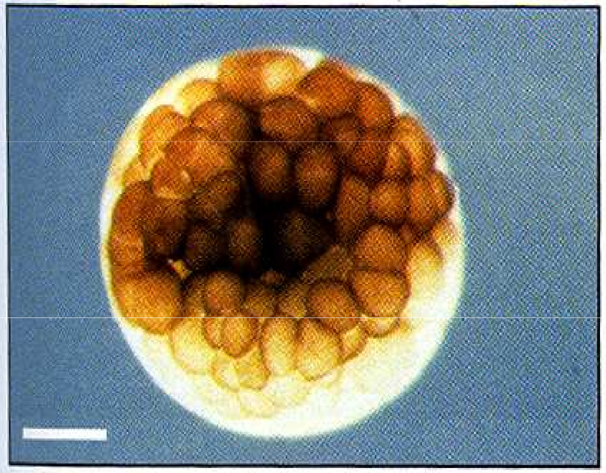
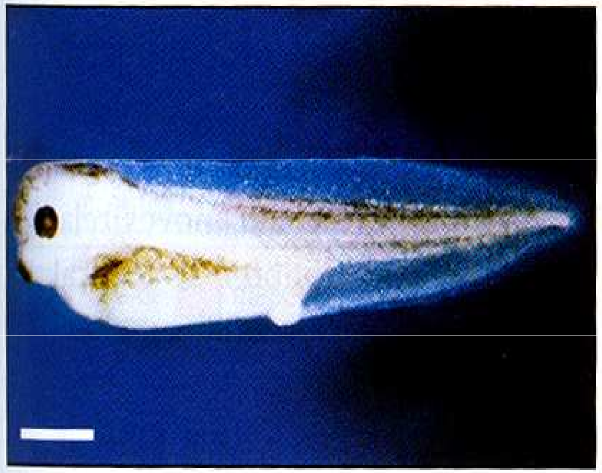
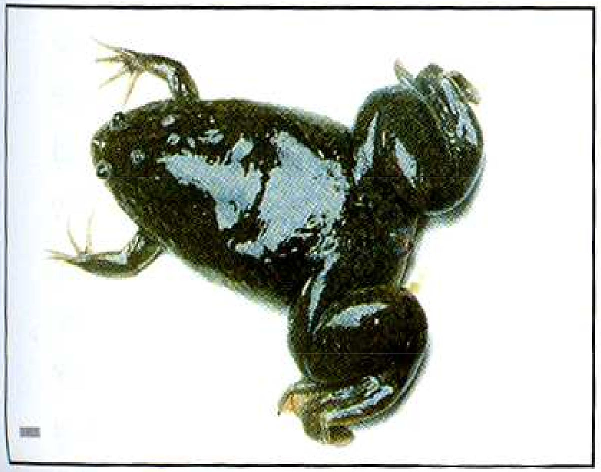
