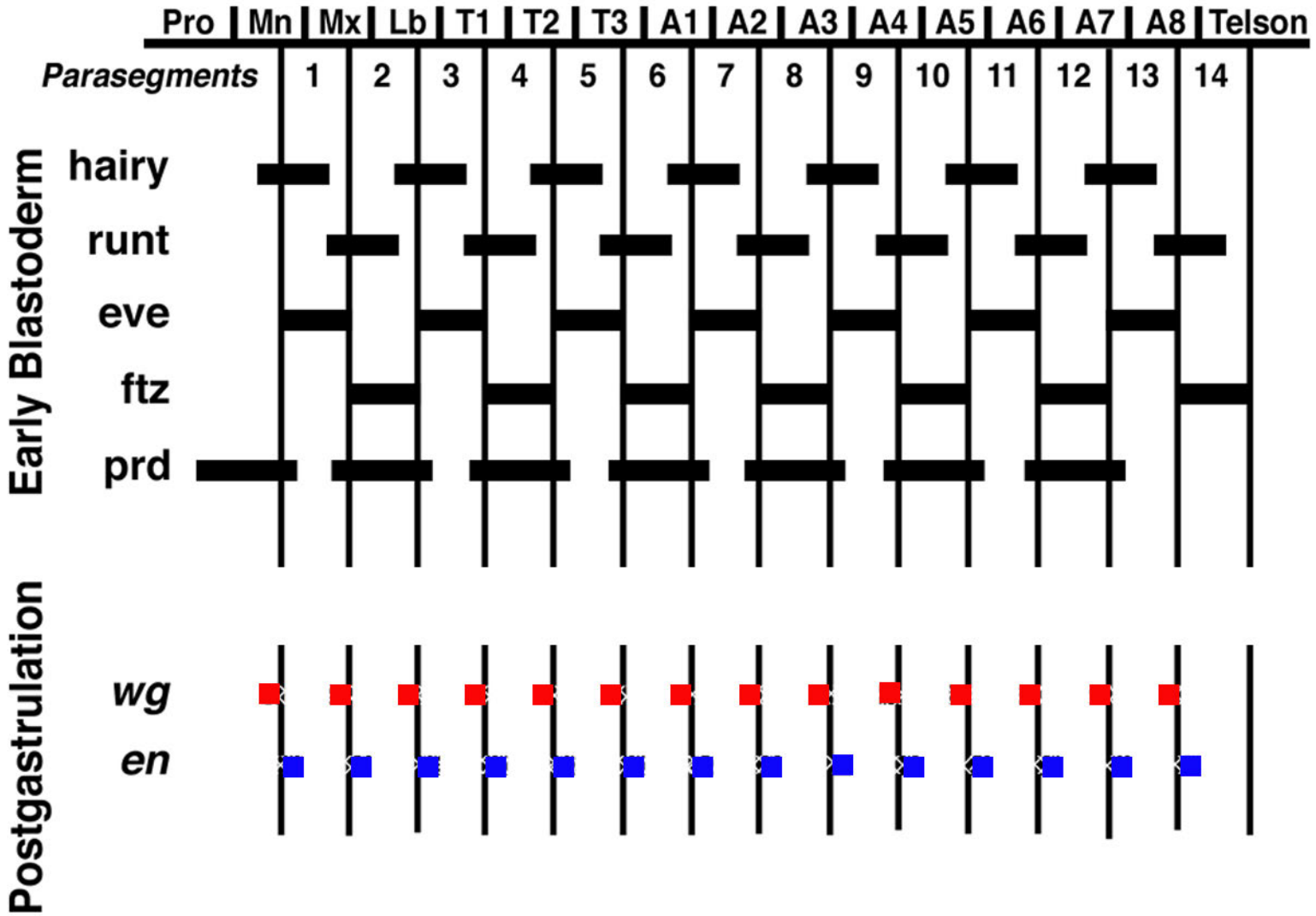
Segment polarity

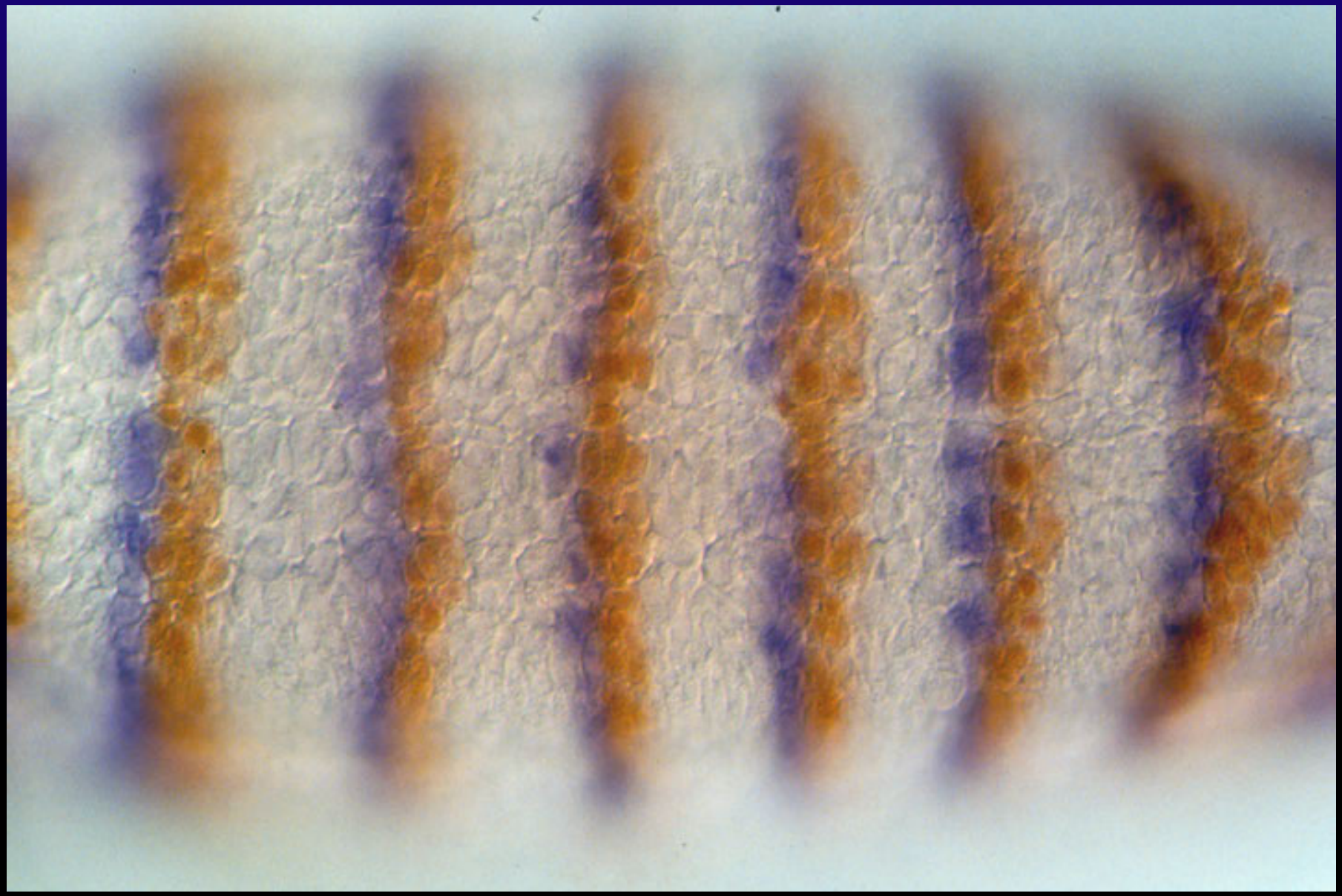
Deletions/duplications in every segment

gooseberry
engrailed
hedgehog
wingless
patched
armadillo

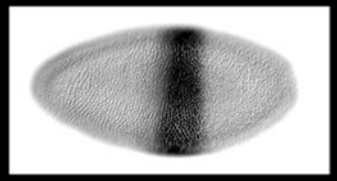
etc.....



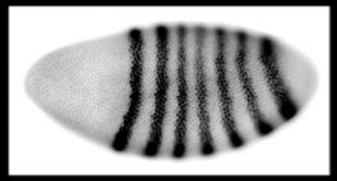

Pro
Mn
Mx
Lb
T1
T2
T3
A1
A2
A3
A4
A5
A6
A7
A8
Telson
Parasegments
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Early Blastoderm
hairy
runt
eve
ftz
prd
Postgastrulation
wg
en



zygotic gap genes



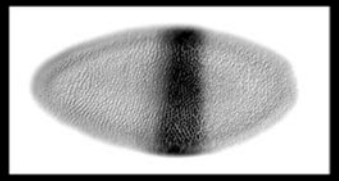
pair-rule genes



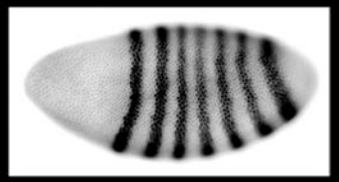
segment-polarity genes

maternal gradients

zygotic gap genes



pair-rule genes



segment-polarity genes

$\frac{ry^-}{ry^+} \times \frac{ry^-}{ry^+}$

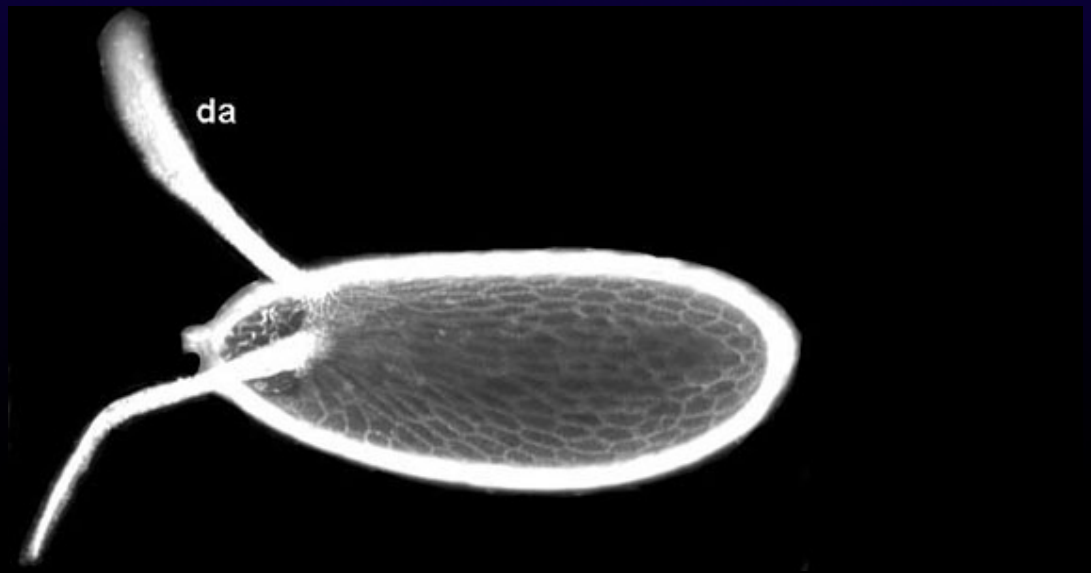
↓

$\frac{1}{4}\ \frac{ry^-}{ry^-}$ $\frac{1}{2}\ \frac{ry^-}{ry^+}$ $\frac{1}{4}\ \frac{ry^+}{ry^+}$

wt (red) eyes

3/4

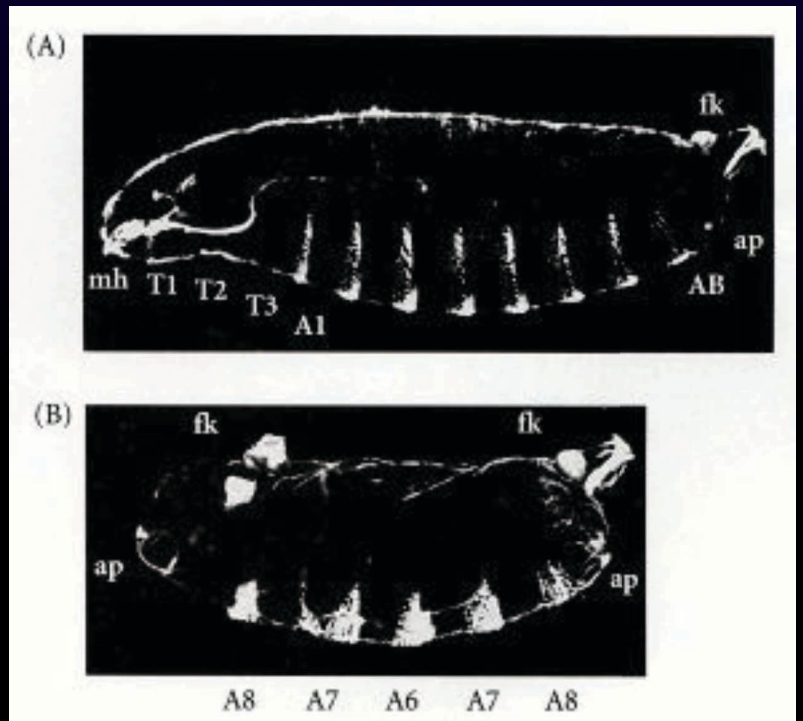
rosy eyes $\frac{1}{4}$


da

Also found maternal effect mutants

Three classes: terminal, anterior missing, posterior missing

Two key ones - bicoid (anterior missing)
and nanos (posterior) missing



Embryos from bcd-/bcd- mothers

Maternal Effect Mutations

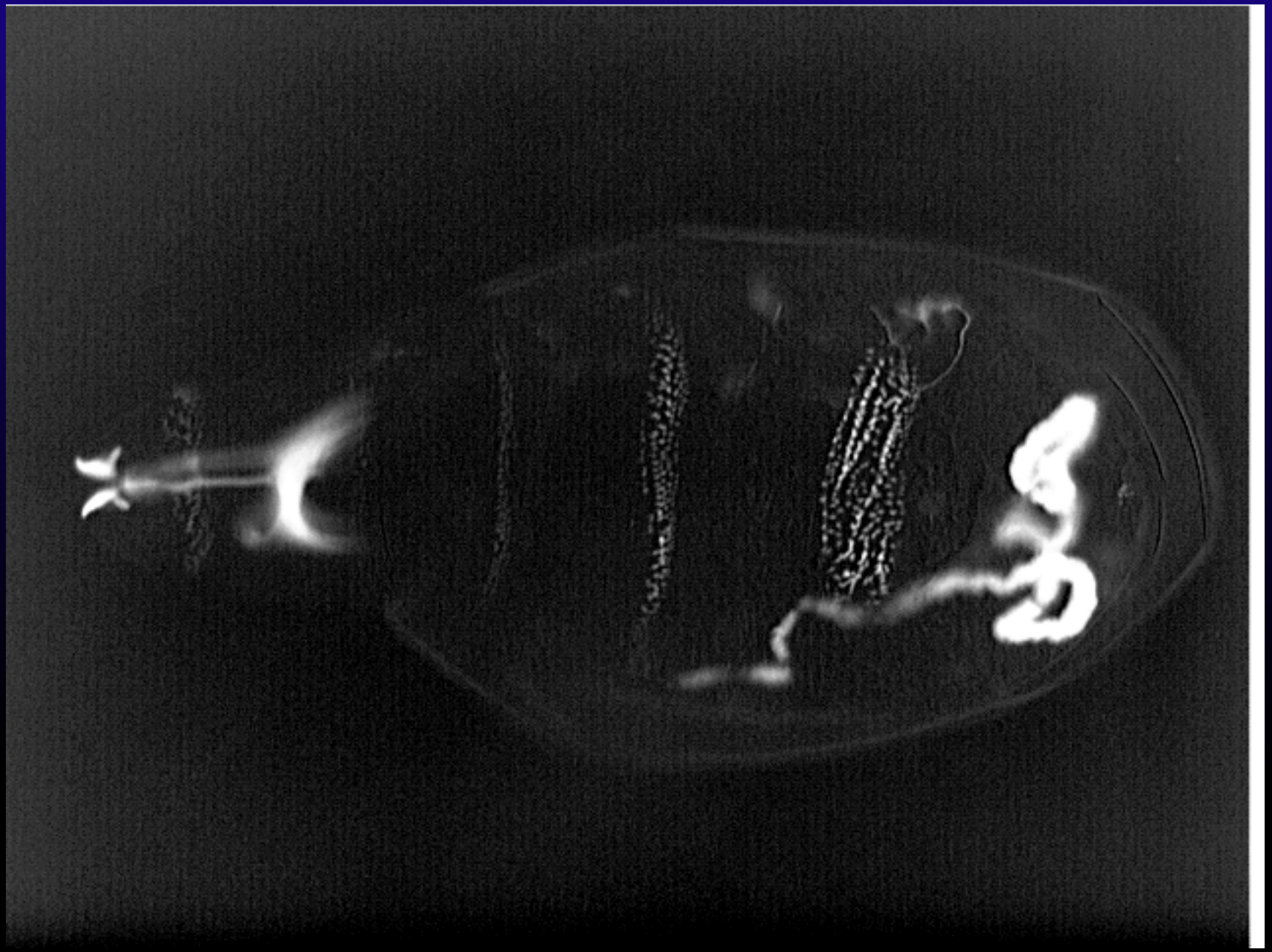
ex. bicoid

$\frac{bcd^-}{bcd^-}$ ← become normal adult flies

But

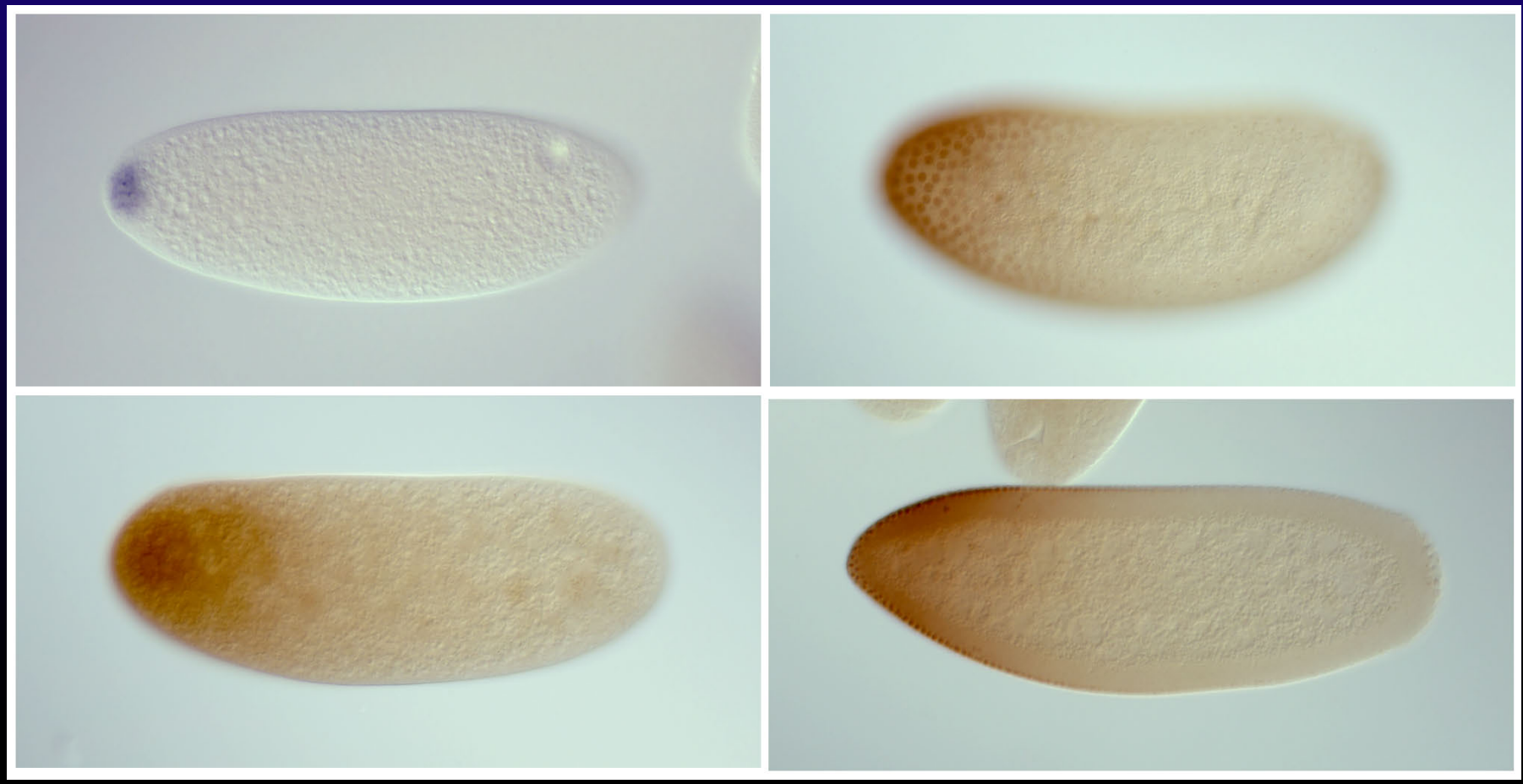
♀ $\frac{bcd^-}{bcd^-}$ → no living embryos