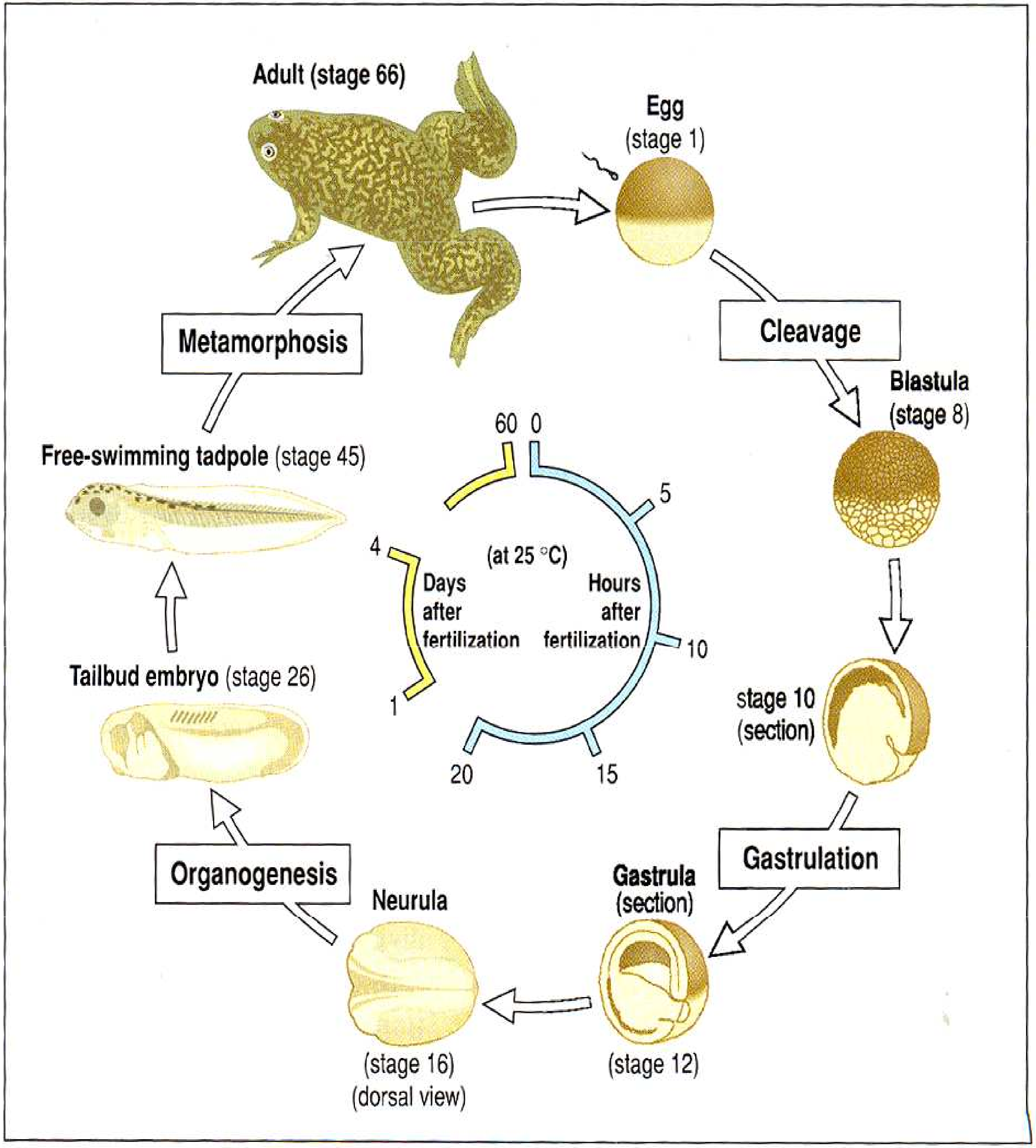
MATERNAL mRNA LOCALIZATION IN THE FROG DEVELOPMENT