→ all embryos are missing anterior segments and abdomen is expanded



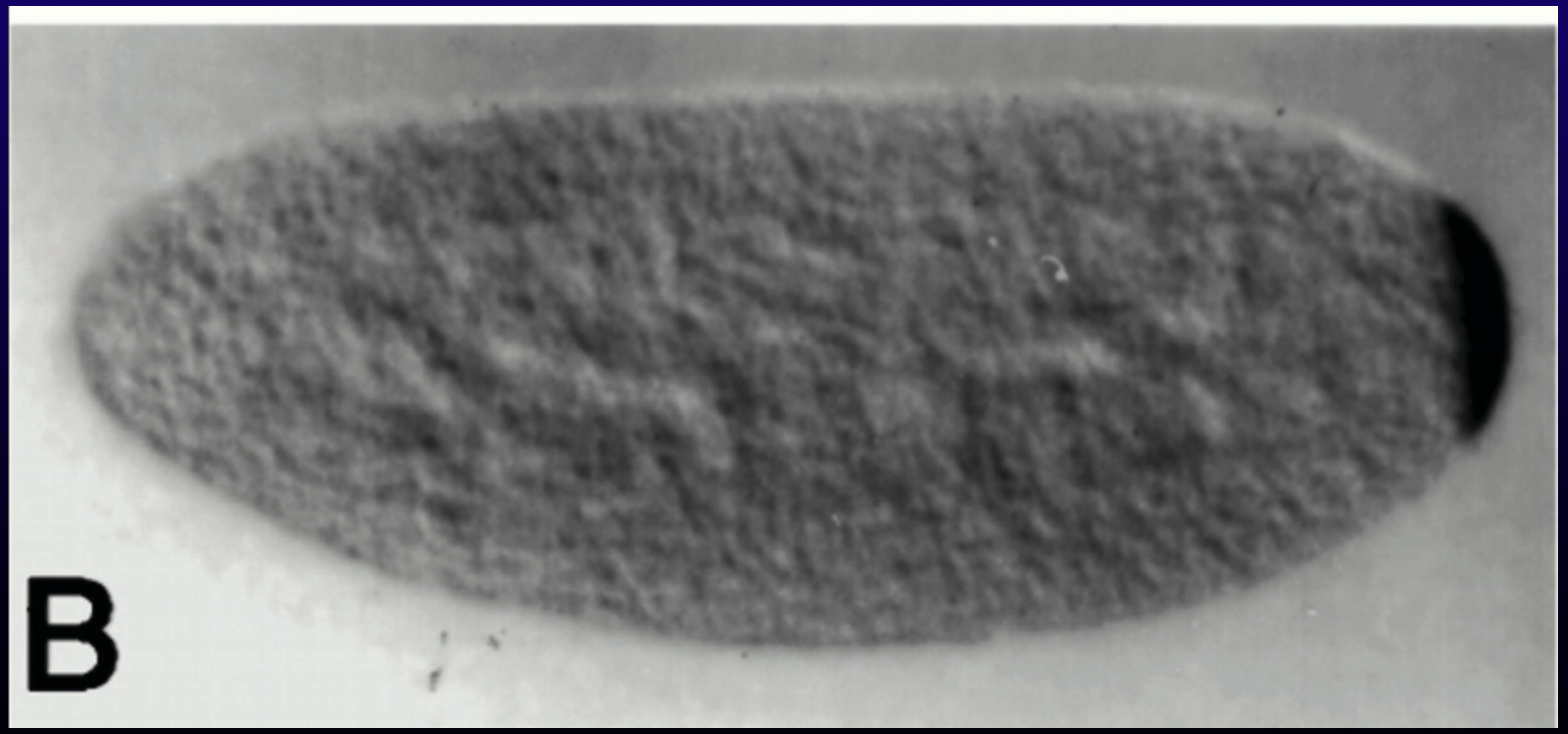
bicoid mRNA

bicoid protein

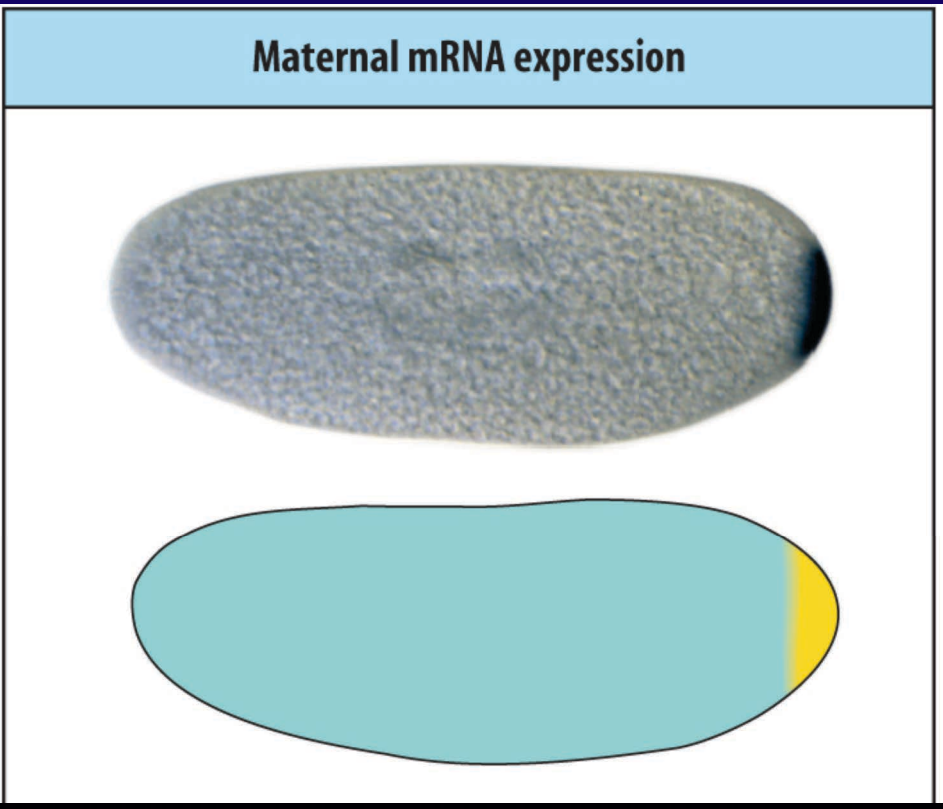


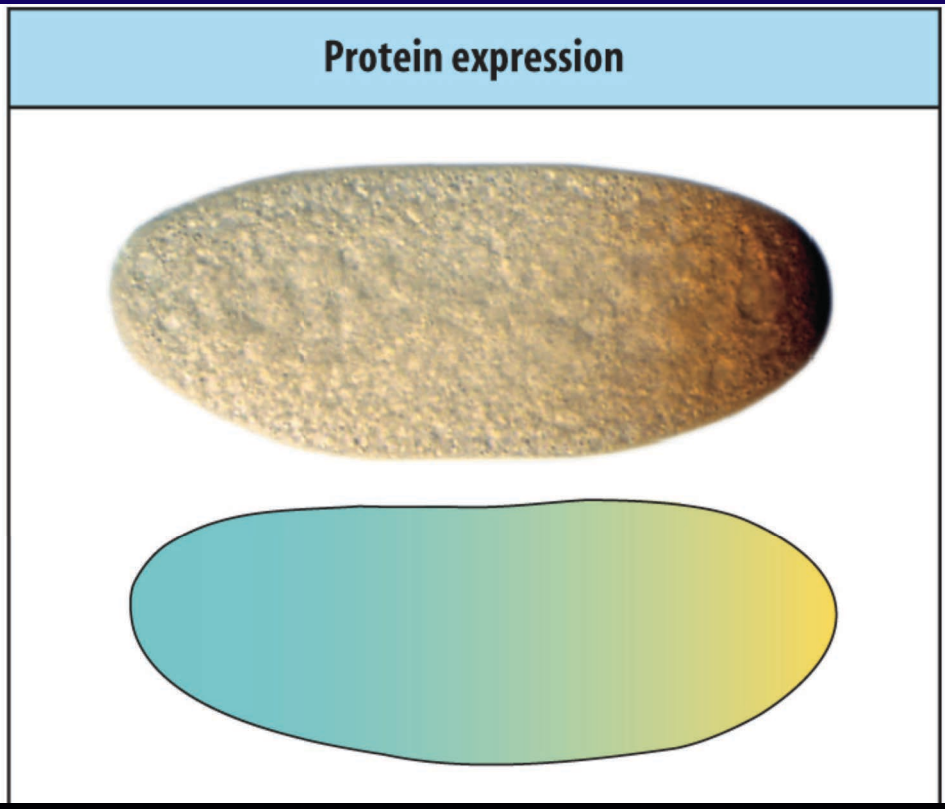
bicoid protein

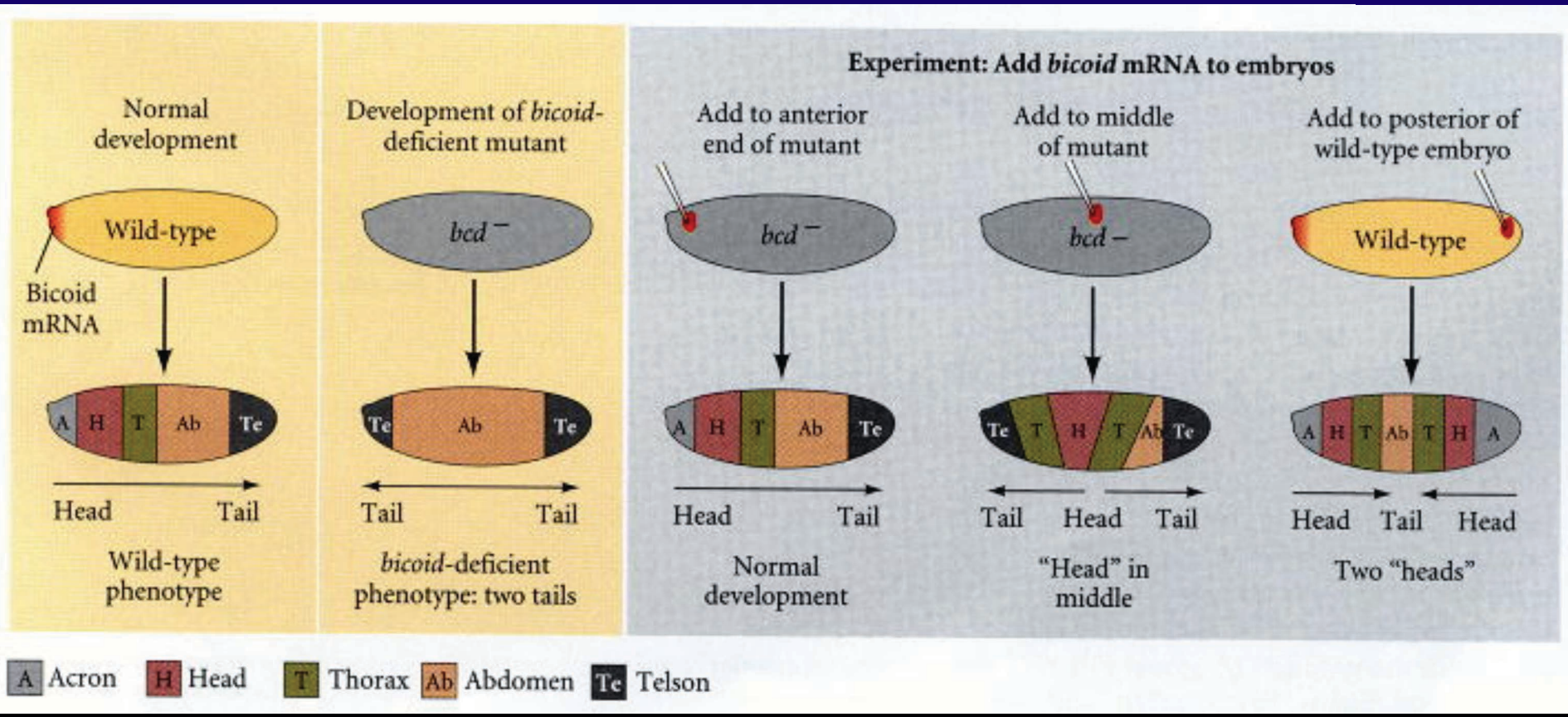
bicoid protein


B

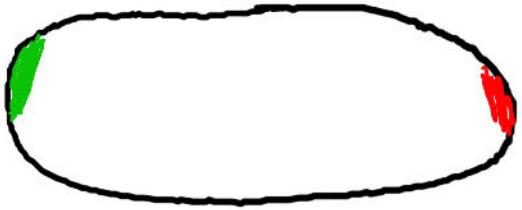
Nanos





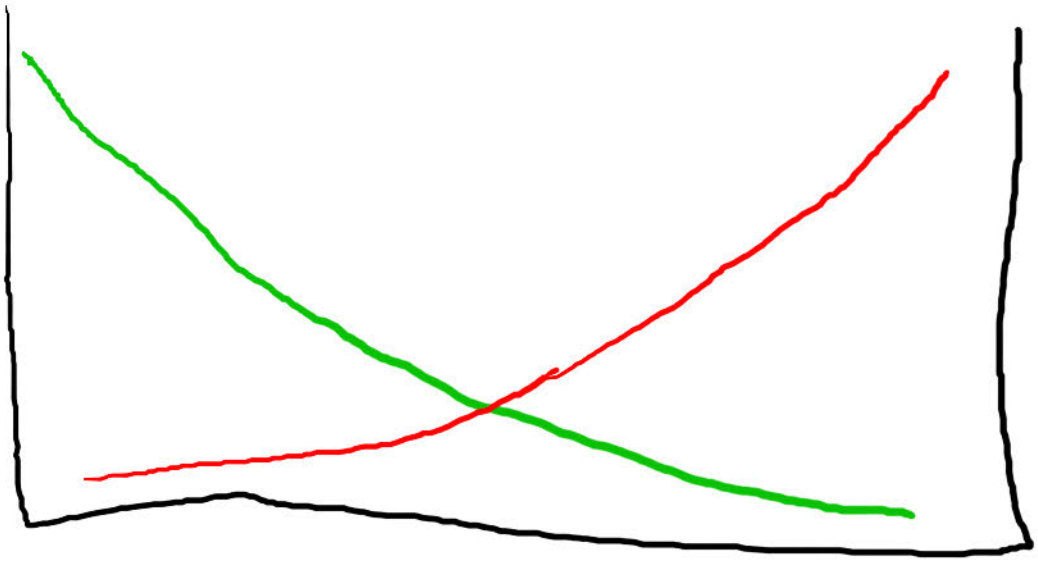

Normal development
Wild-type
Bicoid mRNA
A H T Ab Te
Head Tail
Wild-type phenotype
Development of bicoid-deficient mutant
bcd –
Te Ab Te
Tail Tail
bicoid-deficient phenotype: two tails
Experiment: Add bicoid mRNA to embryos
Add to anterior end of mutant
bcd –
A H T Ab Te
Head Tail
Normal development
Add to middle of mutant
bcd –
Te T H T Ab Te
Tail Head Tail
"Head" in middle
Add to posterior of wild-type embryo
Wild-type
A H T Ab T H A
Head Tail Head
Two "heads"
A Acron
H Head
T Thorax
Ab Abdomen
Te Telson

bicoid mRNA

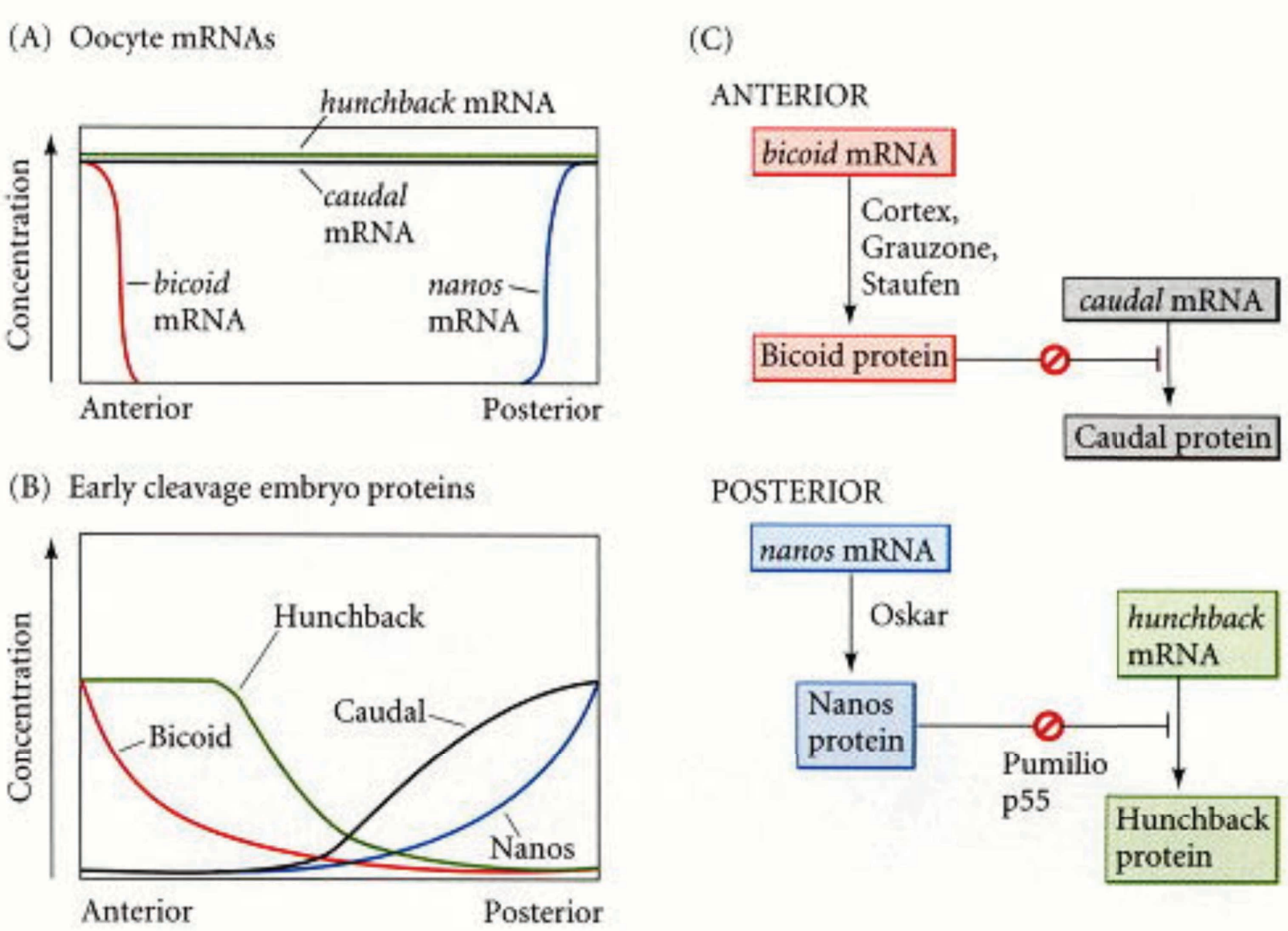


nanos mRNA

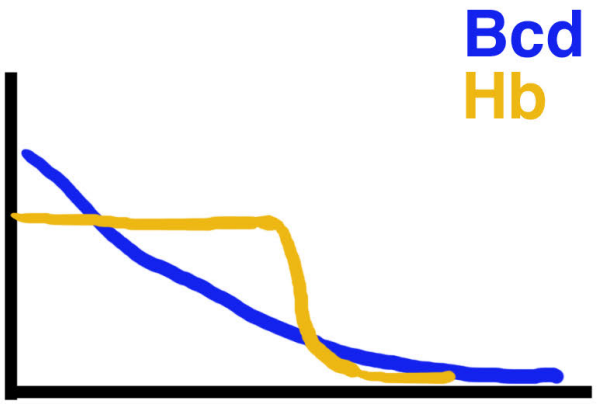
Bicoid protein conc



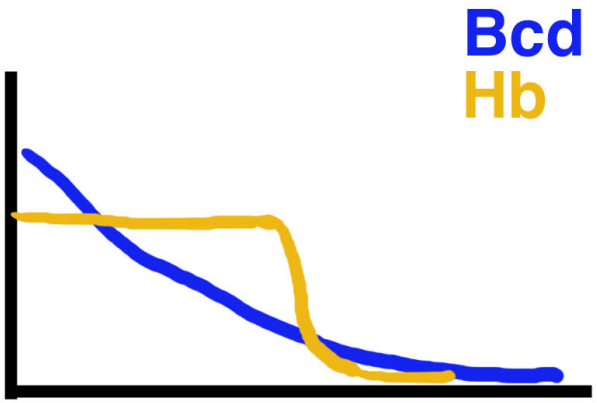
Nanos protein Conc

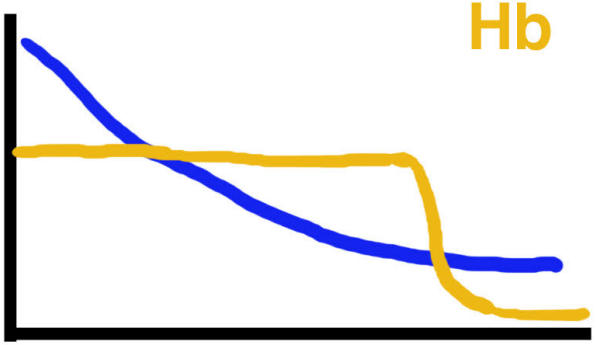

(A) Oocyte mRNAs
hunchback mRNA
caudal mRNA
bicoid mRNA
nanos mRNA
Concentration
Anterior
Posterior
(B) Early cleavage embryo proteins
Hunchback
Caudal
Bicoid
Nanos
Concentration
Anterior
Posterior
(C)
ANTERIOR
bicoid mRNA
Cortex, Grauzone, Staufen
caudal mRNA
Bicoid protein
Caudal protein
POSTERIOR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

WT (2 copies bicoid)



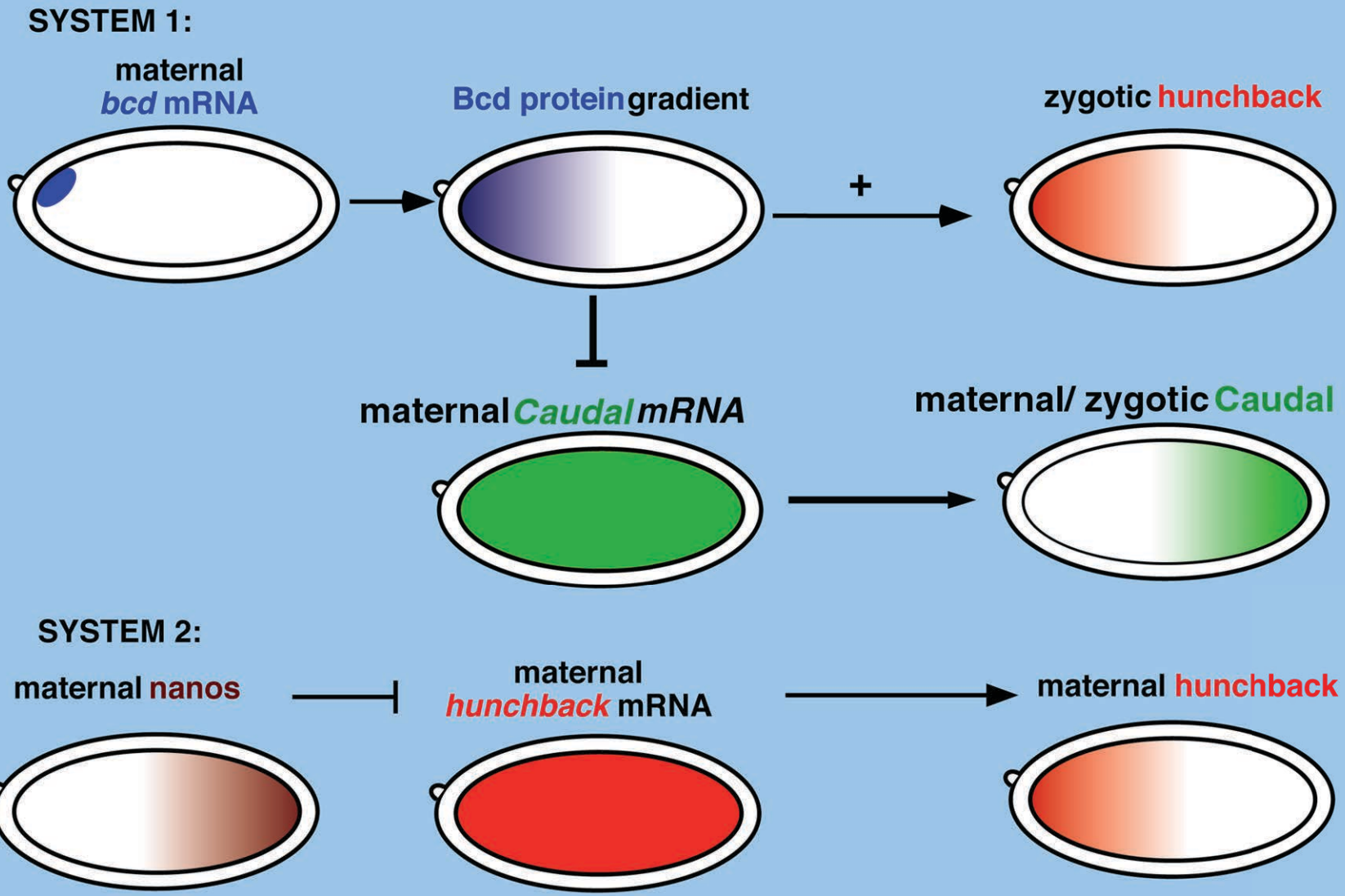
WT (2 copies bicoid)





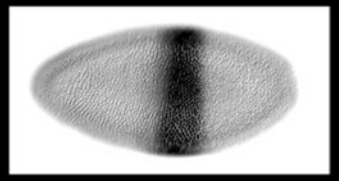
Mutant (4 copies of bicoid)

Drosophila has multiple systems generating AP polarity

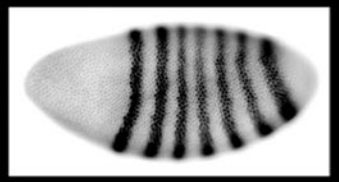


maternal gradients

zygotic gap genes



pair-rule genes



segment-polarity genes

symmetries in the egg

↓

maternal gradients

↓

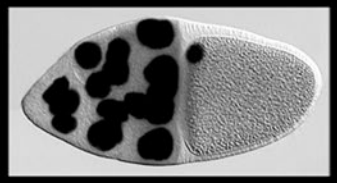
zygotic gap genes

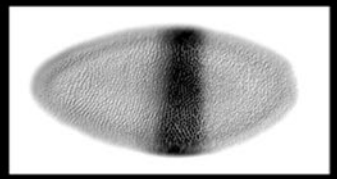
↓

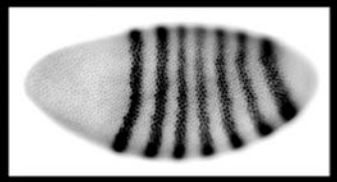
pair-rule genes

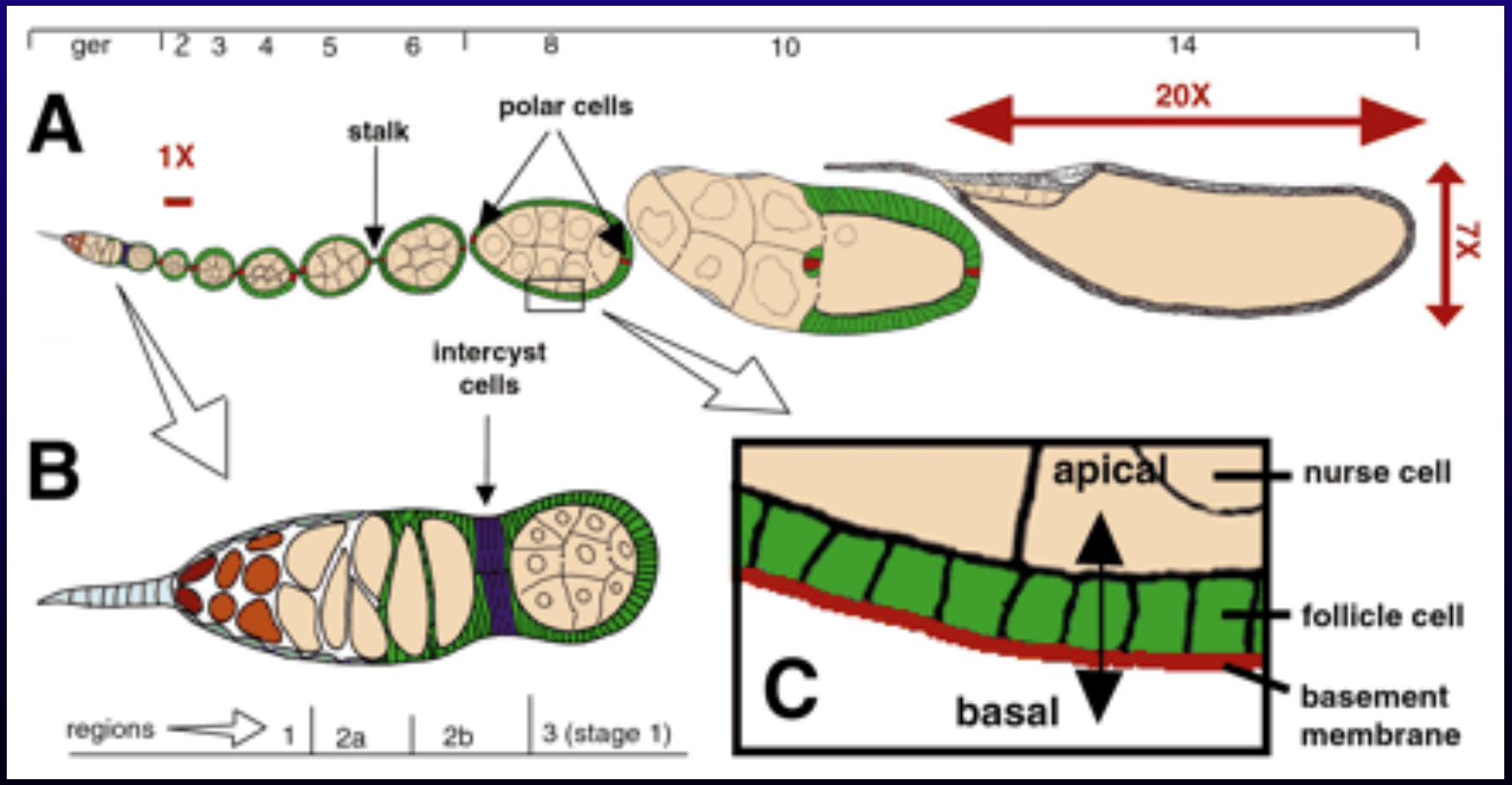
↓

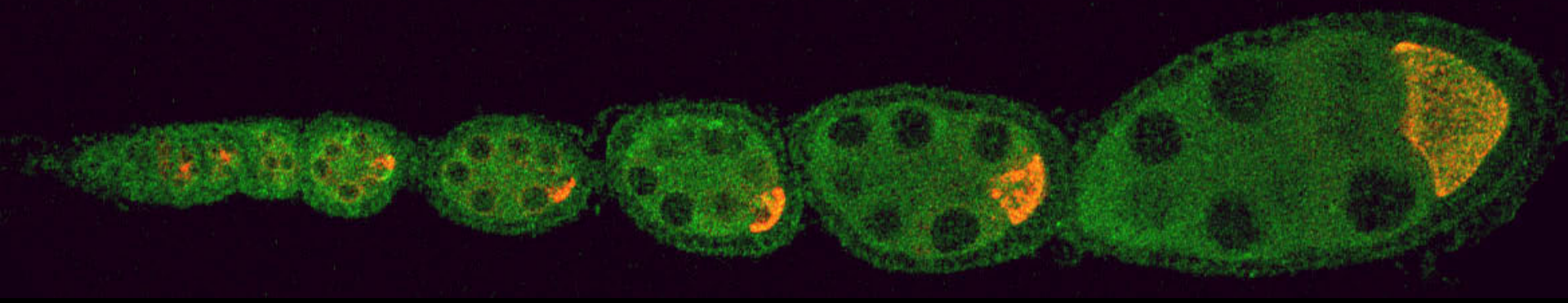
segment-polarity genes

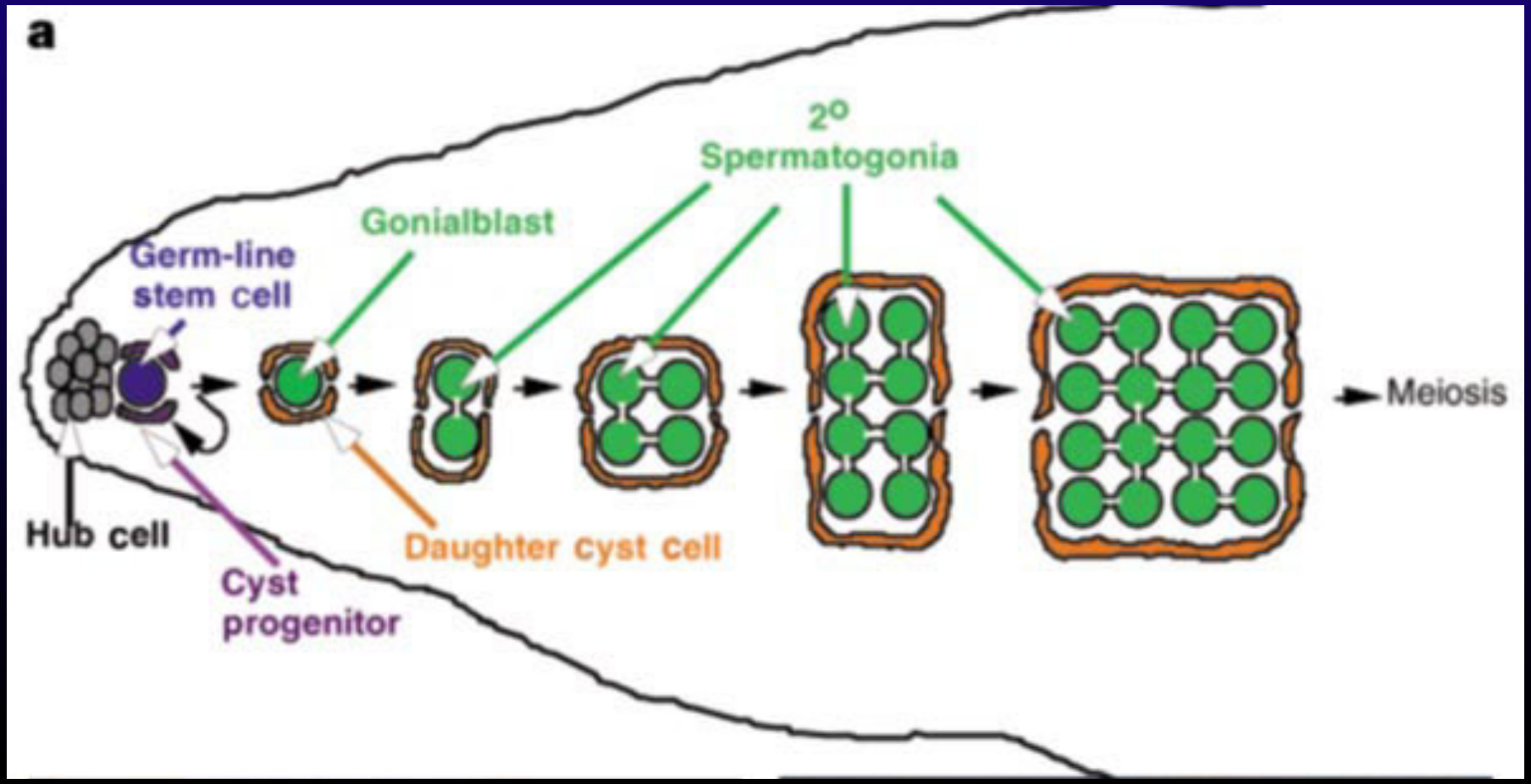


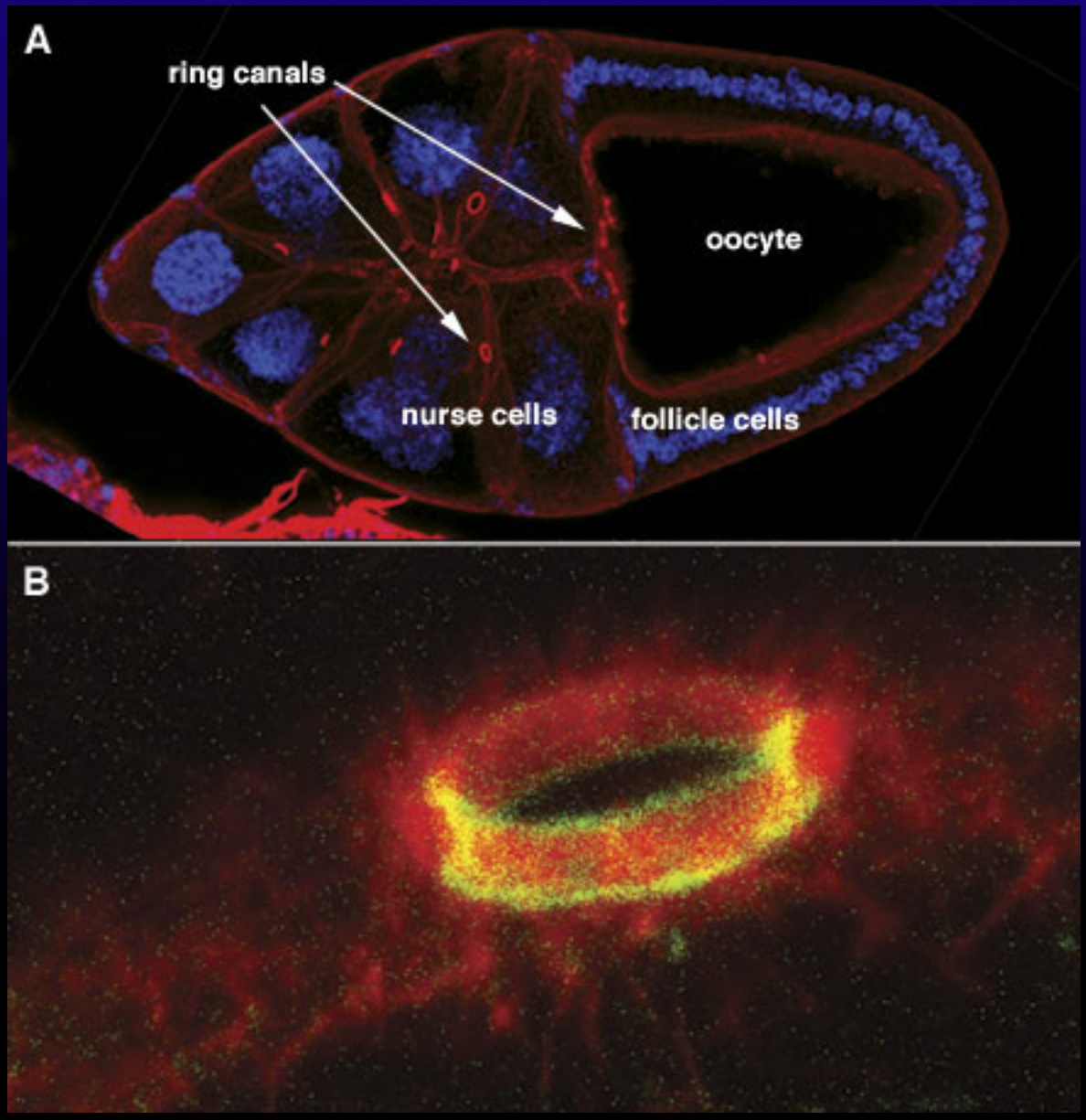


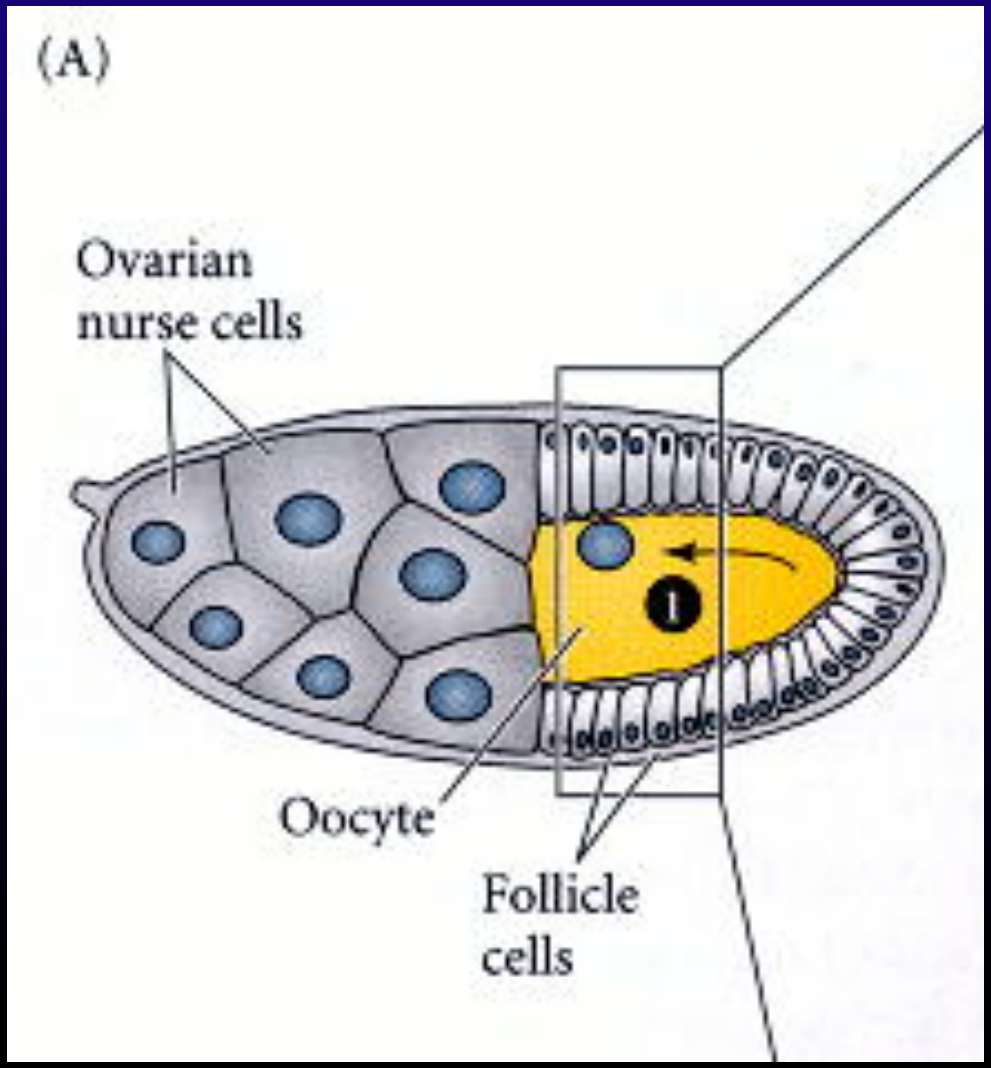



ger
2
3
4
5
6
8
10
14
A
1X
stalk
polar cells
20X
7X
intercyst cells
B
regions
1
2a
2b
3 (stage 1)
C
apical
basal
nurse cell
follicle cell
basement membrane

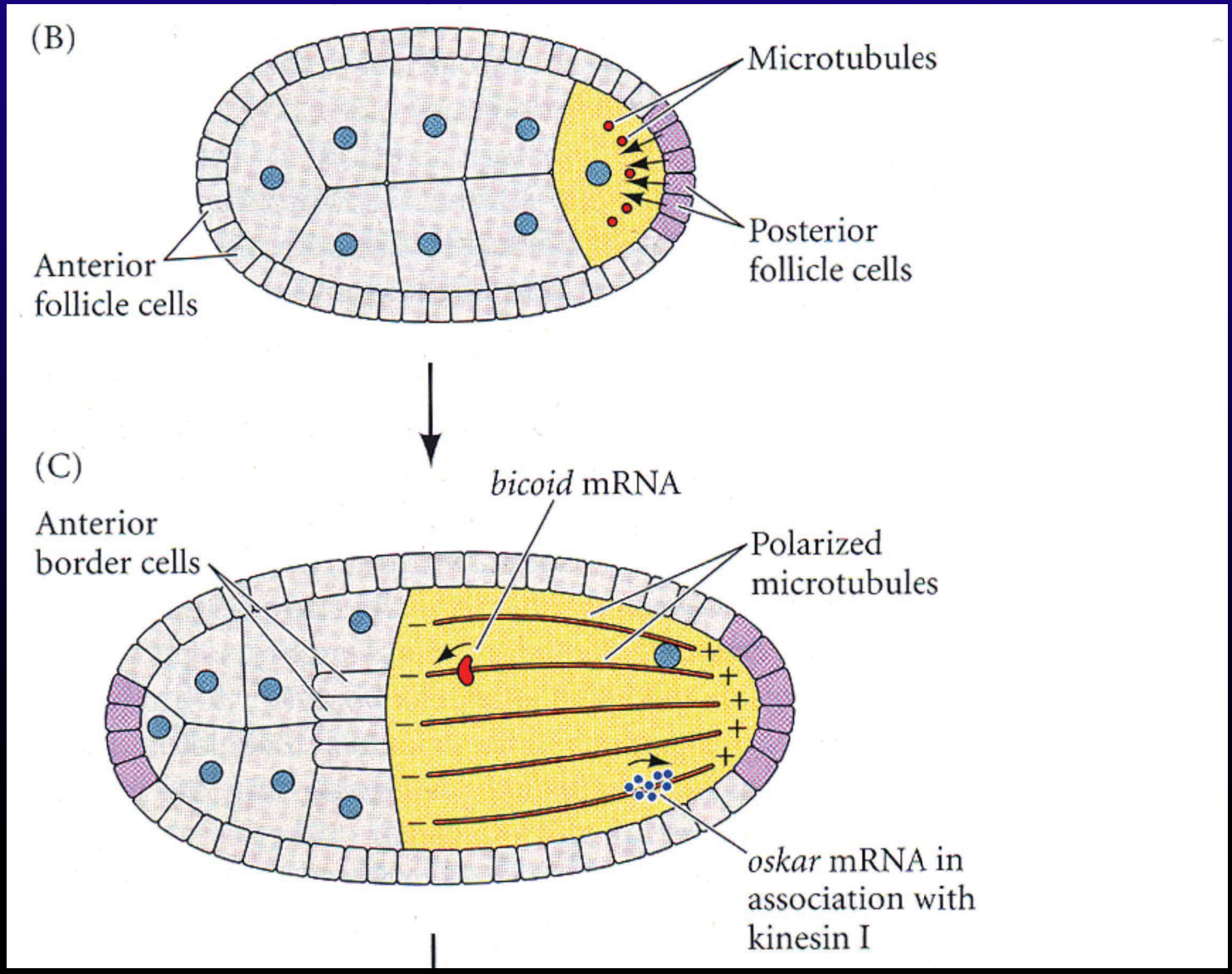


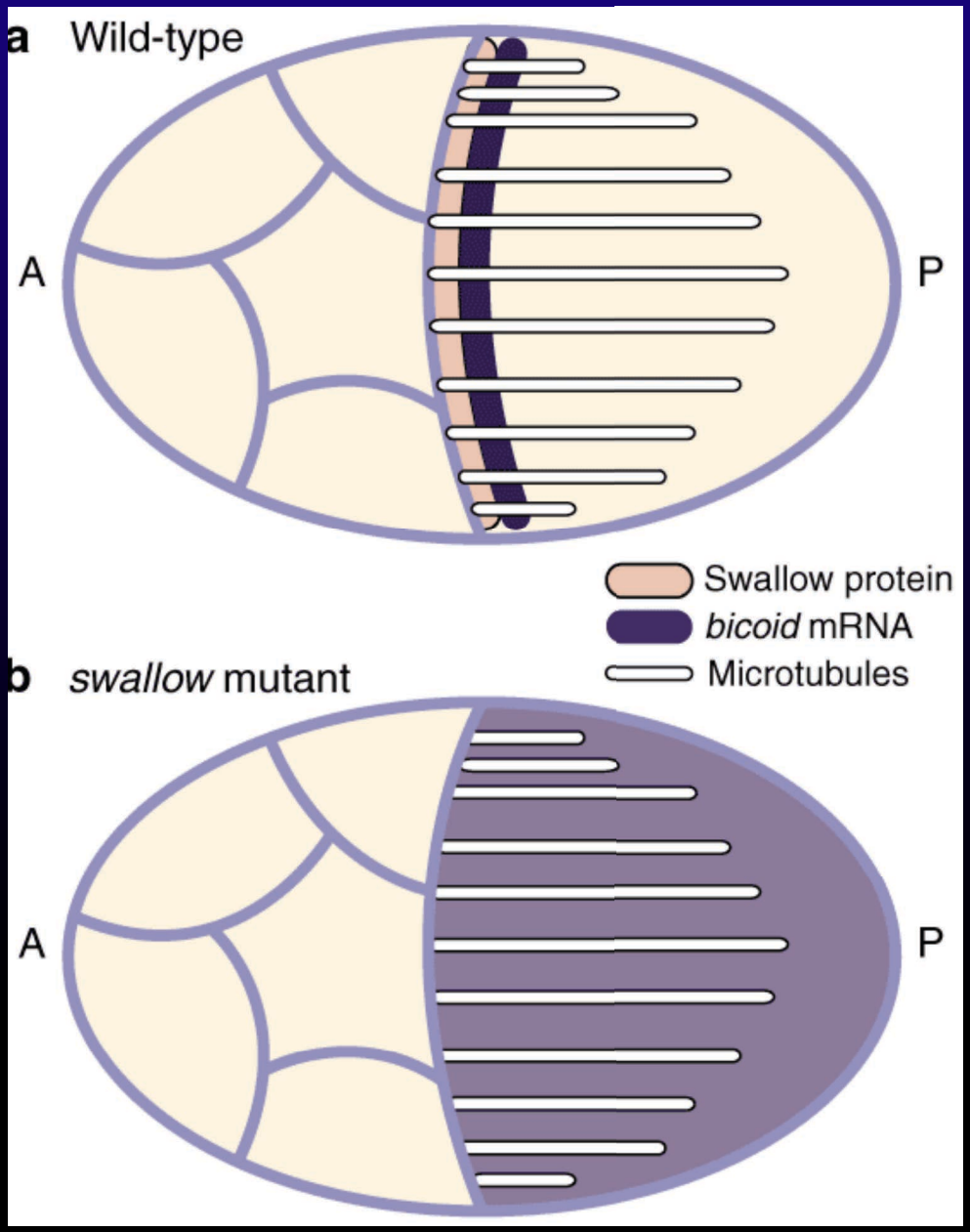

a
2°
Spermatogonia
Gonialblast
Germ-line
stem cell
Meiosis
Hub cell
Daughter cyst cell
Cyst
progenitor


A
ring canals
oocyte
nurse cells
follicle cells
B



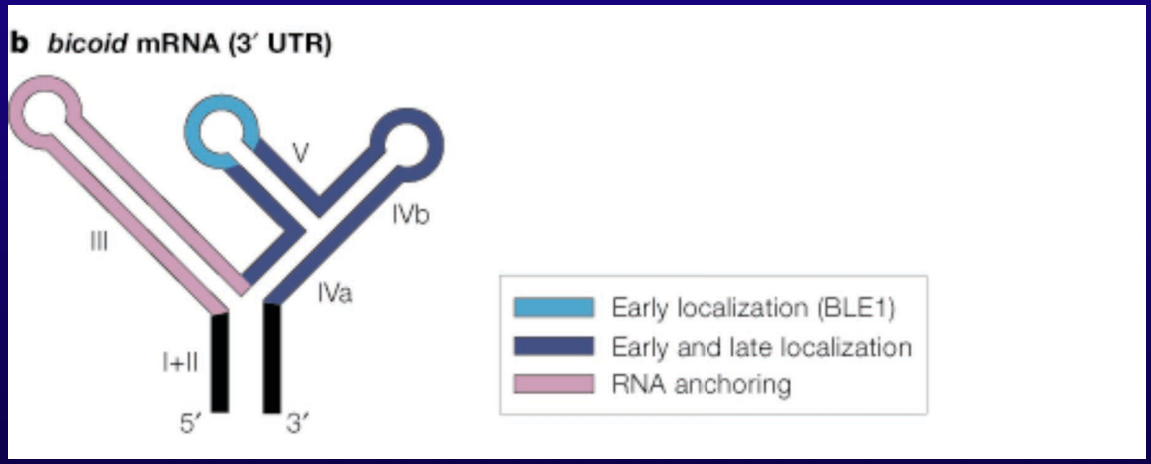
Oocyte nucleus migrates to future anterior dorsal