Putting RNAs at the right place in the right time








Adult (stage 66)
Egg
(stage 1)
Cleavage
Blastula
(stage 8)
stage 10
(section)
Gastrulation
Gastrula
(section)
(stage 12)
Neurula
(stage 16)
(dorsal view)
Organogenesis
Tailbud embryo (stage 26)
Free-swimming tadpole (stage 45)
Metamorphosis
60
0
5
10
15
20
1
4
(at 25 °C)
Days
after
fertilization
Hours
after
fertilization

CLEAVAGE MOVIE

ACCURACY AND REPRODUCIBILITY IN ACQUIRING THE CELLULAR FATE WITHIN THE EMBRYO

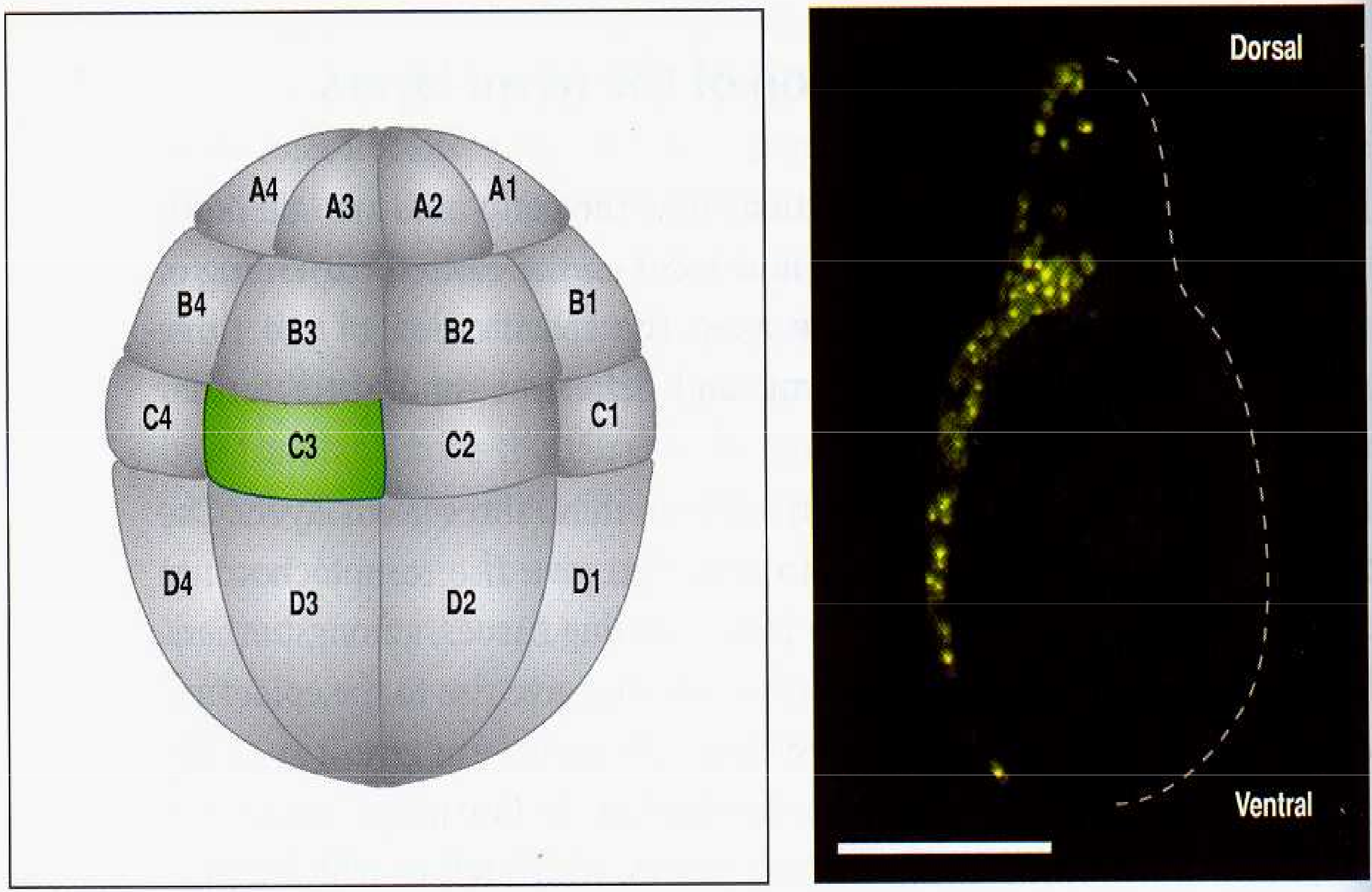
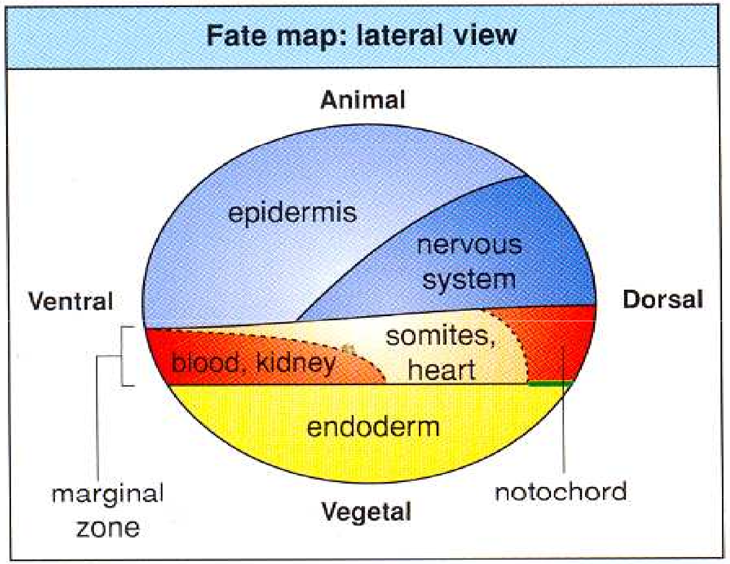


Fig. 3.17 Fate mapping of the early *Xenopus* embryo. Left panel: a single cell in the embryo, C3, is labeled by injecting fluorescein-dextran-amine, which fluoresces green under UV light. Right panel: a cross-section of the embryo, made at the tailbud stage, shows that the labeled cell has given rise to mesoderm cells on one side of the embryo. Scale bar = 0.5 mm.

Photograph courtesy of L. Dale.