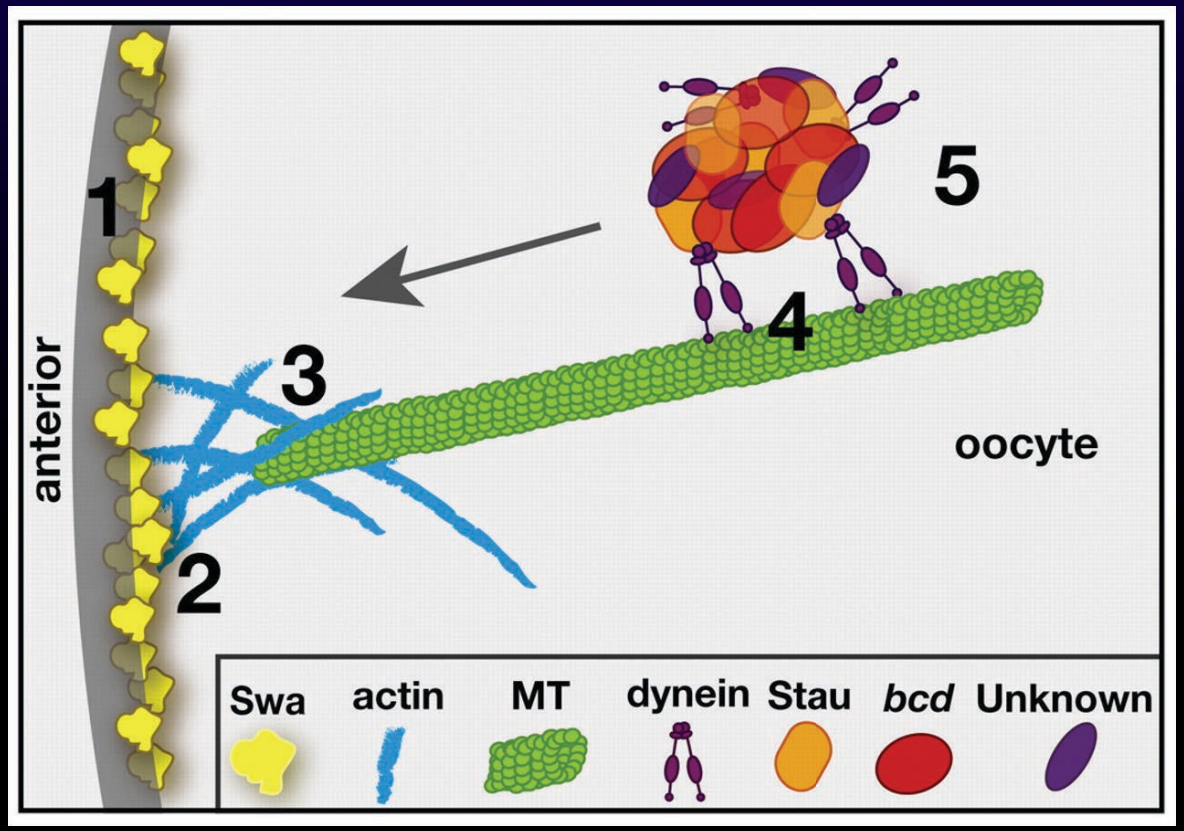

(B)
Microtubules
Posterior
follicle cells
Anterior
follicle cells
(C)
bicoid mRNA
Anterior
border cells
Polarized
microtubules
oskar mRNA in
association with
kinesin I

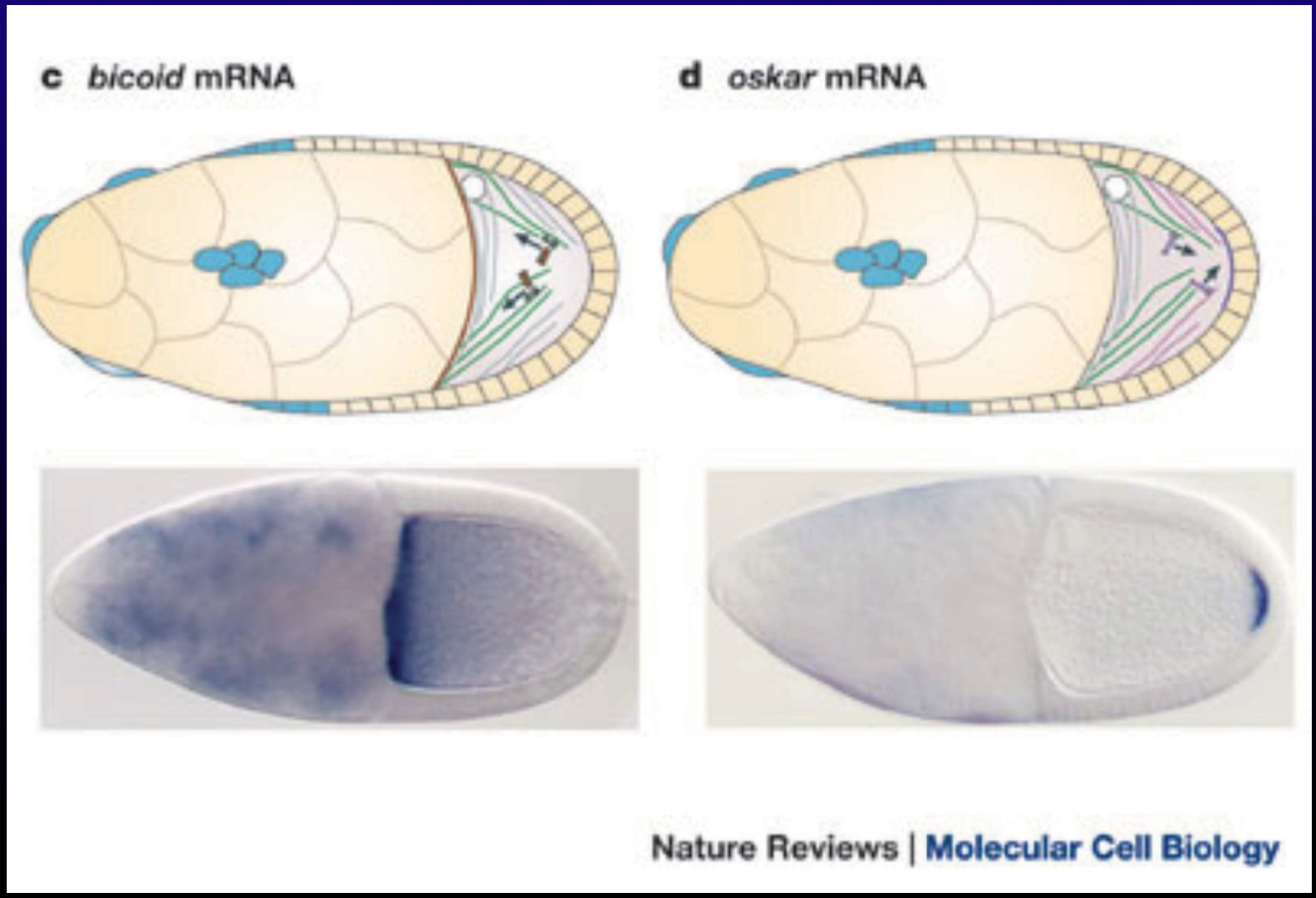


Microtubules are polarized (+/- ends) [+ end is posterior]

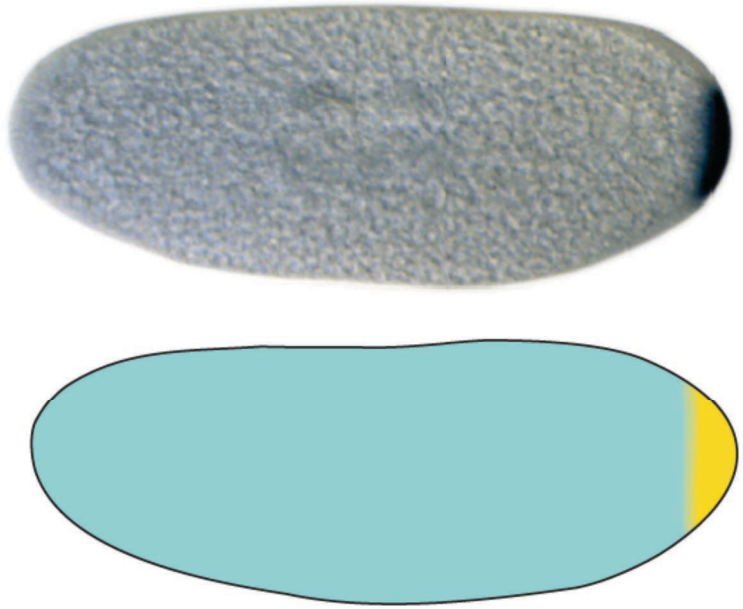
Some motors go in + direction, others go in - direction.

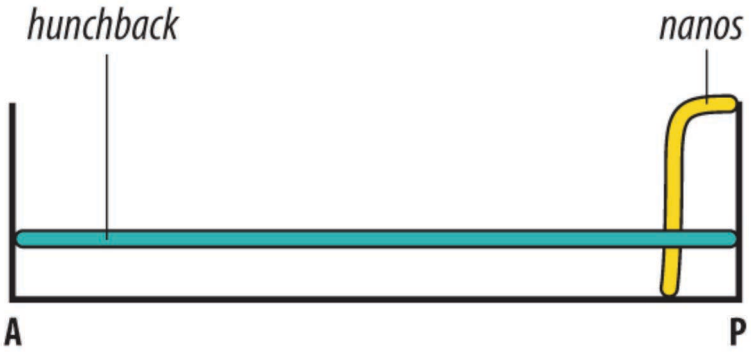

b bicoid mRNA (3′ UTR)
V
IVb
III
IVa
I+II
5′
3′
Early localization (BLE1)
Early and late localization
RNA anchoring


1
2
3
4
5
anterior
oocyte
Swa
actin
MT
dynein
Stau
bcd
Unknown

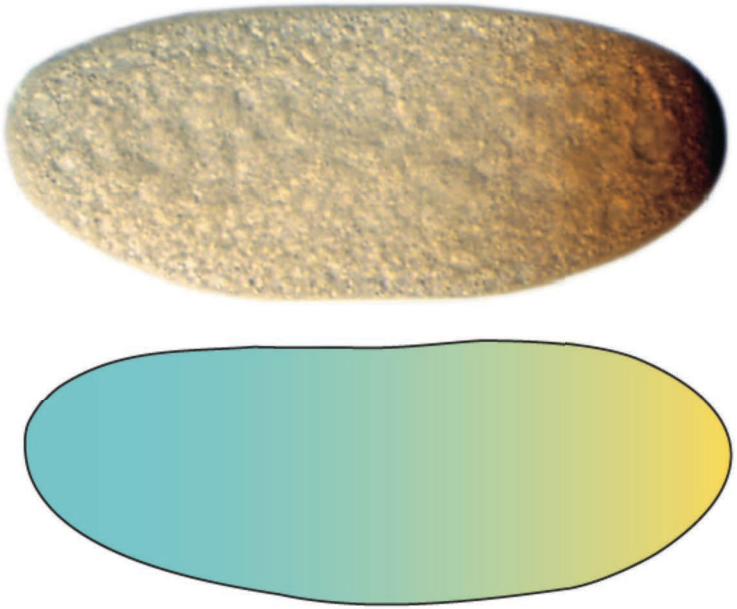

c bicoid mRNA
d oskar mRNA
Nature Reviews | Molecular Cell Biology

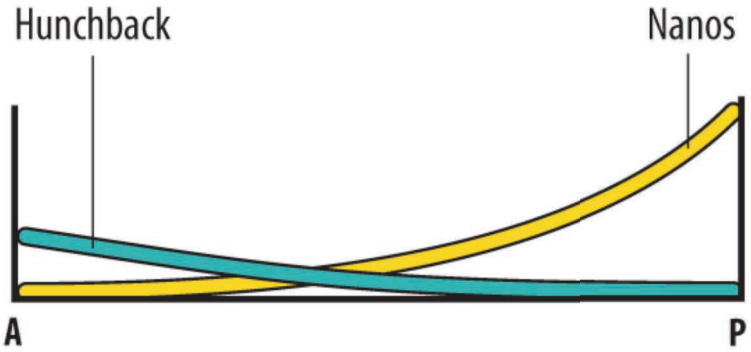
Maternal mRNA expression

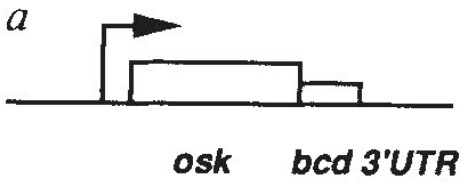


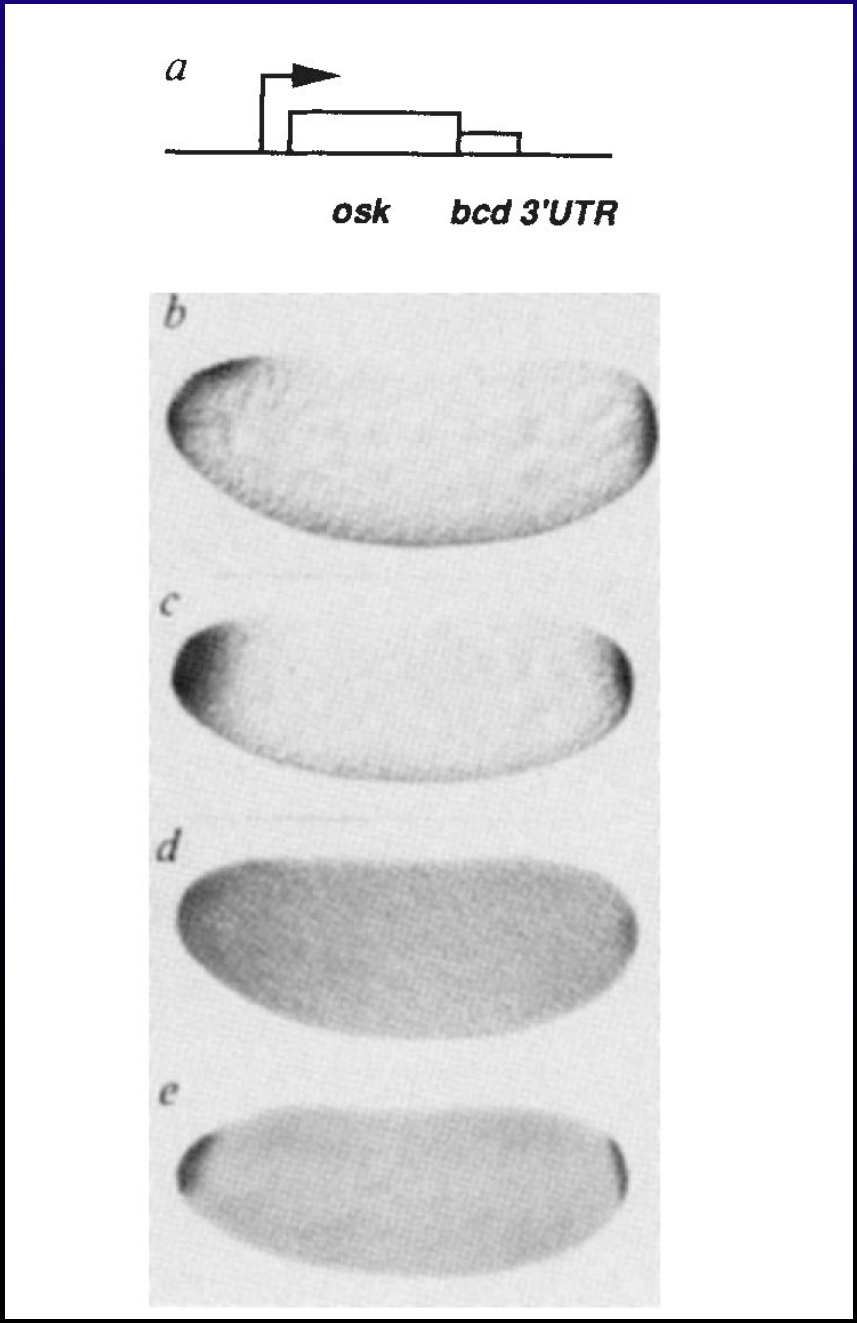


Protein expression






a
osk
bcd 3'UTR



osk mRNA

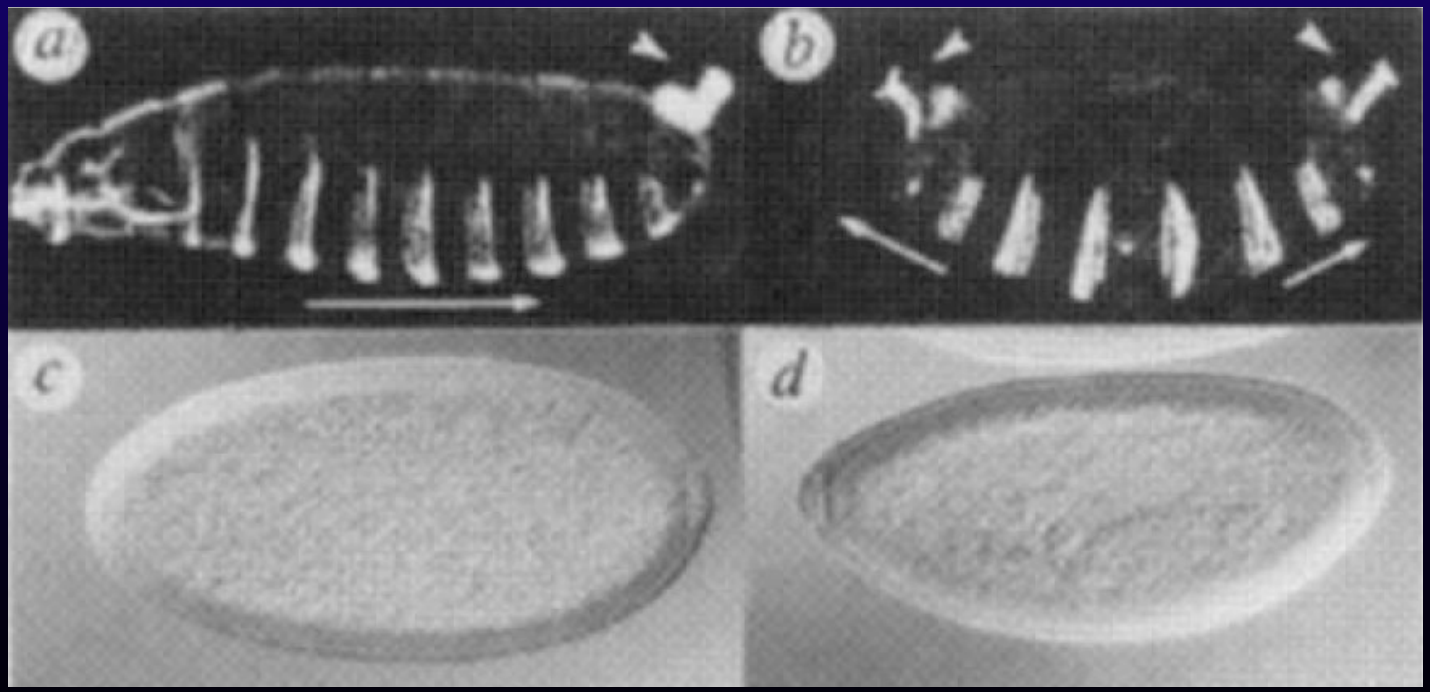
Osk protein

Vasa protein

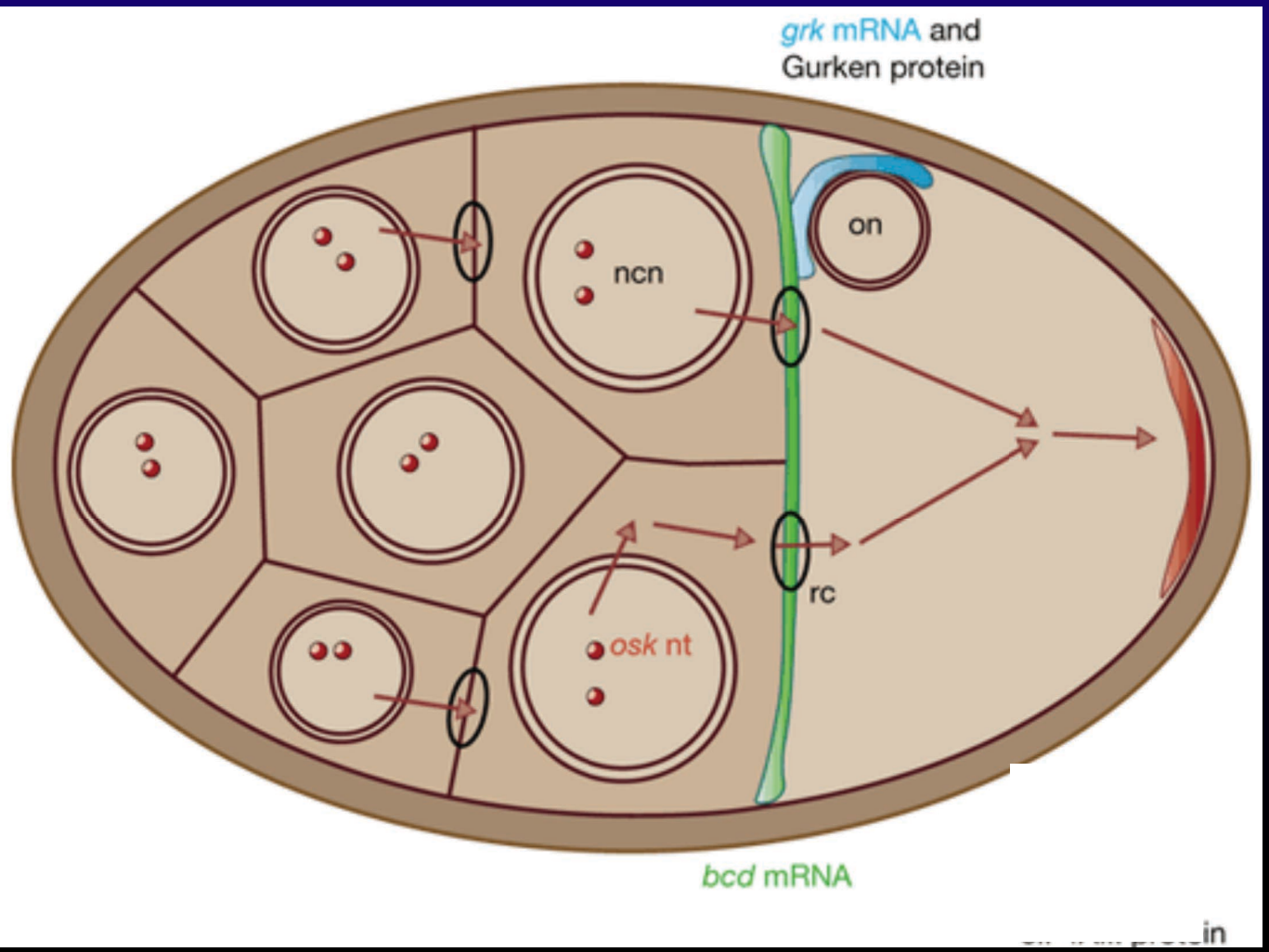
nanos mRNA

WT

Osk at anterior and posterior

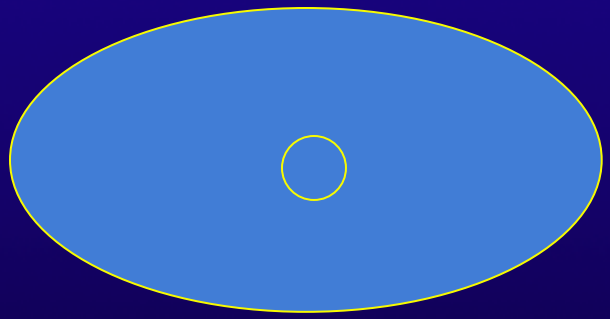


Double abdomen and germ cells at both ends



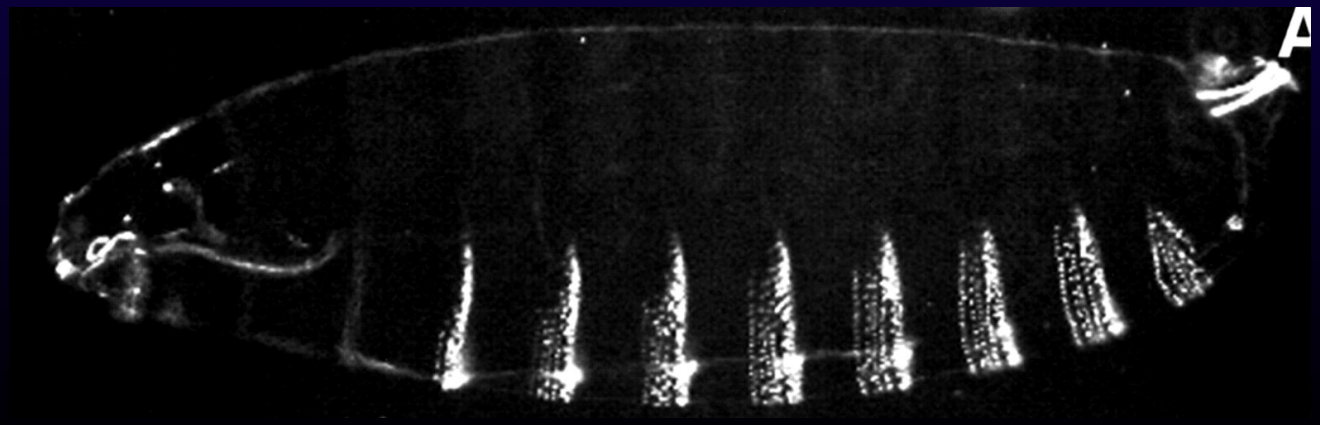
nanos mRNA

bicoid mRNA



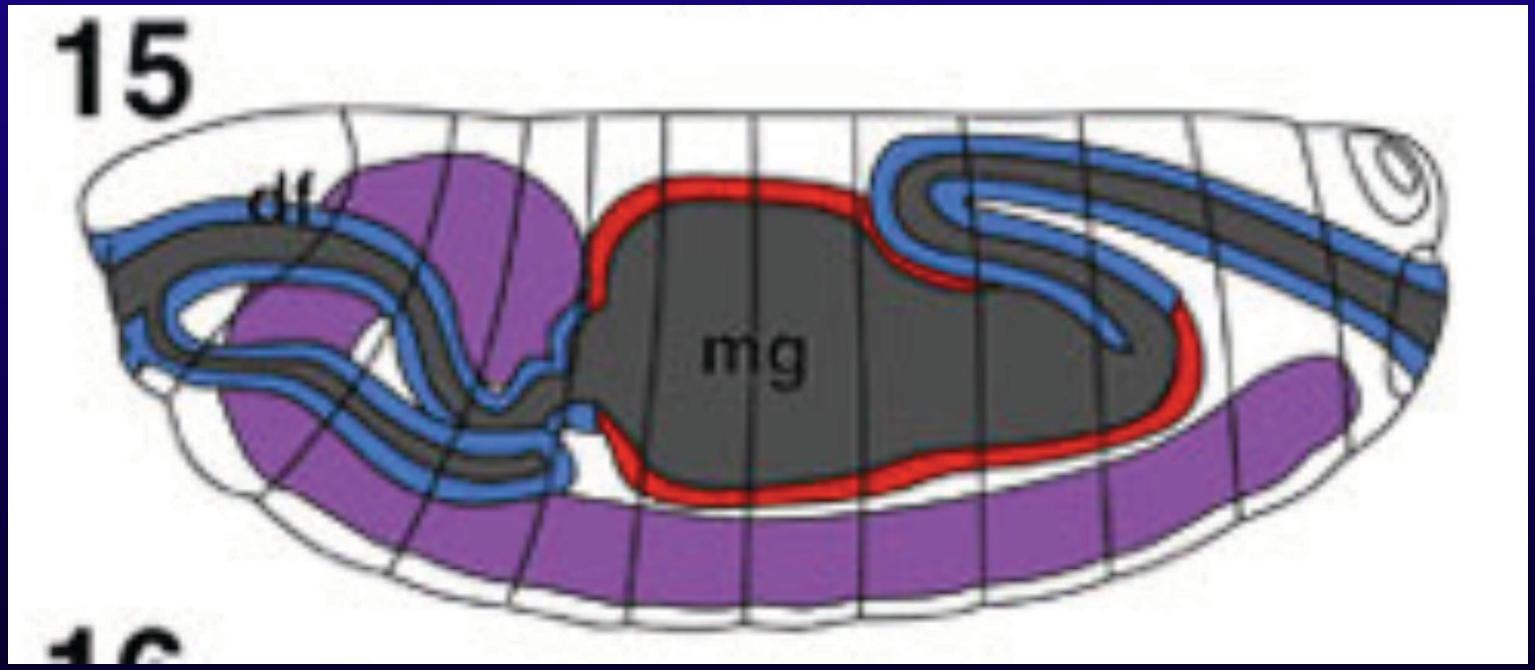
Dorsal

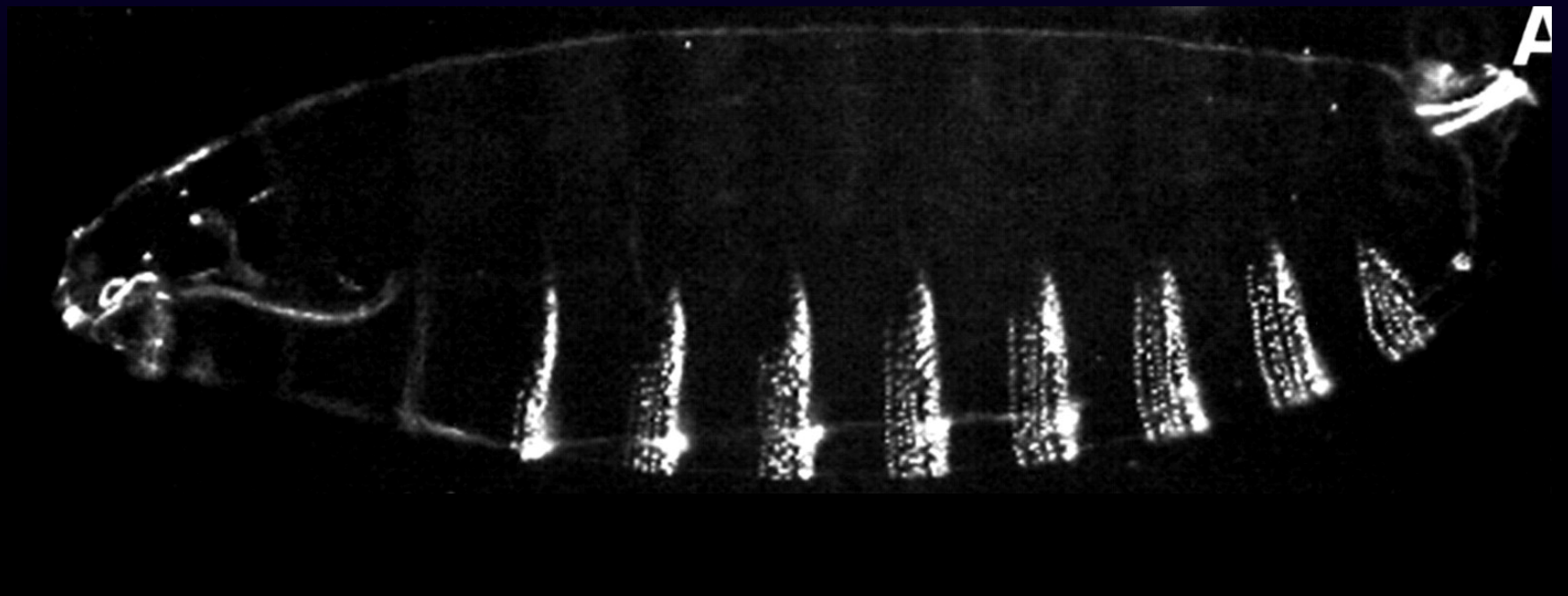
Ant

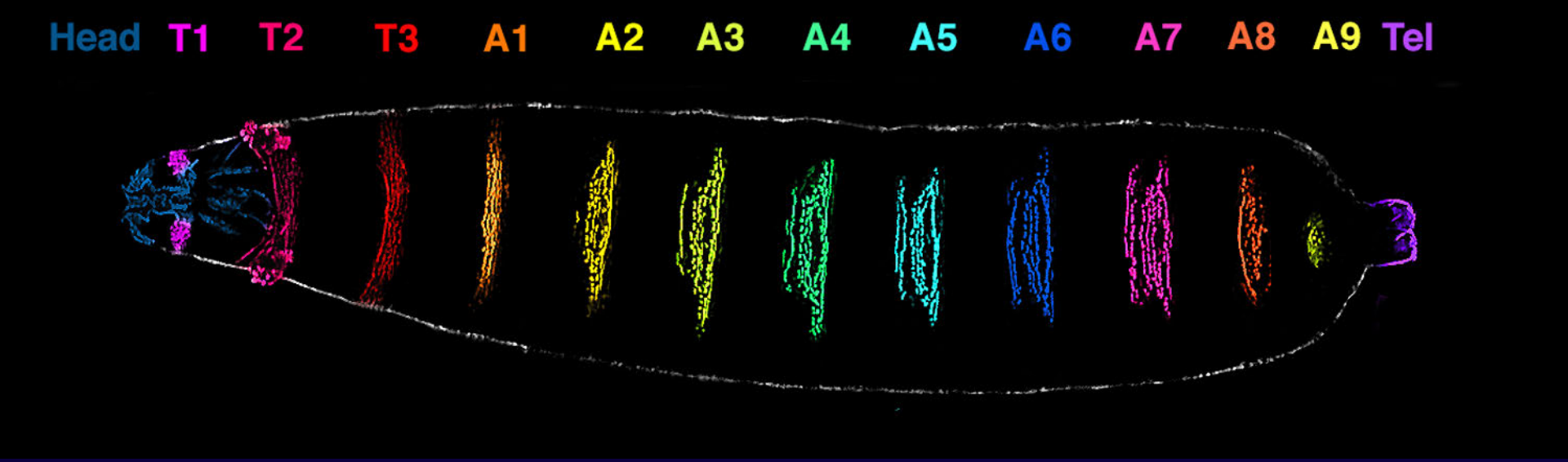


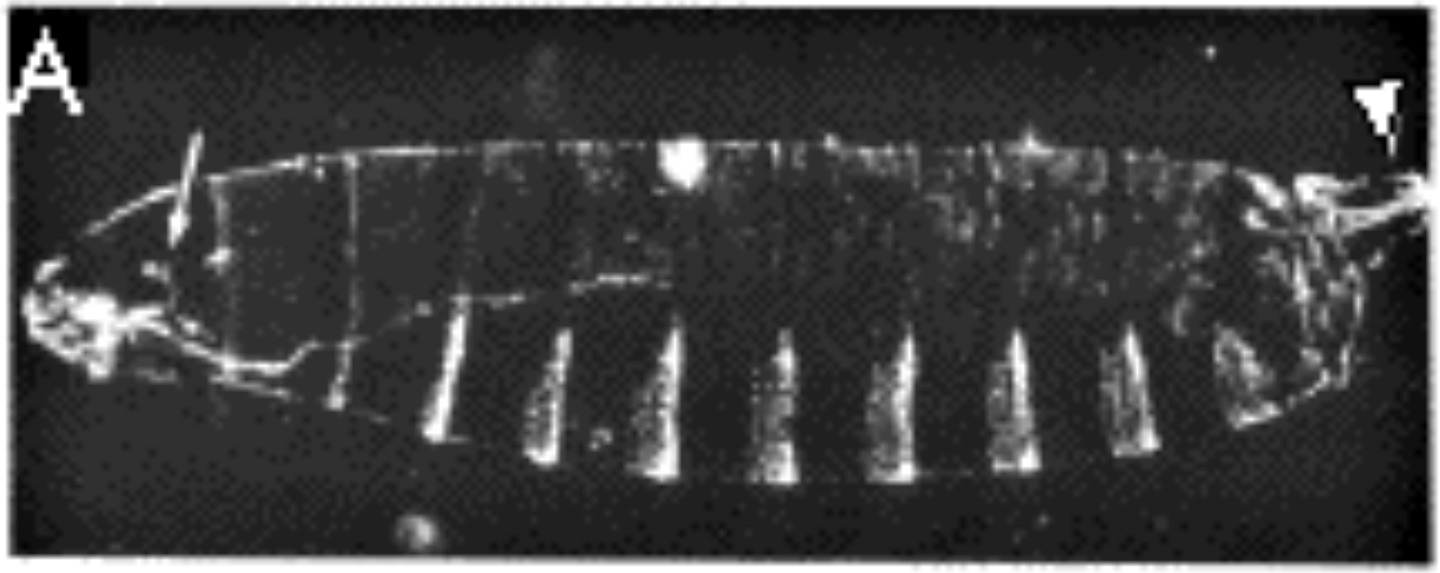
Post

Ventral

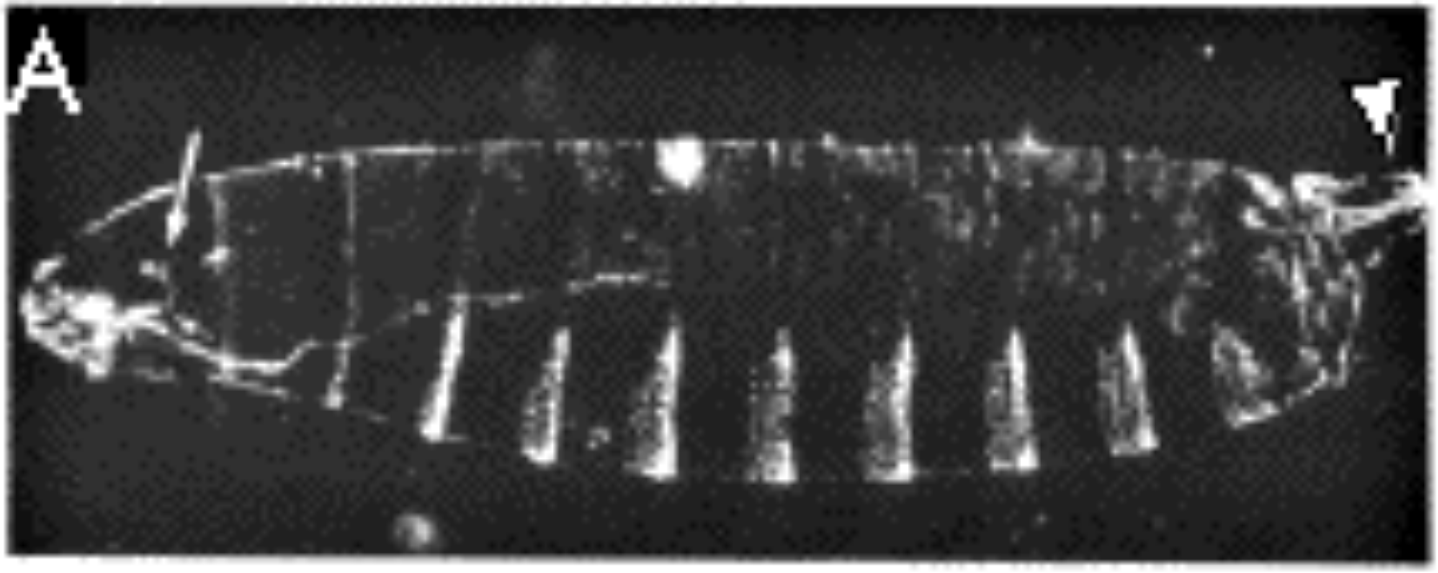

15
dl
mg


A


Head
T1
T2
T3
A1
A2
A3
A4
A5
A6
A7
A8
A9
Tel

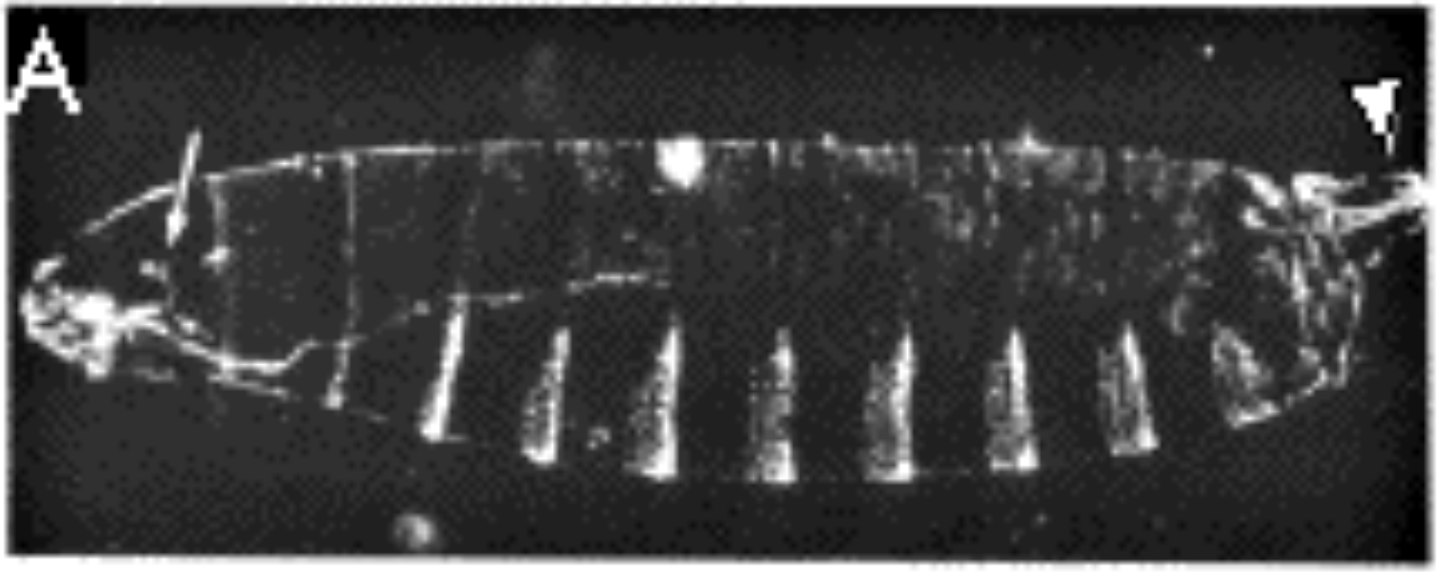

A

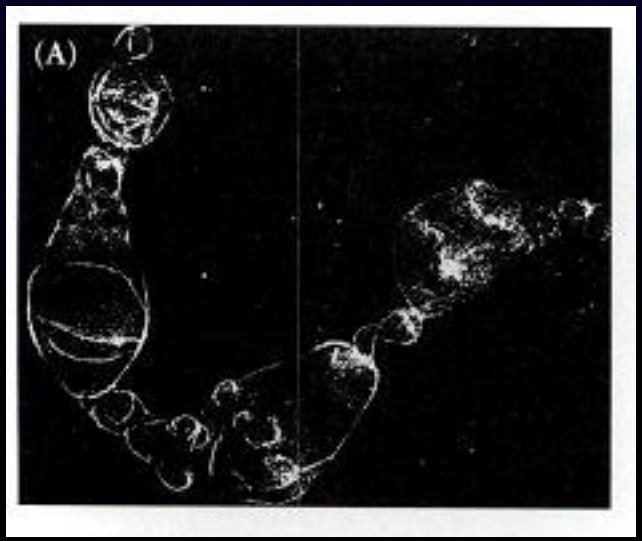
Wild-type



Ventralized (cactus mutant)

Wild-type





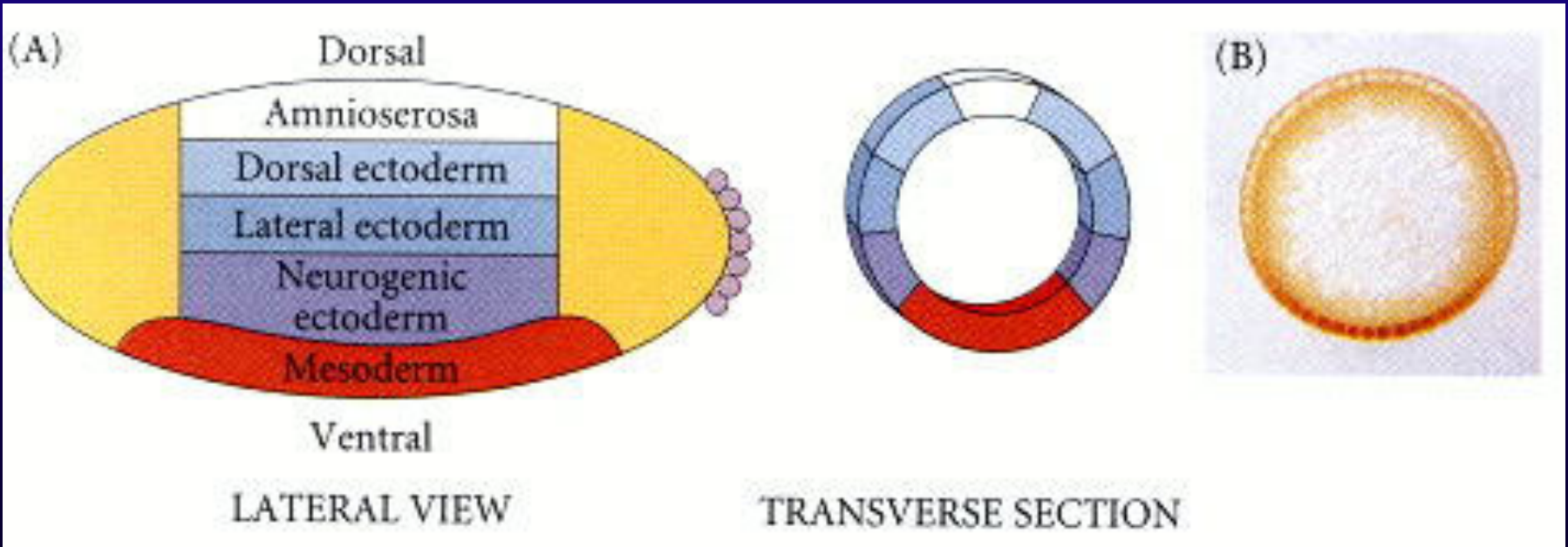
Dorsalized (dorsal mutant)

Ventralize

gurken
torpedo
cactus

Dorsalize

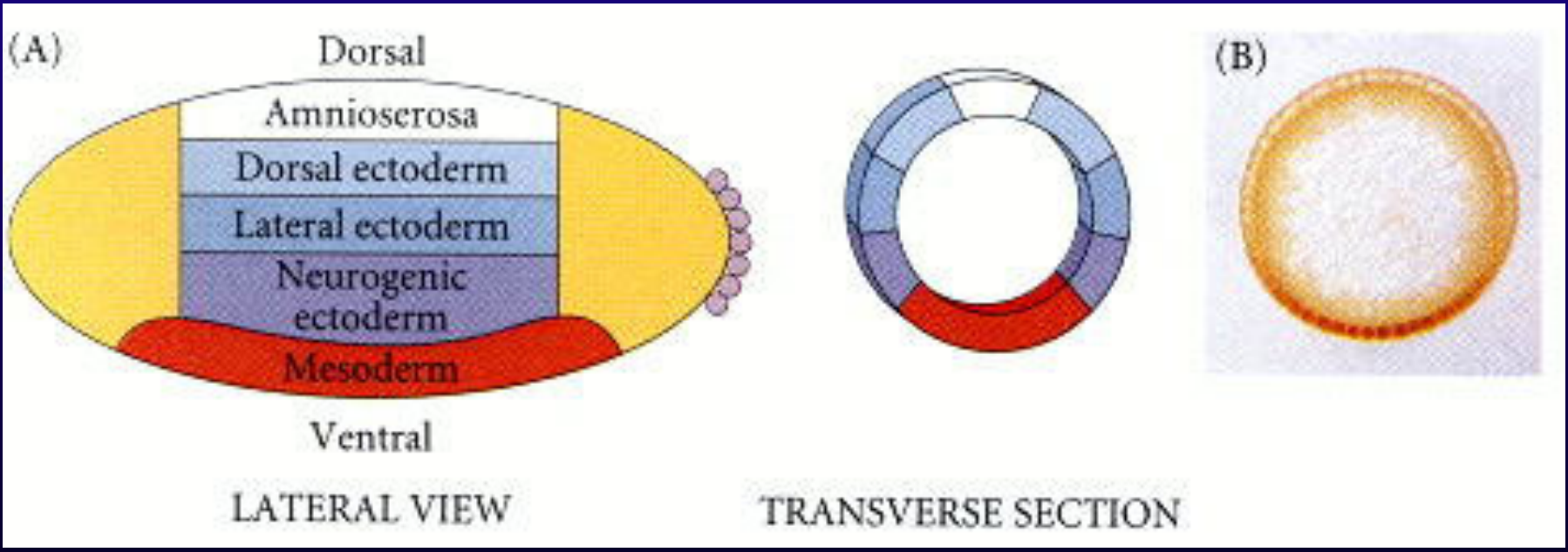
pipe
Gd
snake
easter
spatzle
Toll
pelle
tube
dorsal