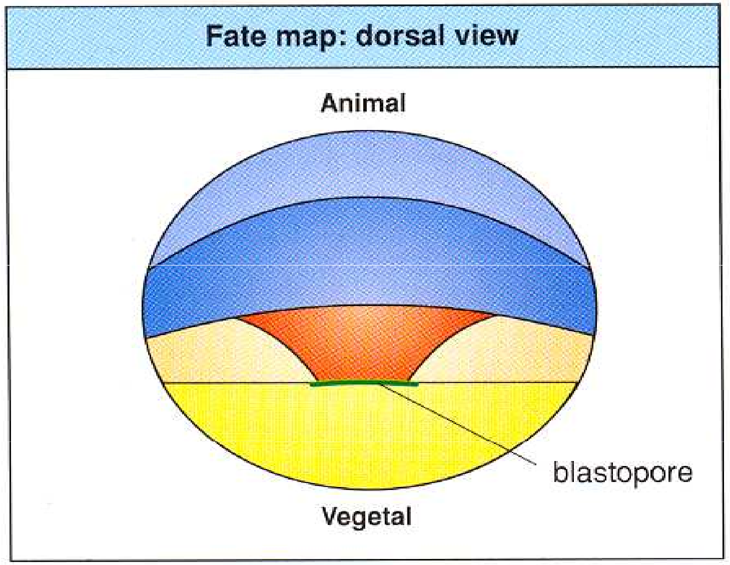
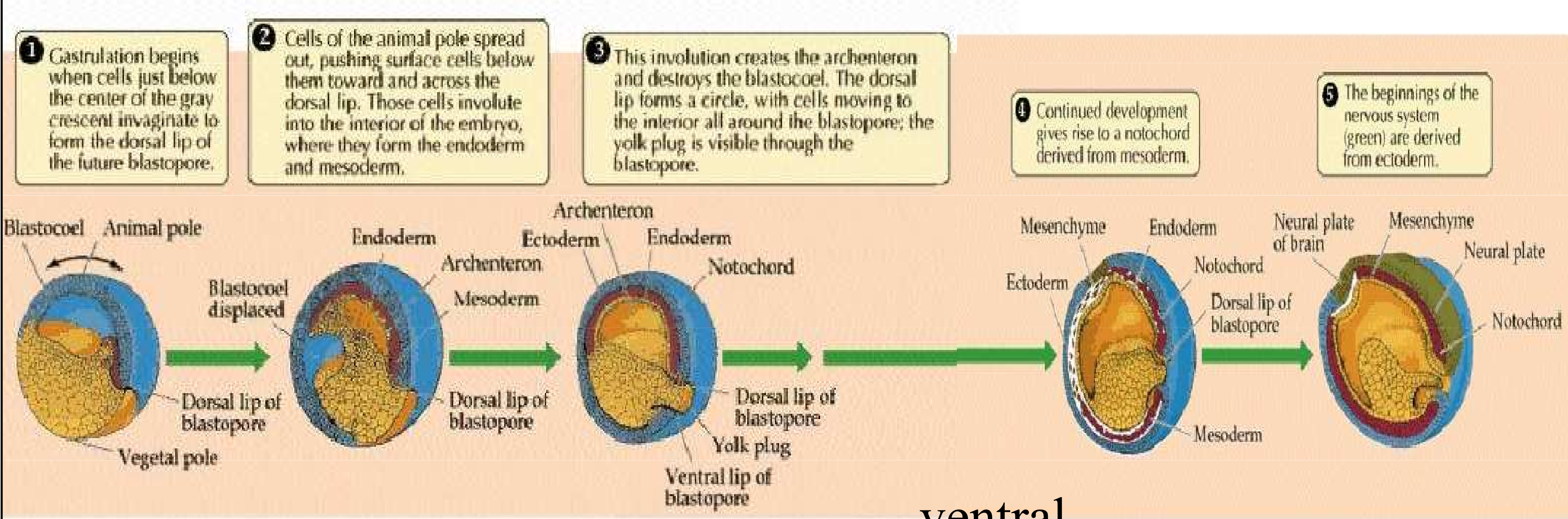
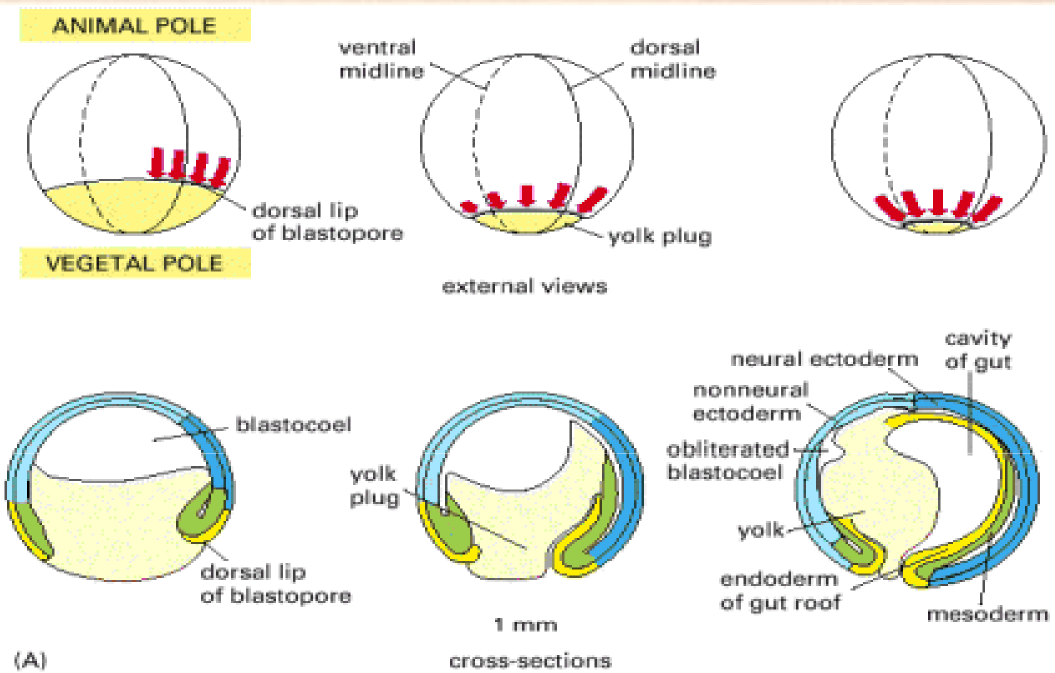


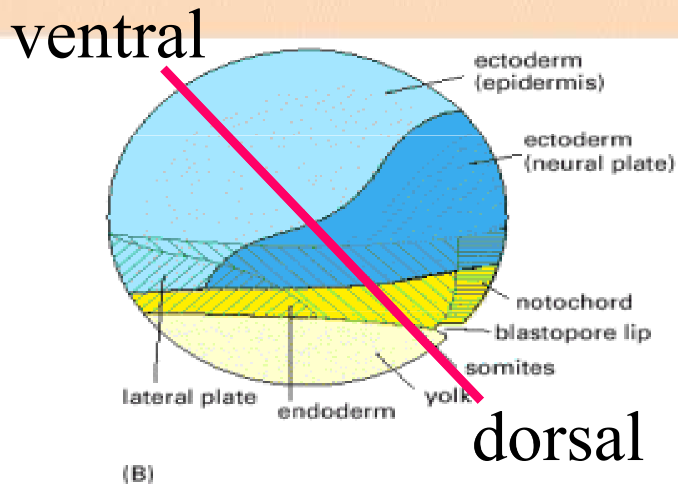
Fig. 3.18 Fate map of a late *Xenopus* blastula. The ectoderm gives rise to the epidermis and nervous system. Along the dorso-ventral axis the mesoderm gives rise to notochord, somites, heart, kidneys, and blood. Note that blood can also form in more dorsal regions. In *Xenopus*, although not in all amphibians, there is also endoderm (not shown here) overlying the mesoderm in the marginal zone.

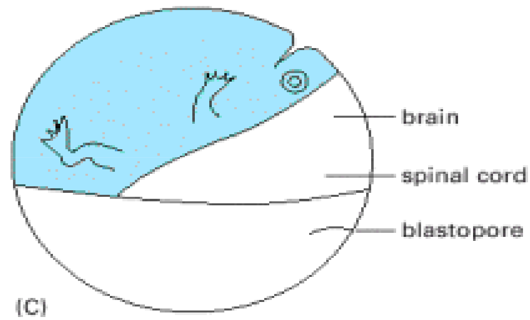
MANY CLEAVAGES MOVIE

GASTRULATION/NEURULATION





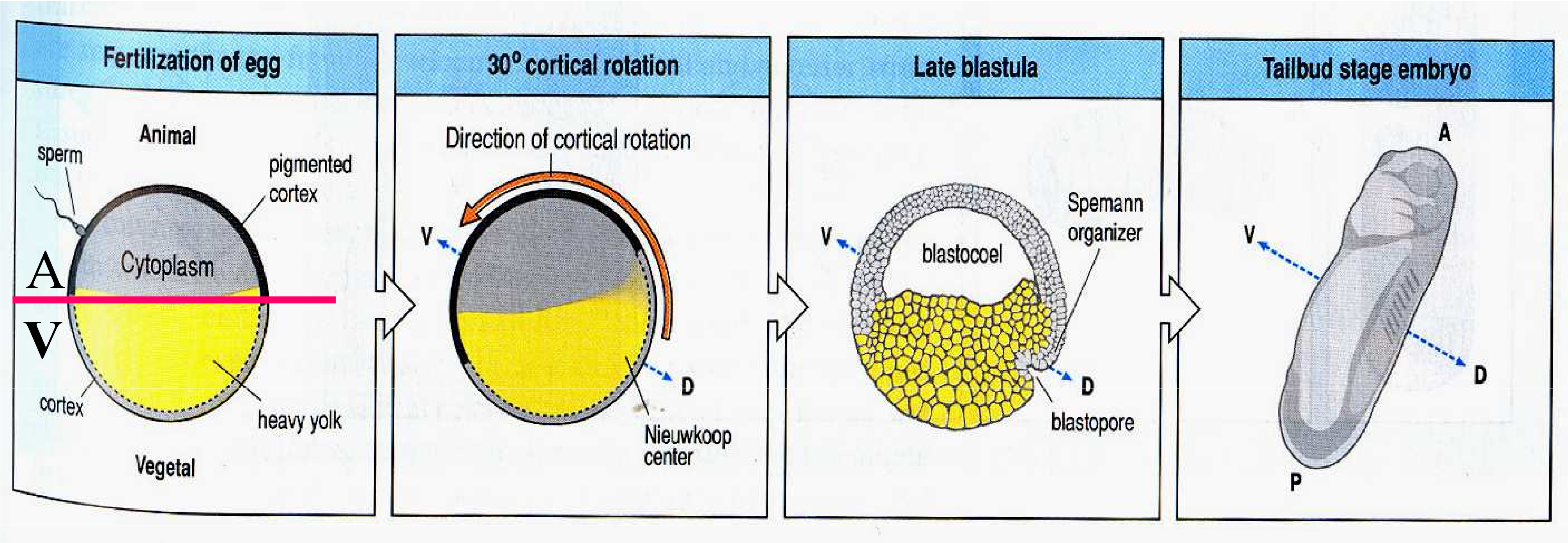




GASTRULATION MOVIE 1 – internal cell movements

GASTRULATION MOVIE 2 – dorsal surface view – blastoporus closure

Dorso-ventral axis is set-up by site of sperm entry



GASTRULATION/NEURULATION – dorsal surface view

Differential mRNA localization to subcelular compartments

-allows for spatial regulation of gene expression
-essential for polarity set-up in oogenesis
-patterning during embryogenesis
-in *Xenopus*: localized maternal mRNAs generate developmental polarity along the animal/vegetal axis.