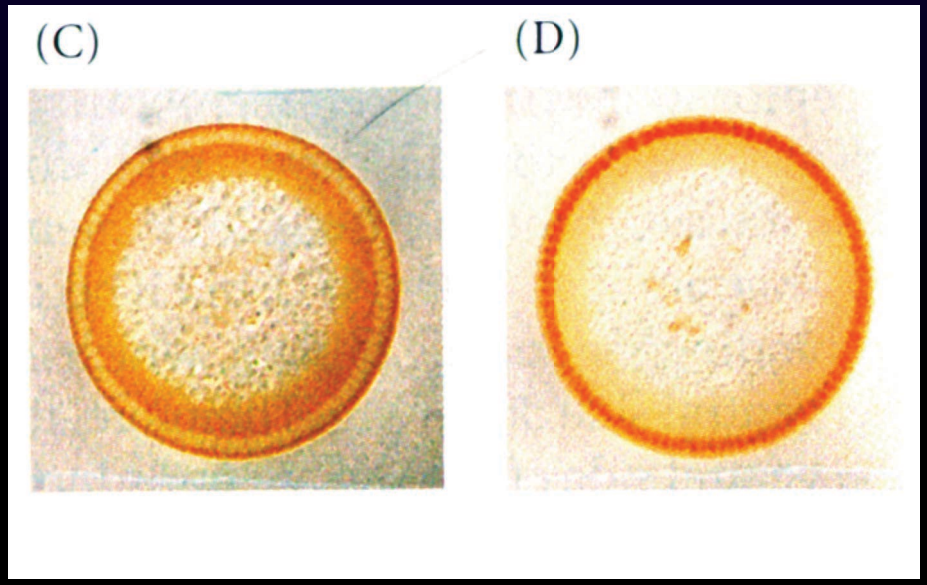


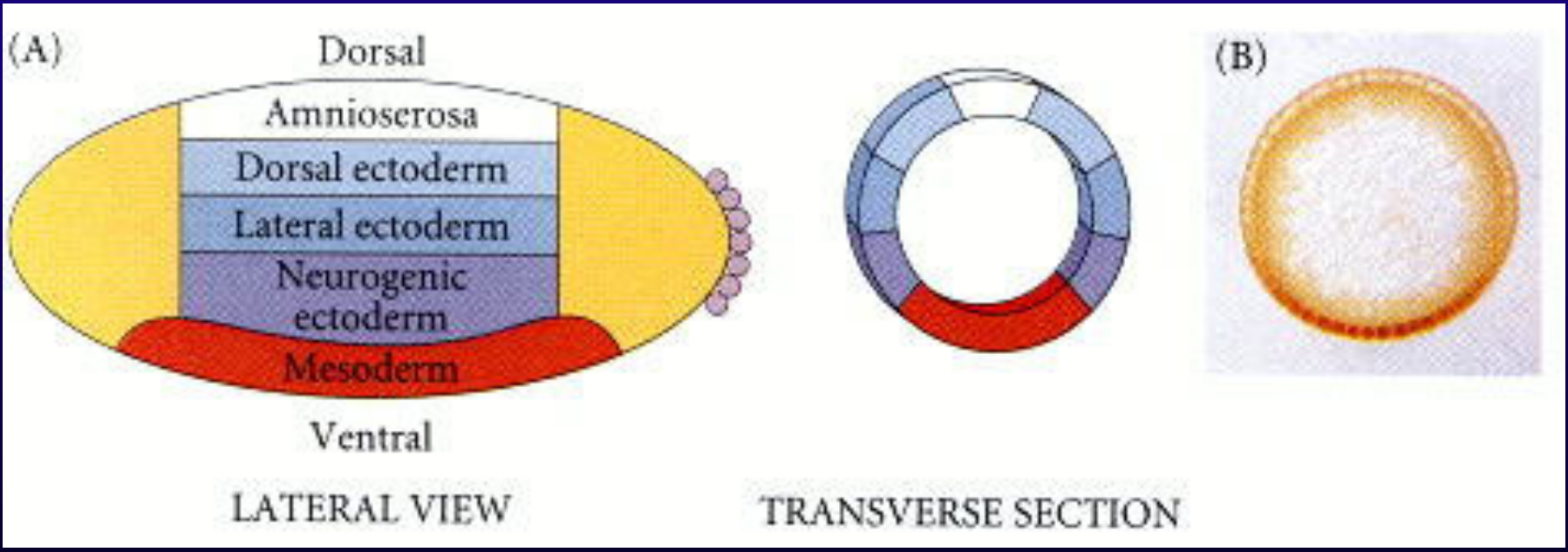
Dorsal protein only enters nucleus
in cells on ventral side

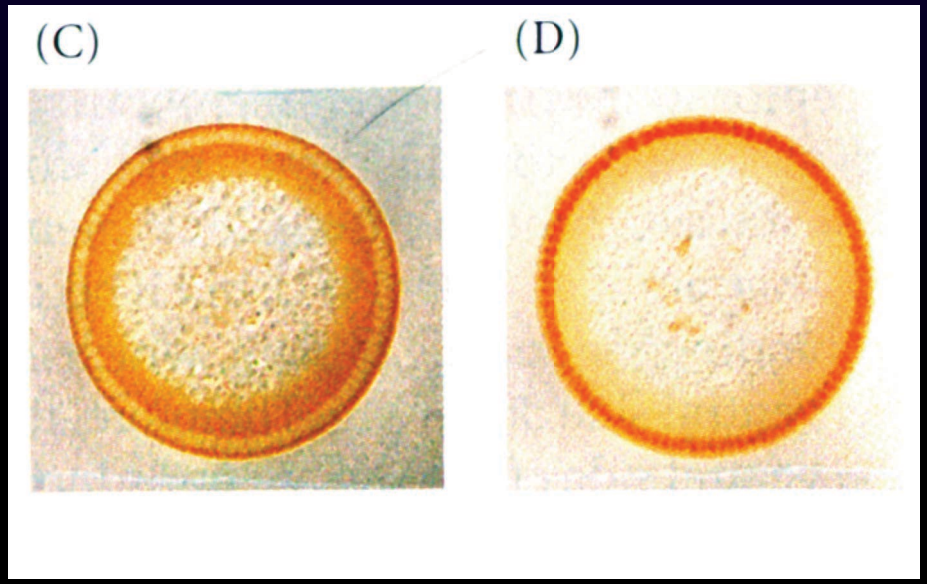
Mother puts dorsal mRNA in the egg uniformly.

The dorsal mRNA is translated into protein everywhere,
but dorsal protein stays in the cytoplasm dorsally,
and enters the nucleus only on the ventral side

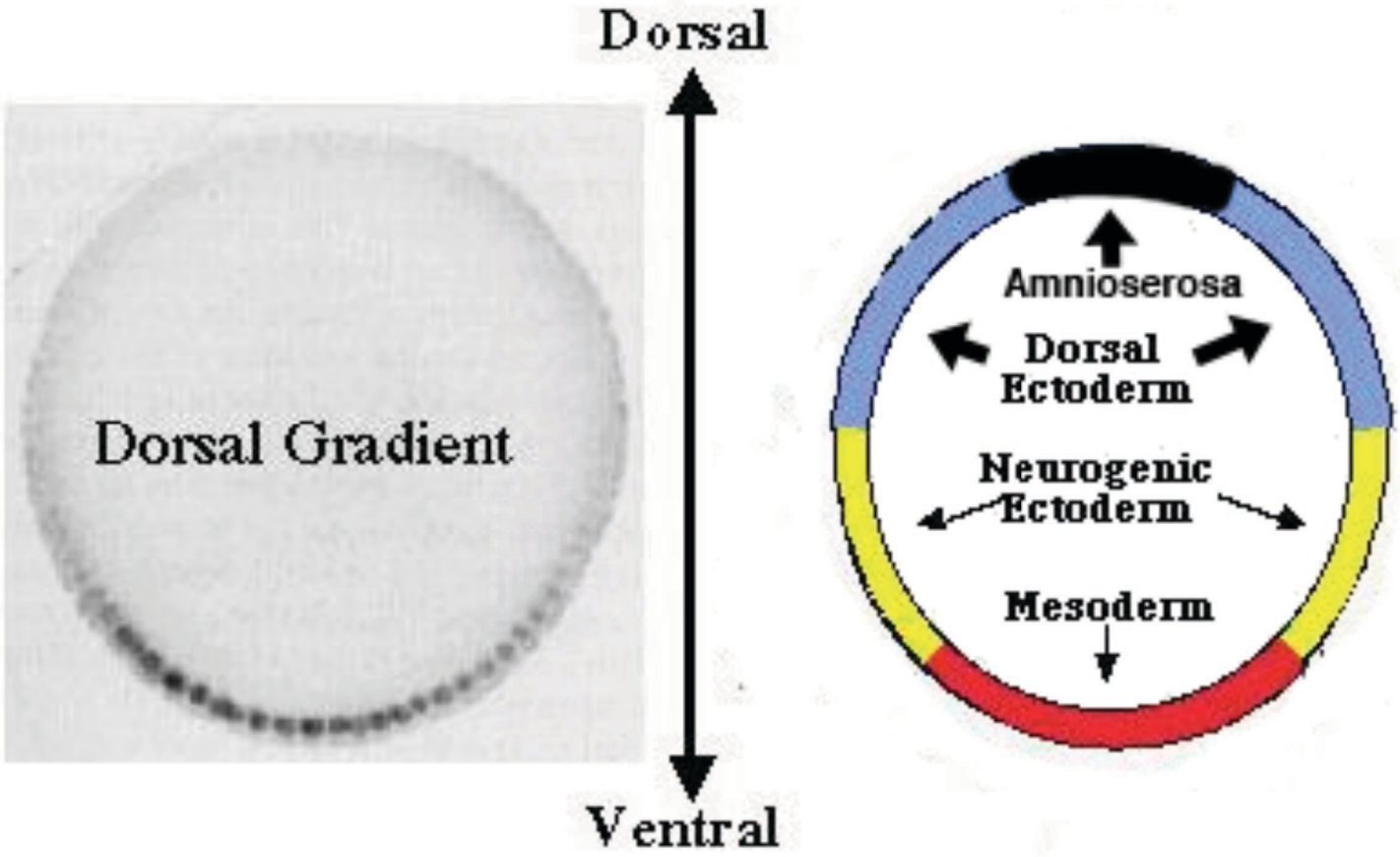

(A)
Dorsal
Amnioserosa
Dorsal ectoderm
Lateral ectoderm
Neurogenic ectoderm
Mesoderm
Ventral
LATERAL VIEW
TRANSVERSE SECTION
(B)

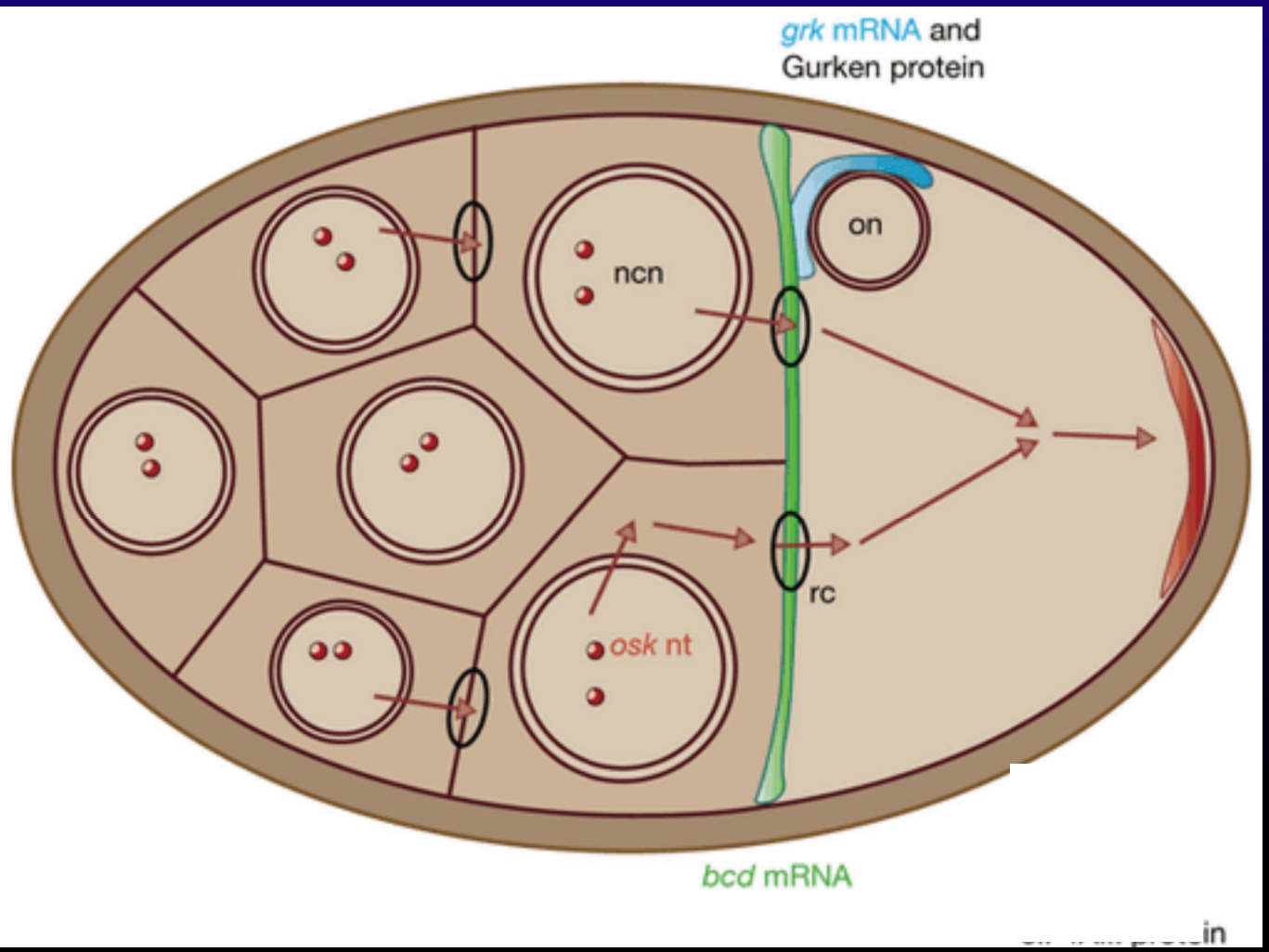

(C)
(D)





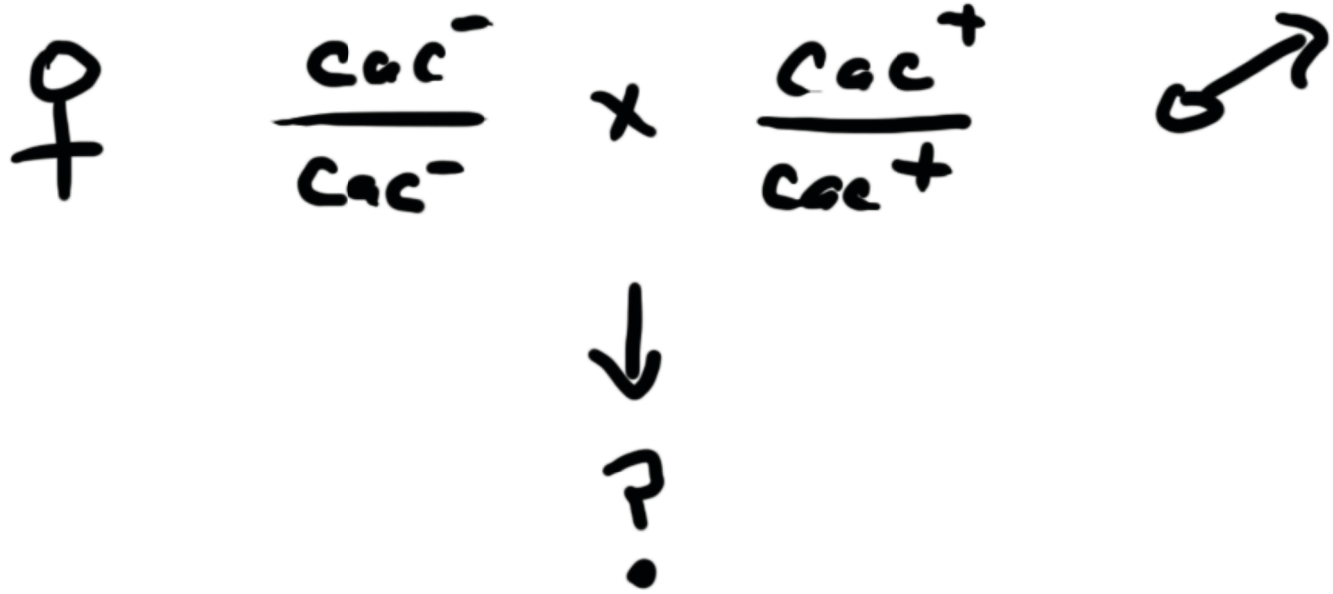
Dorsalized Ventralized

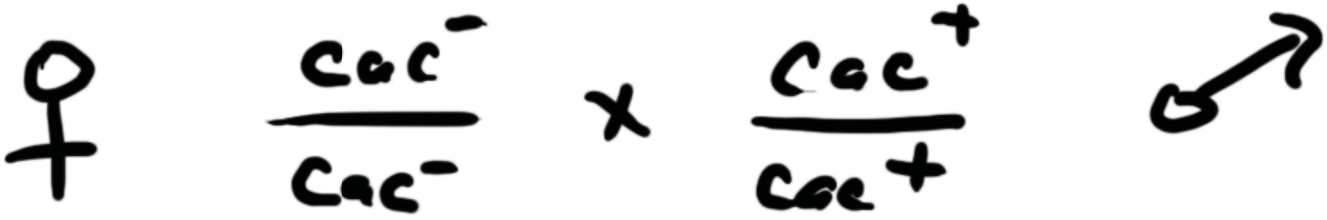

Dorsal
Dorsal Gradient
Ventral
Amnioserosa
Dorsal Ectoderm
Neurogenic Ectoderm
Mesoderm



nanos mRNA

bicoid mRNA


♀
CaC⁻ / CaC⁻
x
CaC⁺ / CaC⁺
♂
?



♀ is sterile ↓

all embryos die

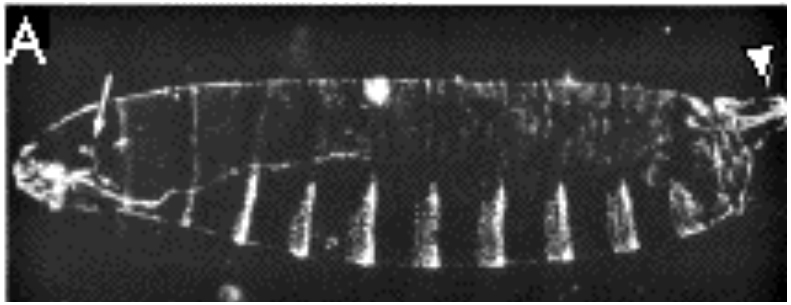
why do they die?