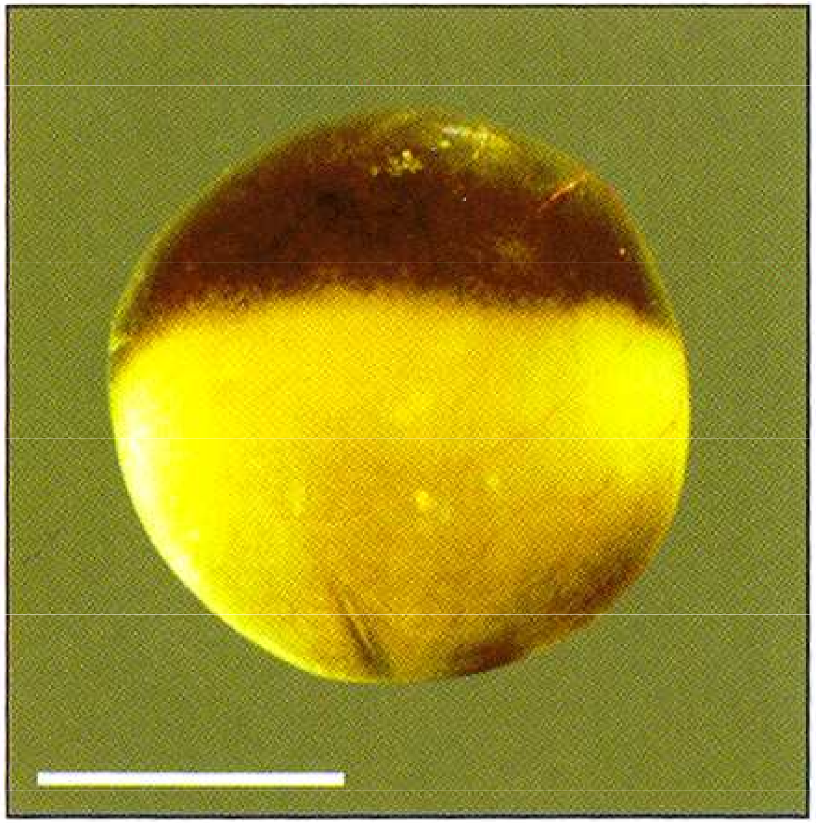
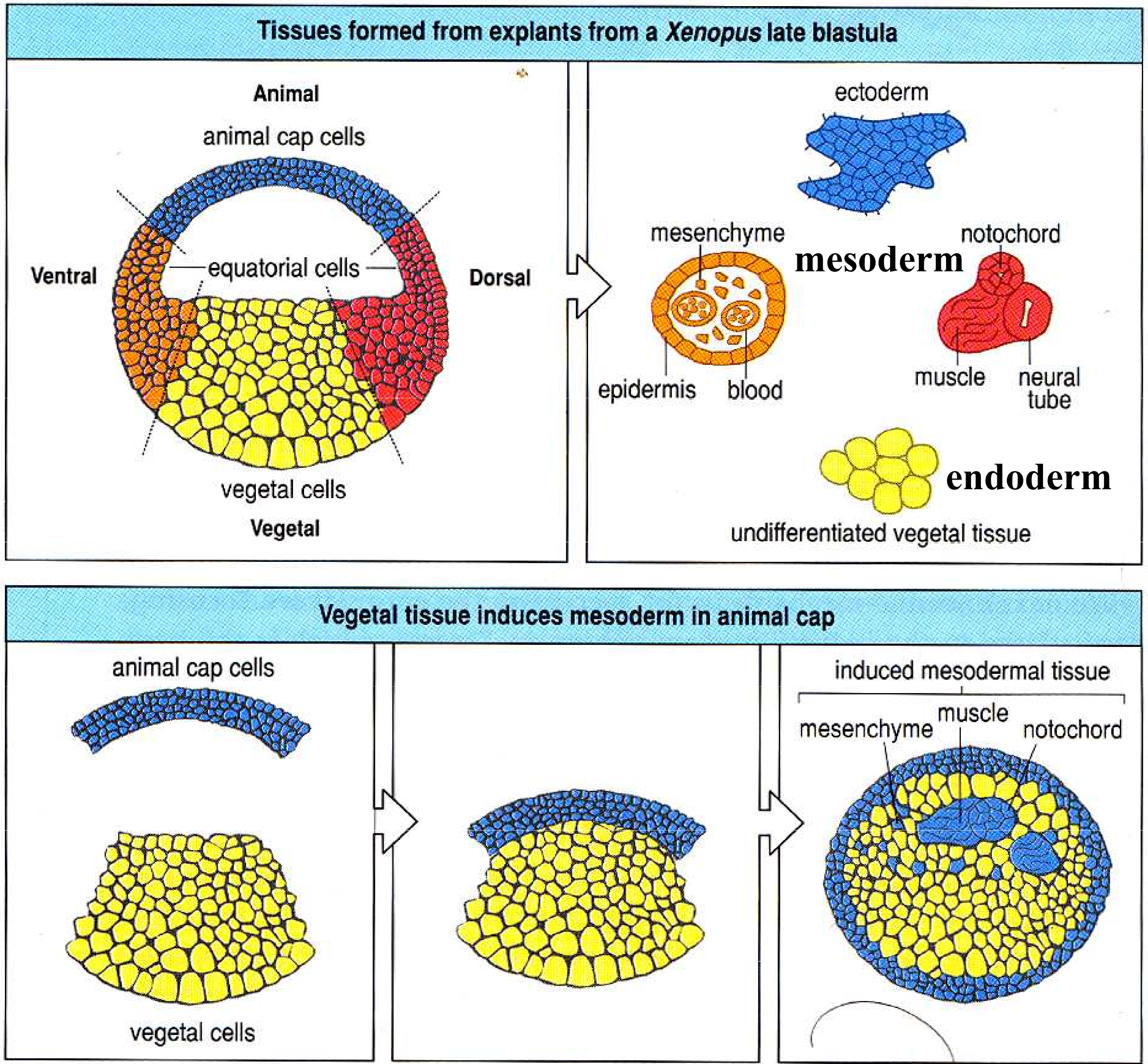


Fig. 2.4 The unfertilized egg of *Xenopus*. The surface of the animal half (top) is pigmented and the paler, vegetal half of the egg is heavy with yolk. Scale bar = 1 mm.

Photograph courtesy of J. Smith.

CELL-TO-CELL SIGNALING vs.MATERNAL FACTORS IN TISSUE SPECIFICATION

Ectoderm and endoderm are specificed by maternal factors in the egg. Versus mesoderm that is induced by vegetal tissue

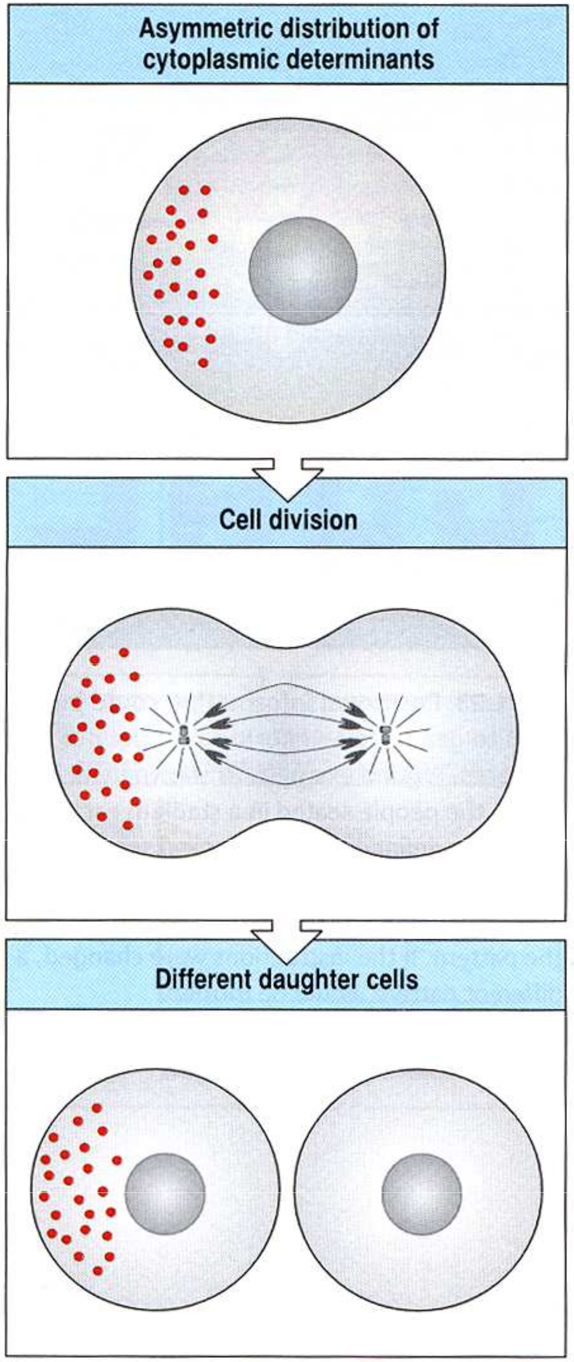


MATERNAL vs. ZYGOTIC REGULATORS

Summary: genes involved in patterning of axes and germ layers

Gene	Maternal/ Zygotic	Type of protein	Where expressed	Effects
activin	Z	TGF-β family	?	mesoderm induction
BMP-4	Z	transcription factor	late blastula	ventralizes mesoderm
Brachyury	Z	transcription factor	early mesoderm	mesoderm development
β-catenin	M	gene regulatory protein	egg	dorsalizing signal
cerberus	Z	secreted	vegetal egg	mesoderm inhibition
chordin	Z	secreted signal molecule	organizer	dorsalizes mesoderm
derriere	Z	TGF-β family	vegetal egg	mesoderm induction
fibroblast growth factor	Z	secreted signal molecule	blastula	ventral mesoderm induction