♀ $\frac{cac^-}{cac^-}$ × $\frac{cac^+}{cac^+}$ ♂

♀ is sterile ↓

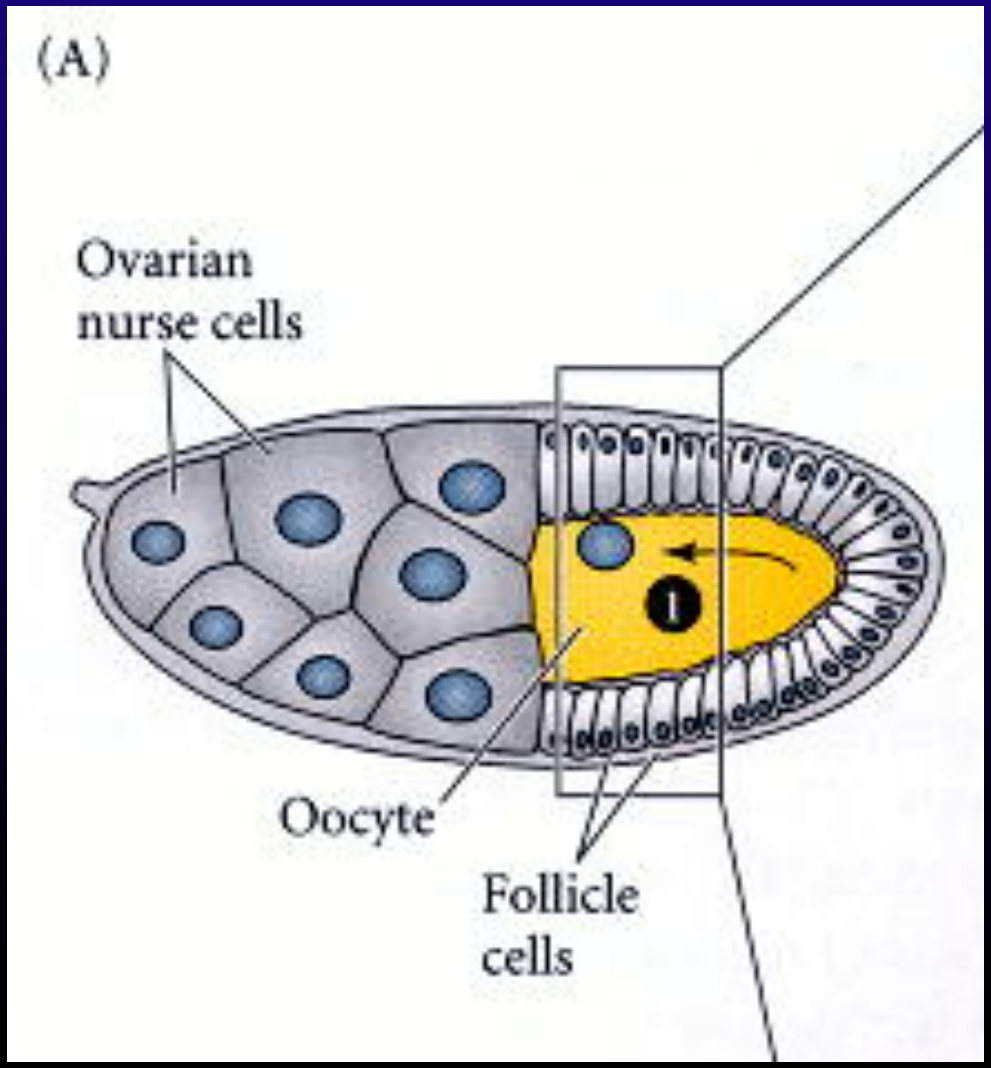
all embryos die

They die because they are all ventralized



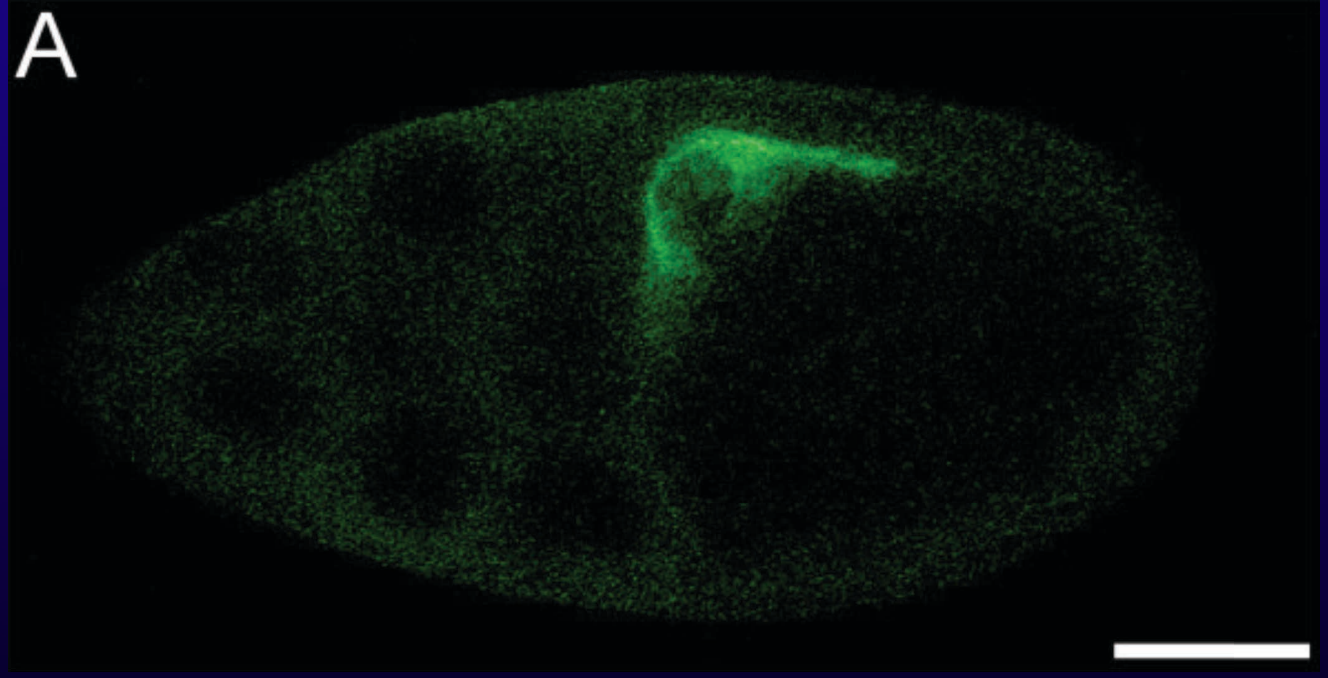
Wildtype

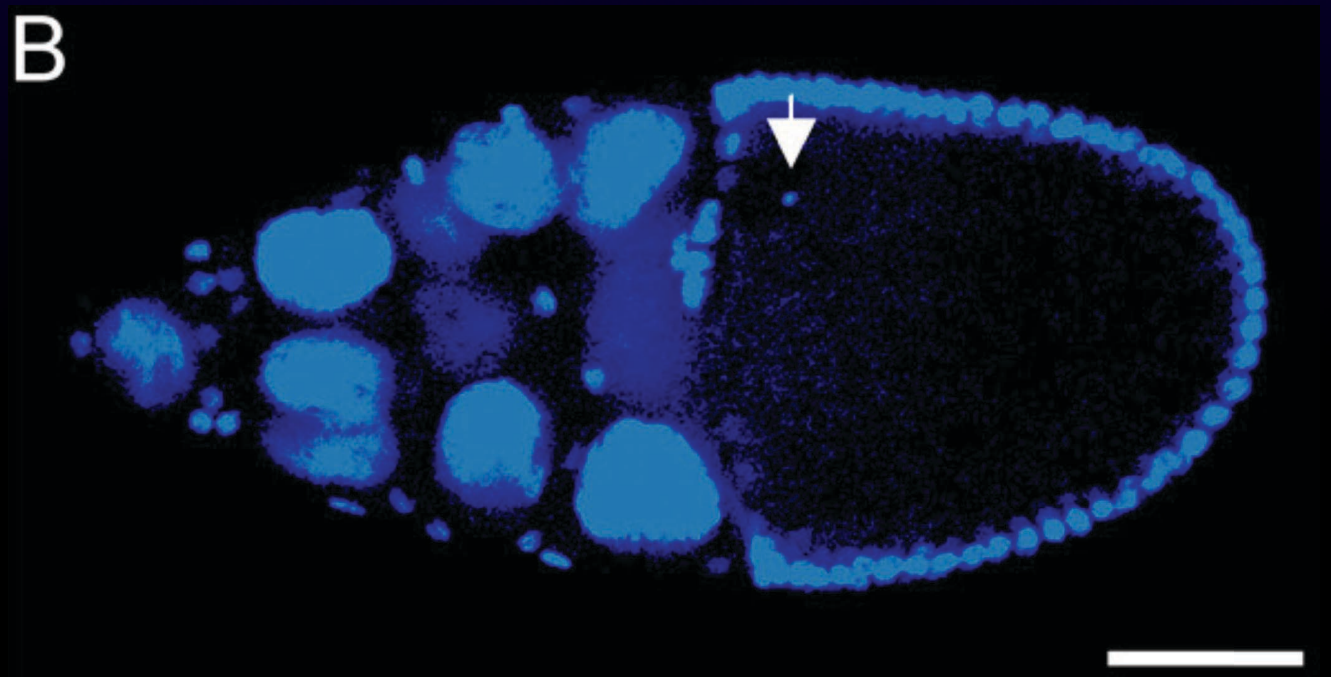
Laid by cac-/cac- mothers



Oocyte nucleus migrates to future anterior dorsal

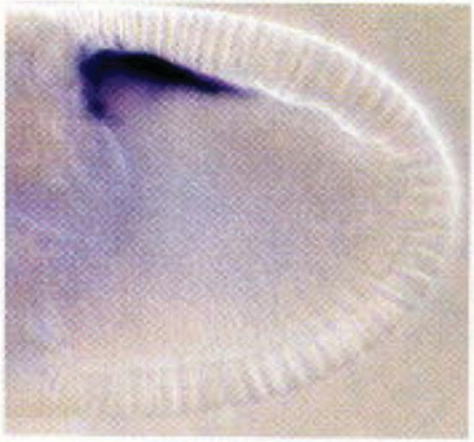
Makes gurken mRNA which stays localized


A


B

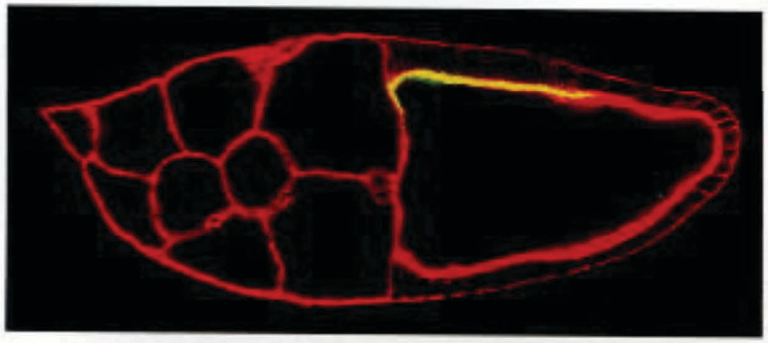
(A)

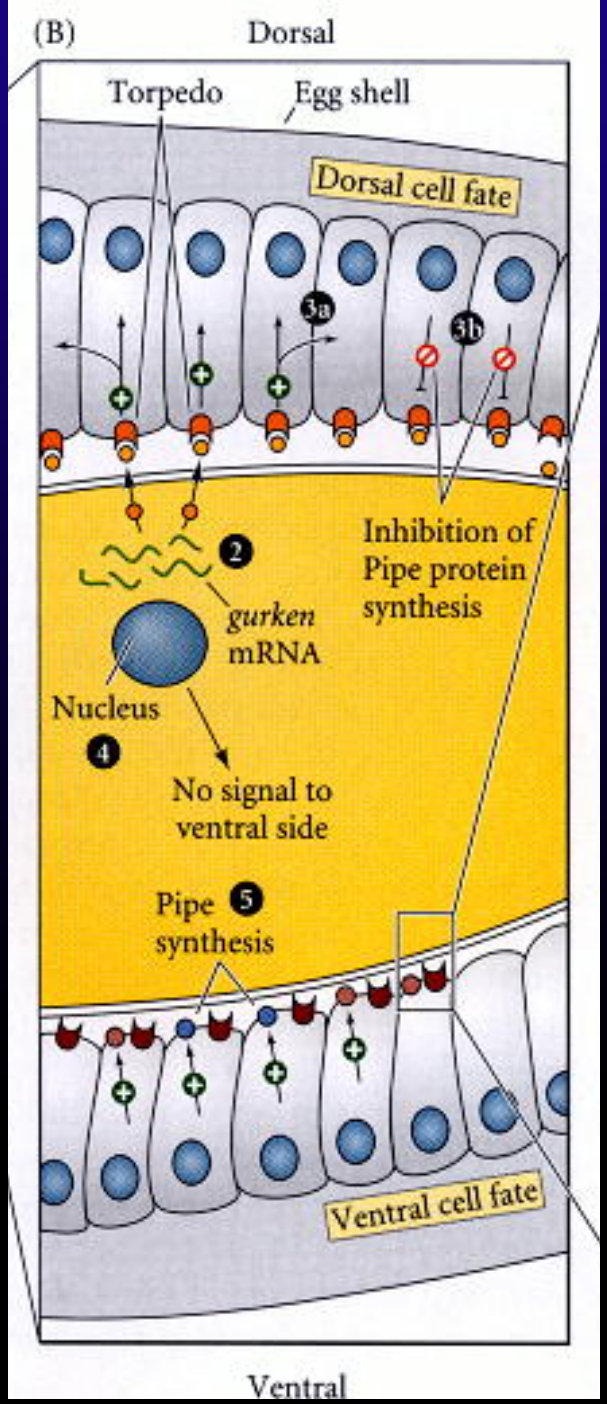
(B)



(C)

(D)



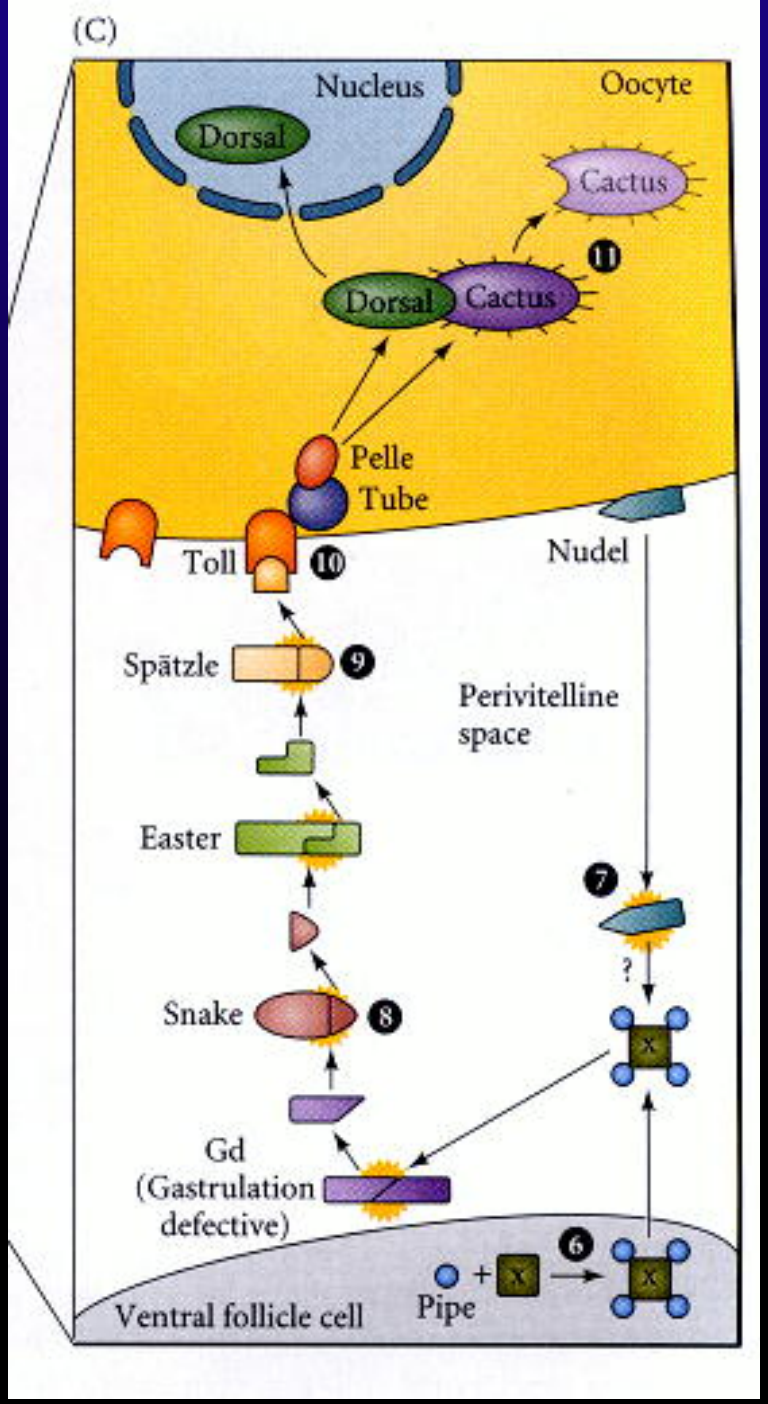


2. Gurken protein received by Torpedo receptor on follicle cells

3. Torpedo signal inhibits synthesis of Pipe protein.

4. Gurken protein never goes to the ventral side, and thus

5. These ventral follicle cells make Pipe protein

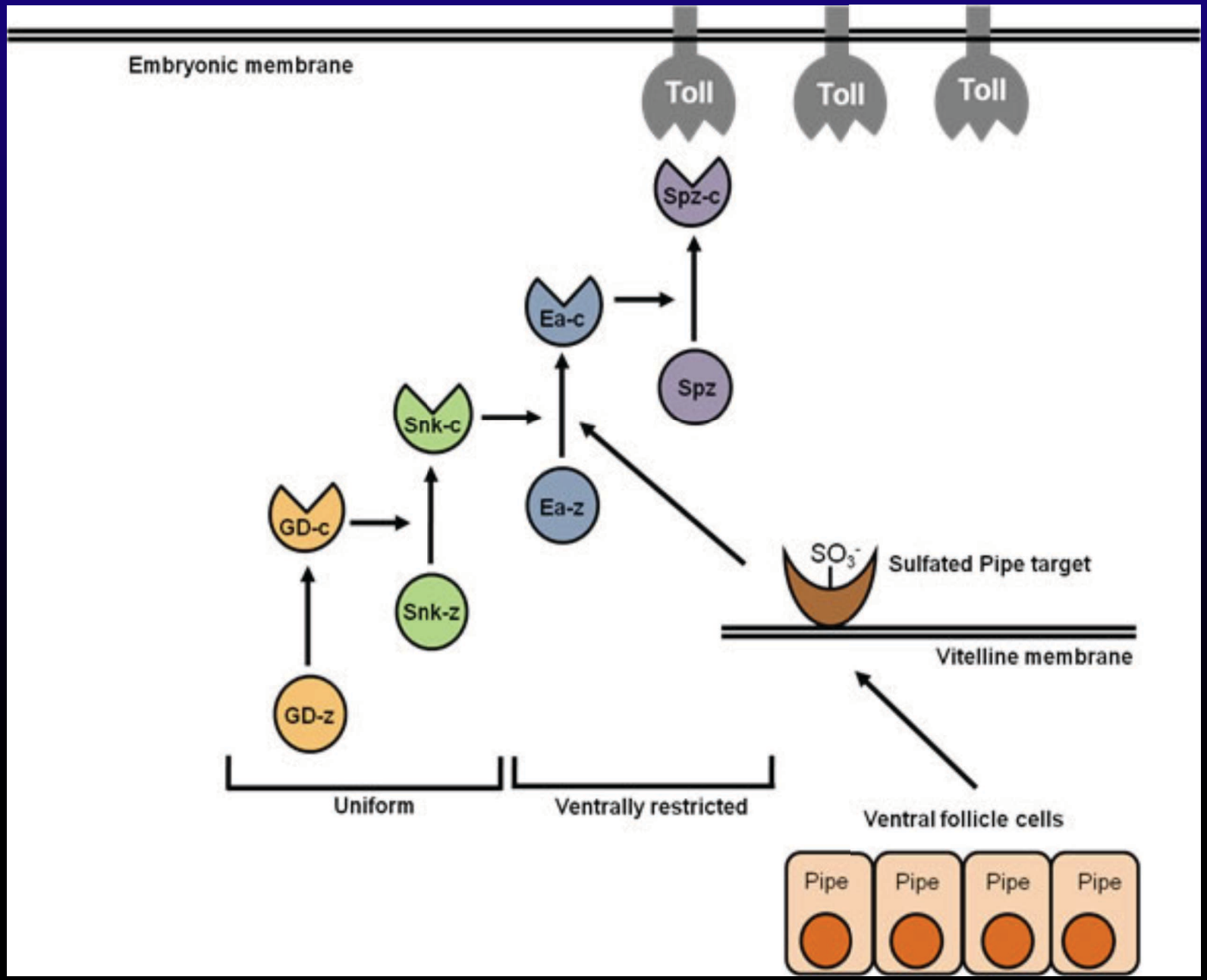


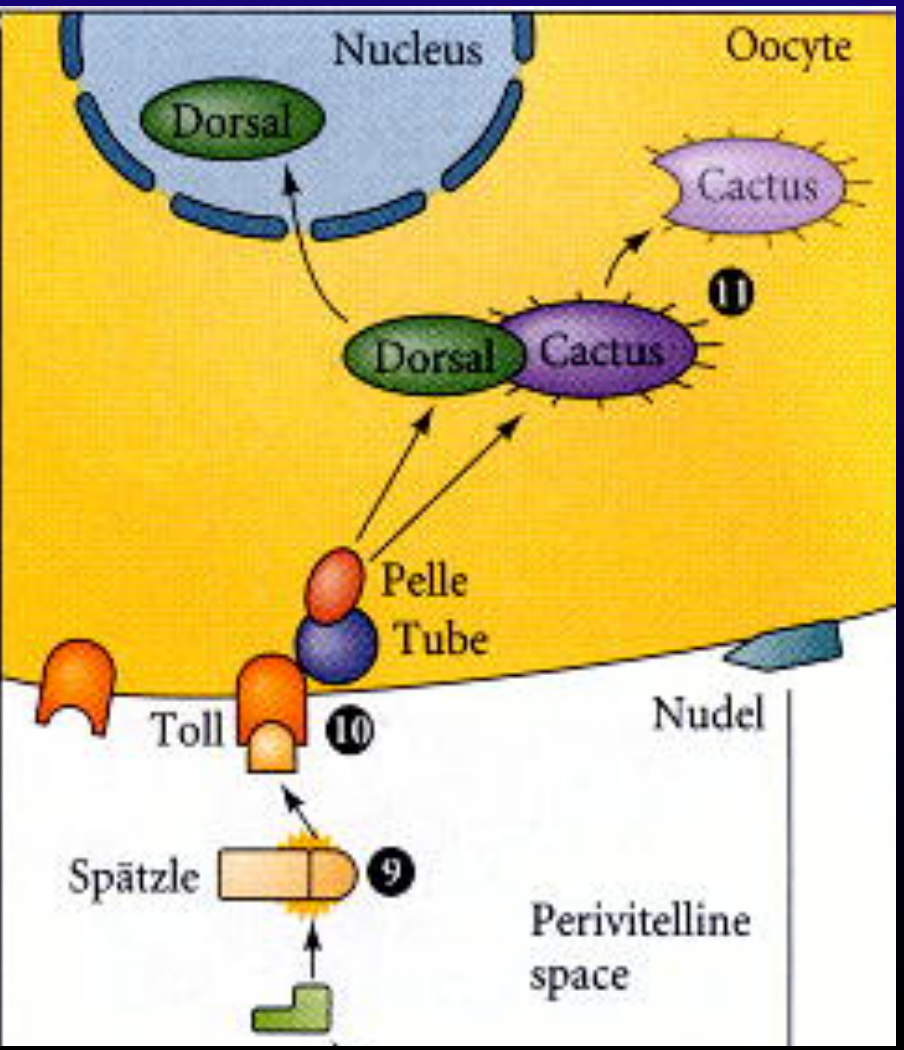
6. In ventral follicle cells, Pipe modifies an unknown factor X

7. Factor X + Nudel act together to split (cleave) Gd, which "activates" Gd.

8. Activated Gd cleaves and activates Easter.

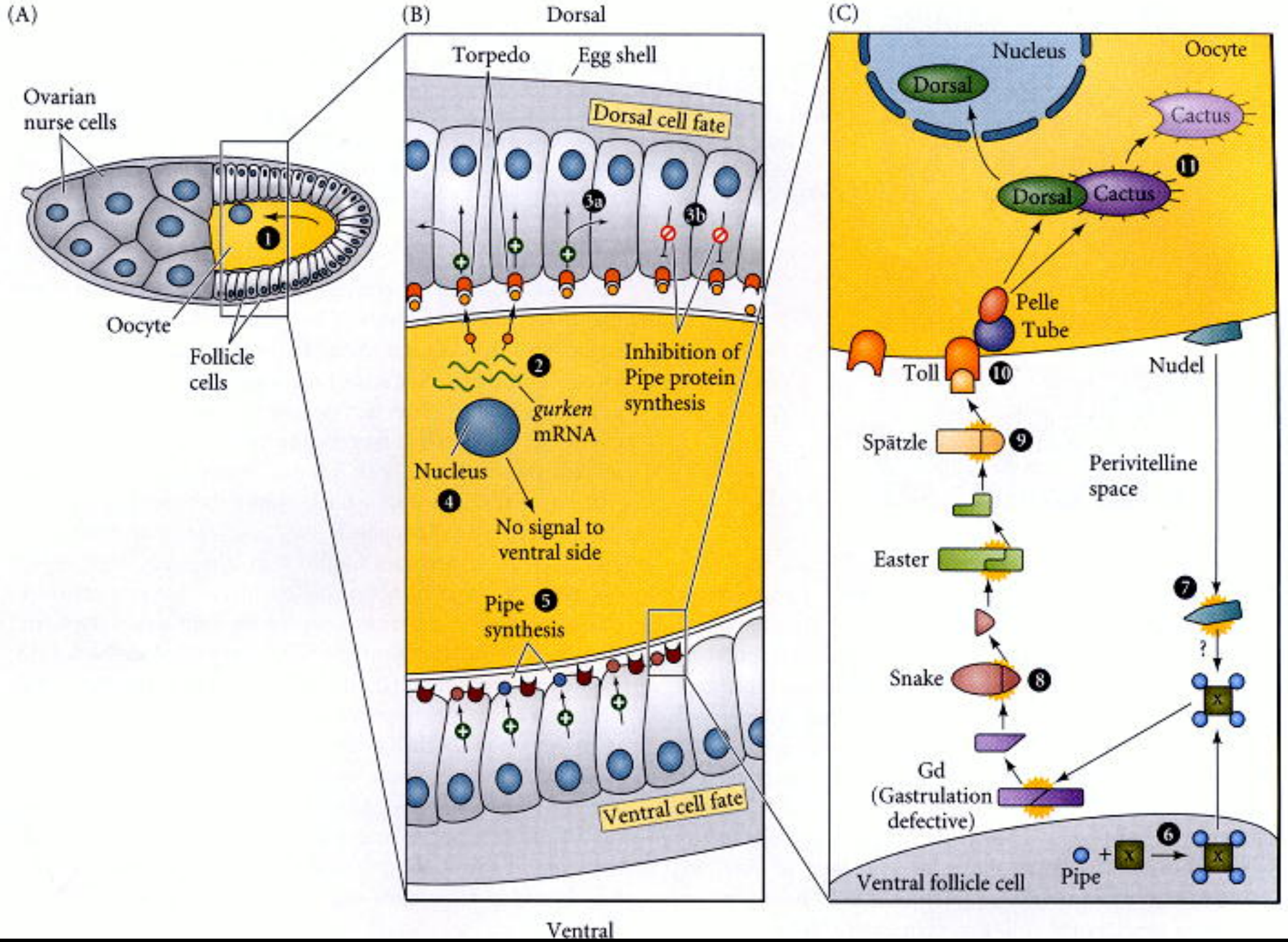
9. Activated Easter cleaves and activates Spätzle, which then Binds the Toll receptor.

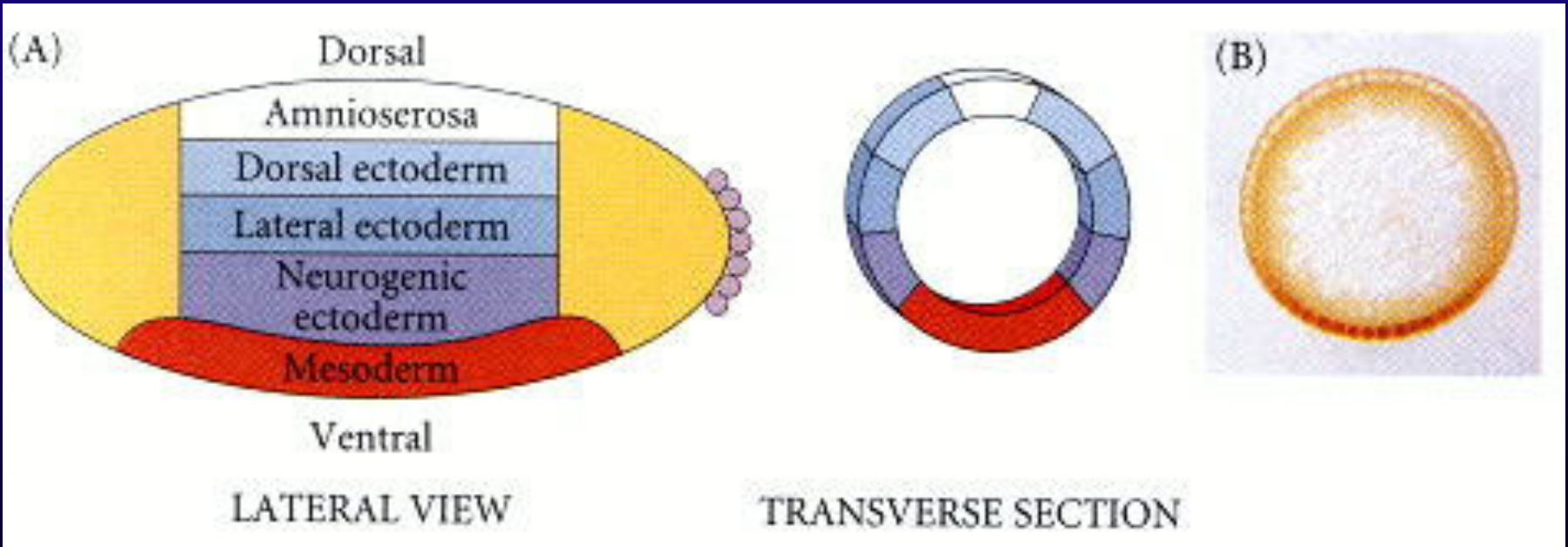

Embryonic membrane
Toll
Toll
Toll
Spz-c
Ea-c
Spz
Snk-c
Ea-z
GD-c
Snk-z
GD-z
SO_3^-
Sulfated Pipe target
Vitelline membrane
Uniform
Ventrally restricted
Ventral follicle cells
Pipe
Pipe
Pipe
Pipe



10. Toll activation activates Tube and Pelle. Tube and Pelle phosphorylate the Cactus protein. This causes the Cactus protein to rapidly degrade, releasing Dorsal protein.

11. Now Dorsal protein can leave the cytoplasm and go into the nucleus

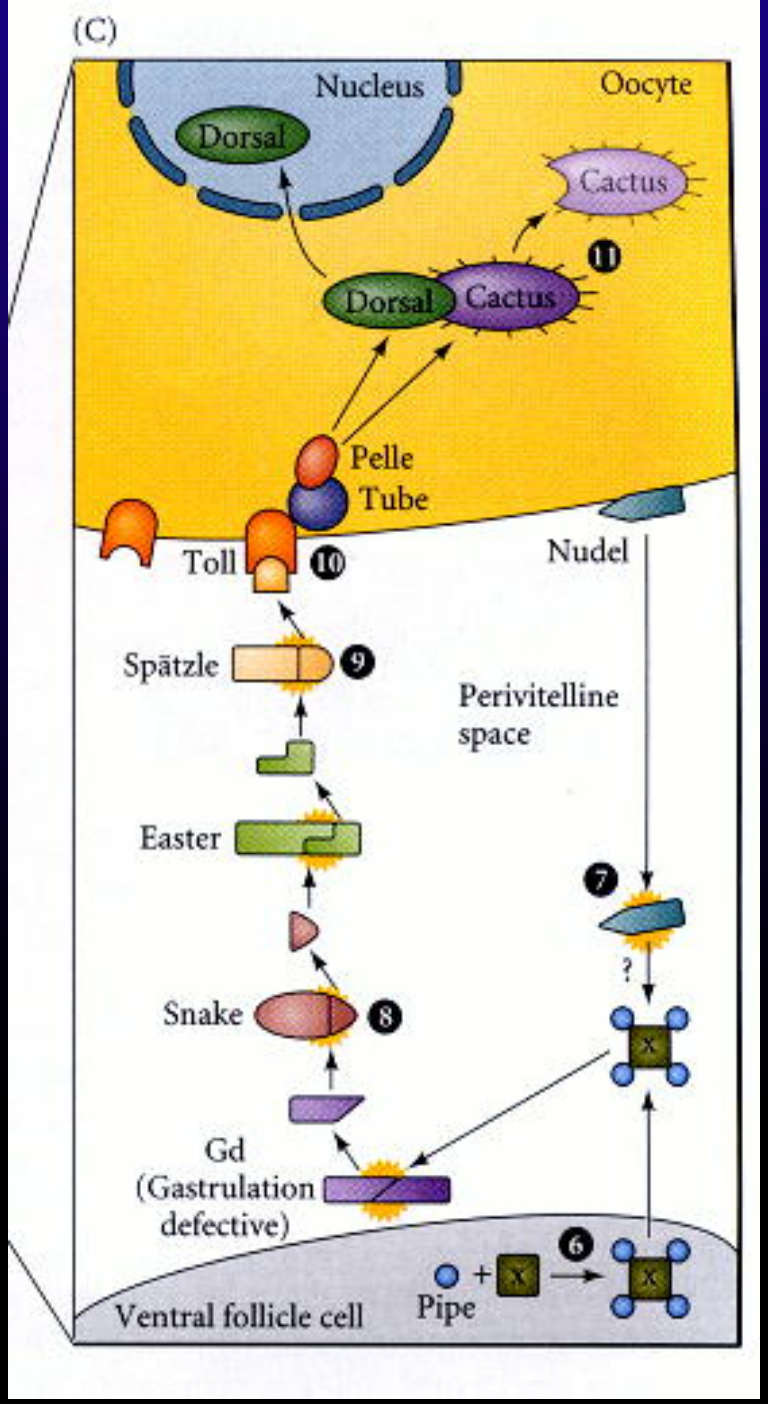

(A)
Ovarian nurse cells
Oocyte
Follicle cells
(B)
Dorsal
Torpedo
Egg shell
Dorsal cell fate
Inhibition of Pipe protein synthesis
gurken mRNA
Nucleus
No signal to ventral side
Pipe synthesis
Ventral cell fate
Ventral
(C)
Nucleus
Oocyte
Dorsal
Cactus
Dorsal
Cactus
Pelle
Tube
Toll
Nudel
Spätzle
Perivitelline space
Easter
Snake
Gd (Gastrulation defective)
Ventral follicle cell
Pipe



Dorsal protein only enters nucleus
in cells on ventral side

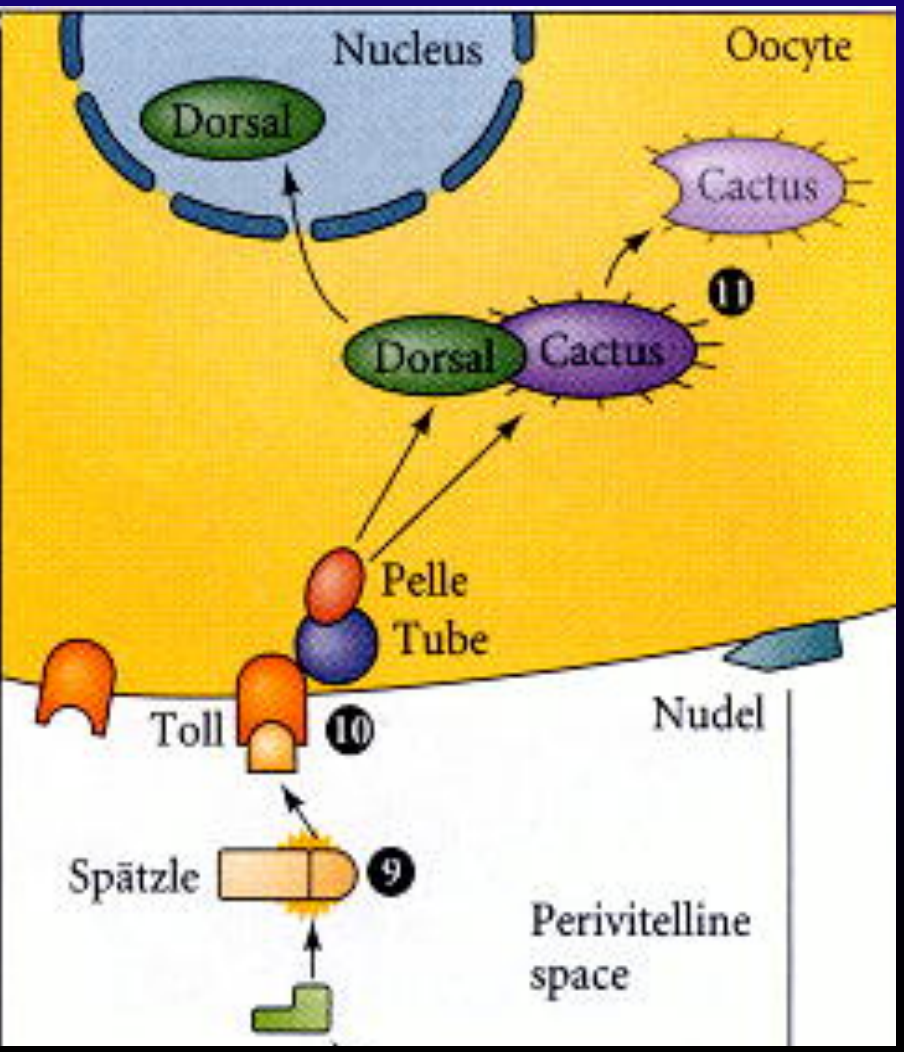
Mother puts dorsal mRNA in the egg uniformly.

The dorsal mRNA is translated into protein everywhere,
but dorsal protein stays in the cytoplasm dorsally,
and enters the nucleus only on the ventral side



What is the phenotype of eggs laid by a female lacking a functional easter gene?

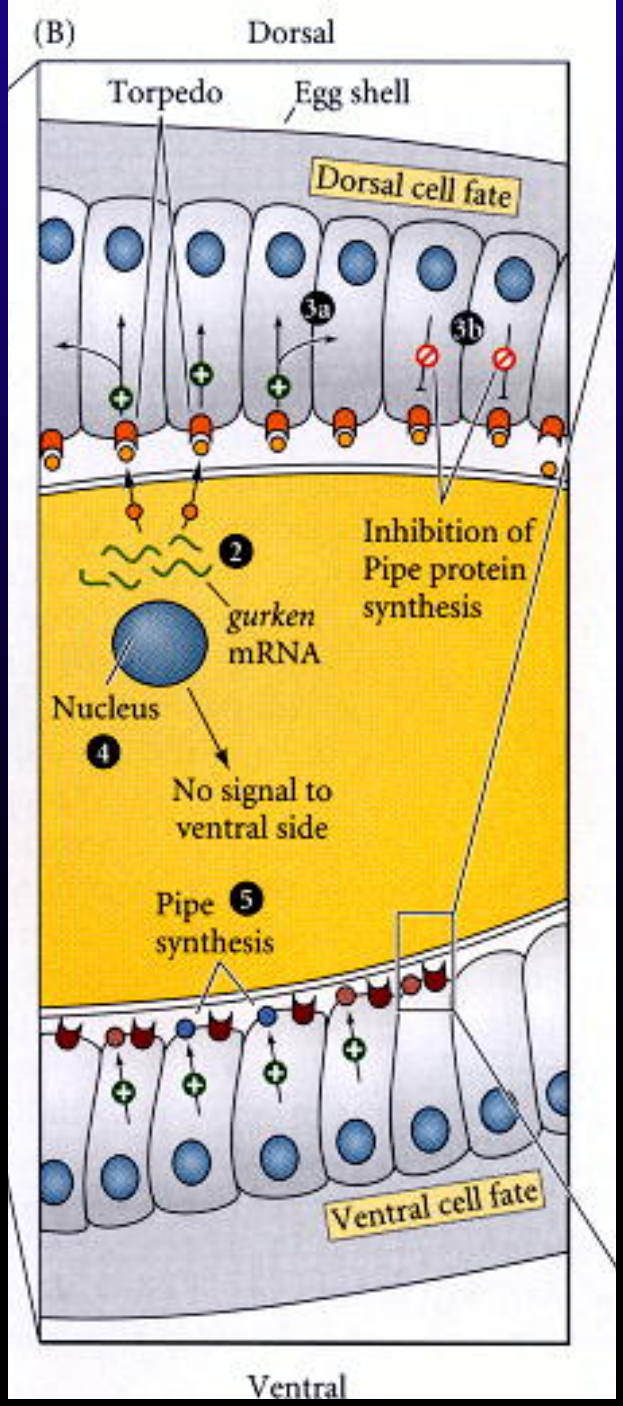
What would happen if you made pre-cleaved Easter protein *in vitro* (in a test tube), and injected that protein into the space around the developing embryo?



You discover an allele of Toll that is dominant (Toll-D).

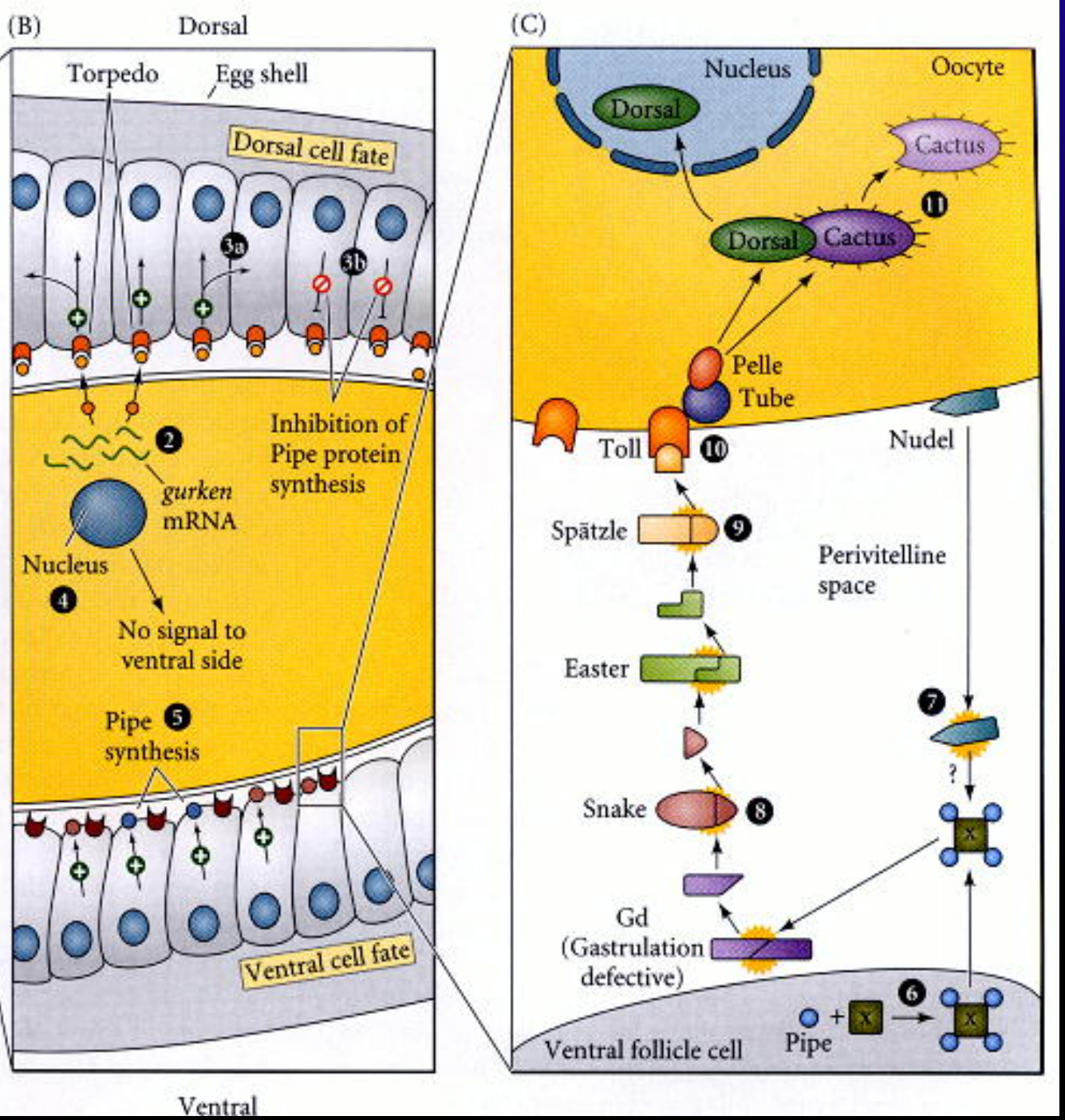
The phenotype of embryos produced by mothers carrying a copy of Toll-D is ventralized.

How could this happen?



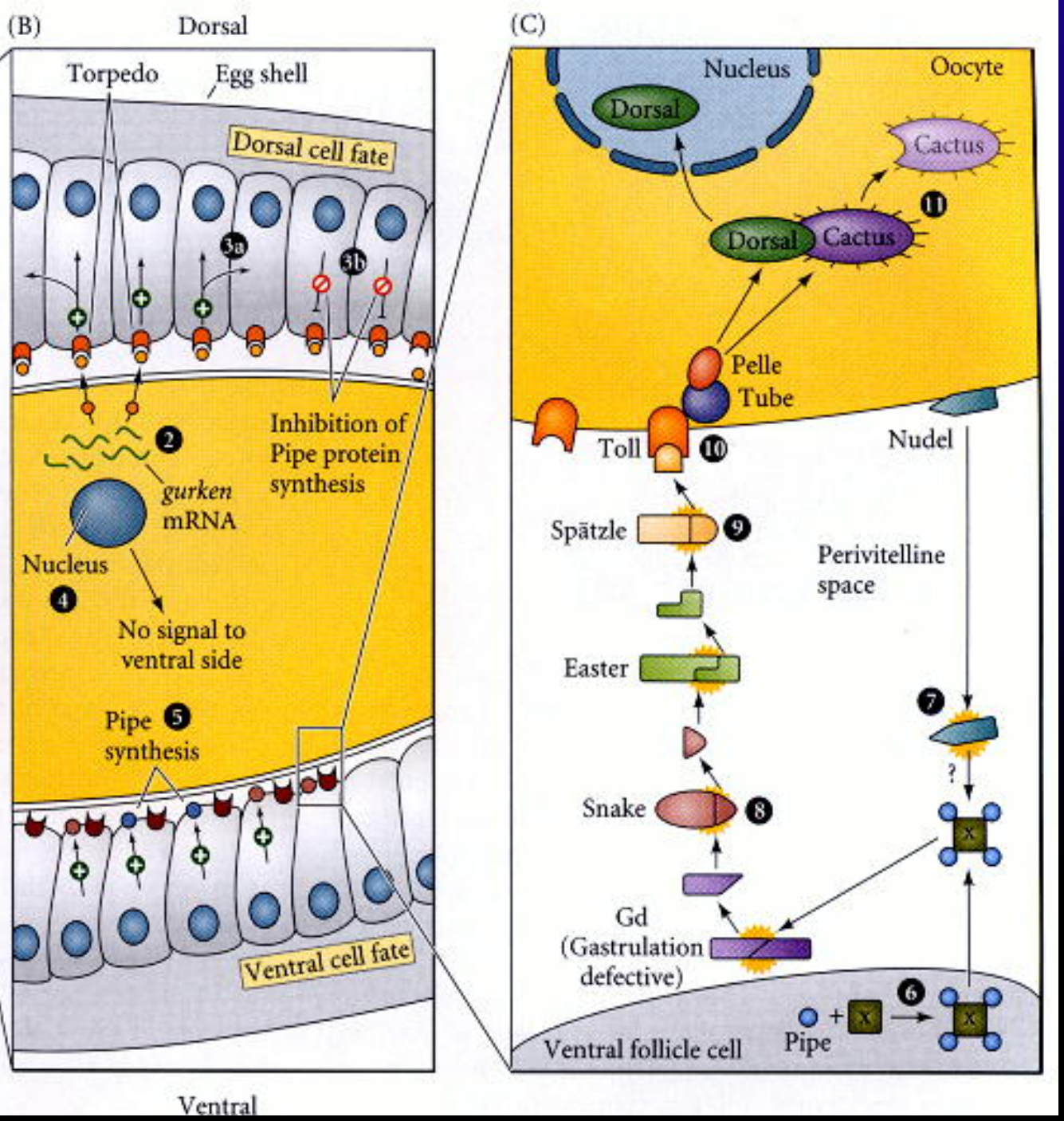
What is the phenotype of eggs laid by a female lacking a functional gurken gene?

What would happen if gurken mRNA and protein were uniformly distributed in the developing egg?



Ventralize
gurken
torpedo
Toll-D
cactus

Dorsalize
pipe
Gd
snake
easter
spatzle
Toll
pelle
tube
dorsal



gurken/torpedo

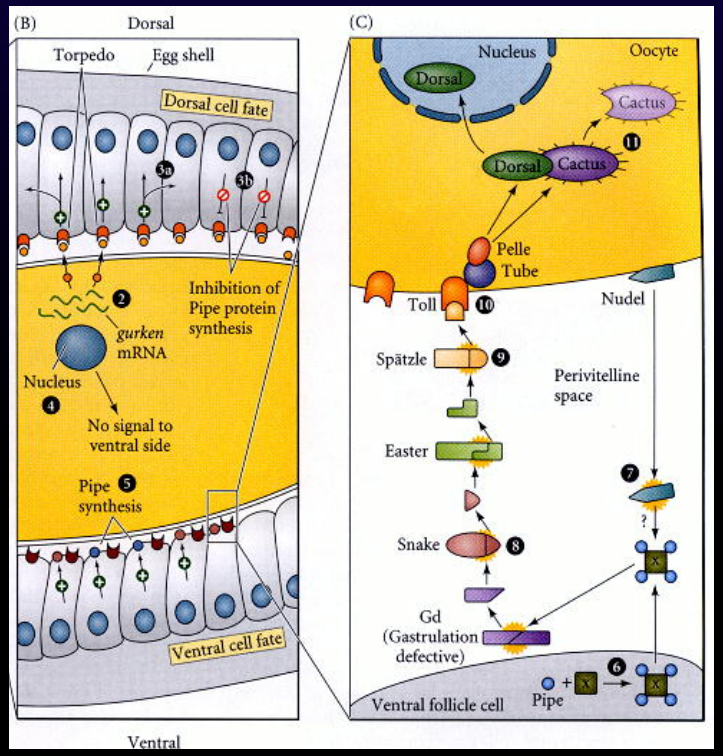
pipe
Gd
snake
easter
spatzle

Toll/Toll-D

tube
Pelle
cactus

dorsal

How did people figure out the order?



gurken/torpedo

pipe
Gd
snake
easter
spatzle

Toll/Toll-D

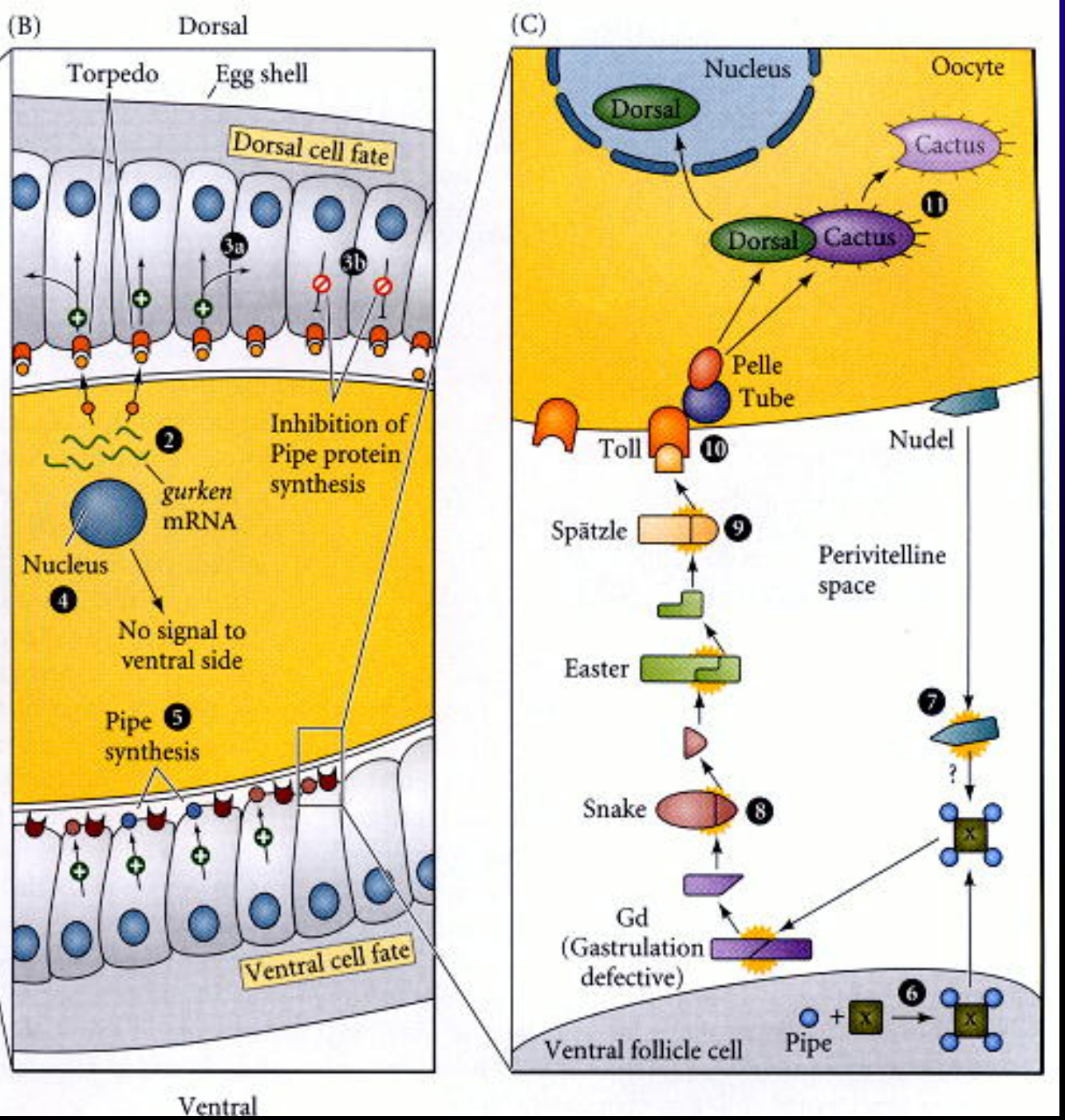
tube
Pelle
cactus

dorsal

Make double mutants

Genetic epistasis

Tells you order of a pathway



Ventralize
gurken
torpedo
Toll-D
cactus

Dorsalize
pipe
Gd
snake
easter
spatzle
Toll
pelle
tube
dorsal