goosecoid	Z	transcription factor	organizer	organizer function
GSK-3	M	protein kinase	egg	suppresses dorsalizing signals
HNF-3β	Z	transcription factor	organizer	organizer development
noggin	M/Z	secreted	organizer	dorsalizes mesoderm
Pintallavis	Z	transcription factor	organizer	?
siamois	Z	transcription factor	dorsal blastula	dorsalizing signal
VegT	M	transcription factor	vegetal egg	induces endoderm and mesoderm signals
Vg-1	M	TGF-β family	vegetal egg	mesoderm induction
Xlim-1	Z	transcription factor	organizer	?
Xnot	Z	transcription factor	organizer	notochord specification
Xnr-1	Z	secreted	vegetal egg	mesoderm induction
Xnr-2	Z	secreted	vegetal egg	mesoderm induction
Xnr-4	Z	secreted	vegetal egg	mesoderm induction
Xwnt-11	M	Wnt family	vegetal egg	mesoderm induction
Xwnt-8	Z	Wnt family	propective mesoderm	ventralizes mesoderm

Vg1 (TGFβ family ligand)



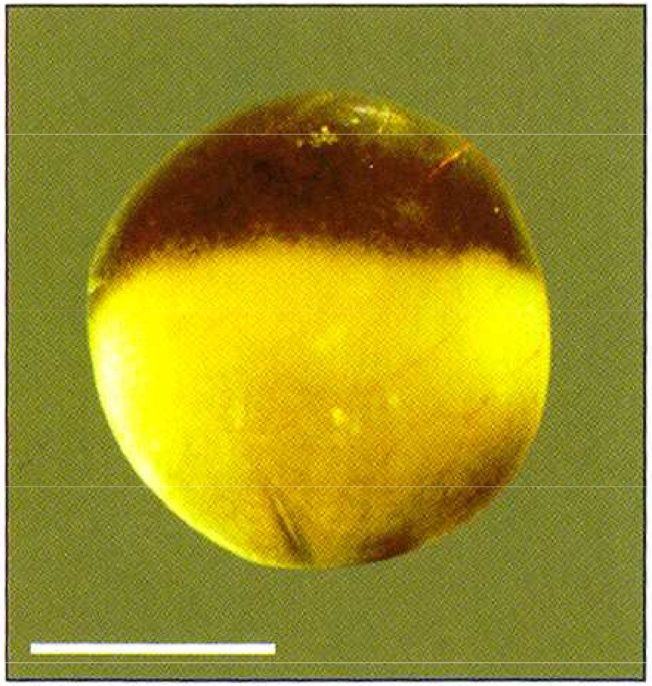


Fig. 2.4 The unfertilized egg of *Xenopus*. The surface of the animal half (top) is pigmented and the paler, vegetal half of the egg is heavy with yolk. Scale bar = 1 mm.

Photograph courtesy of J. Smith.

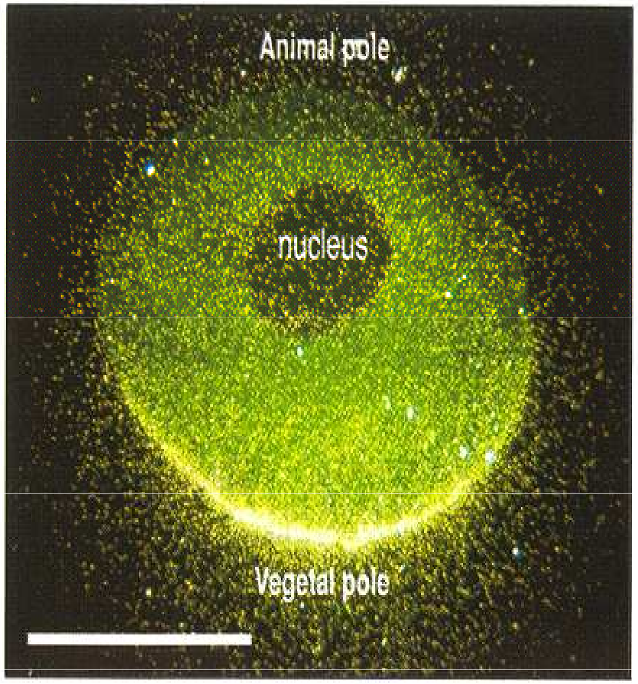
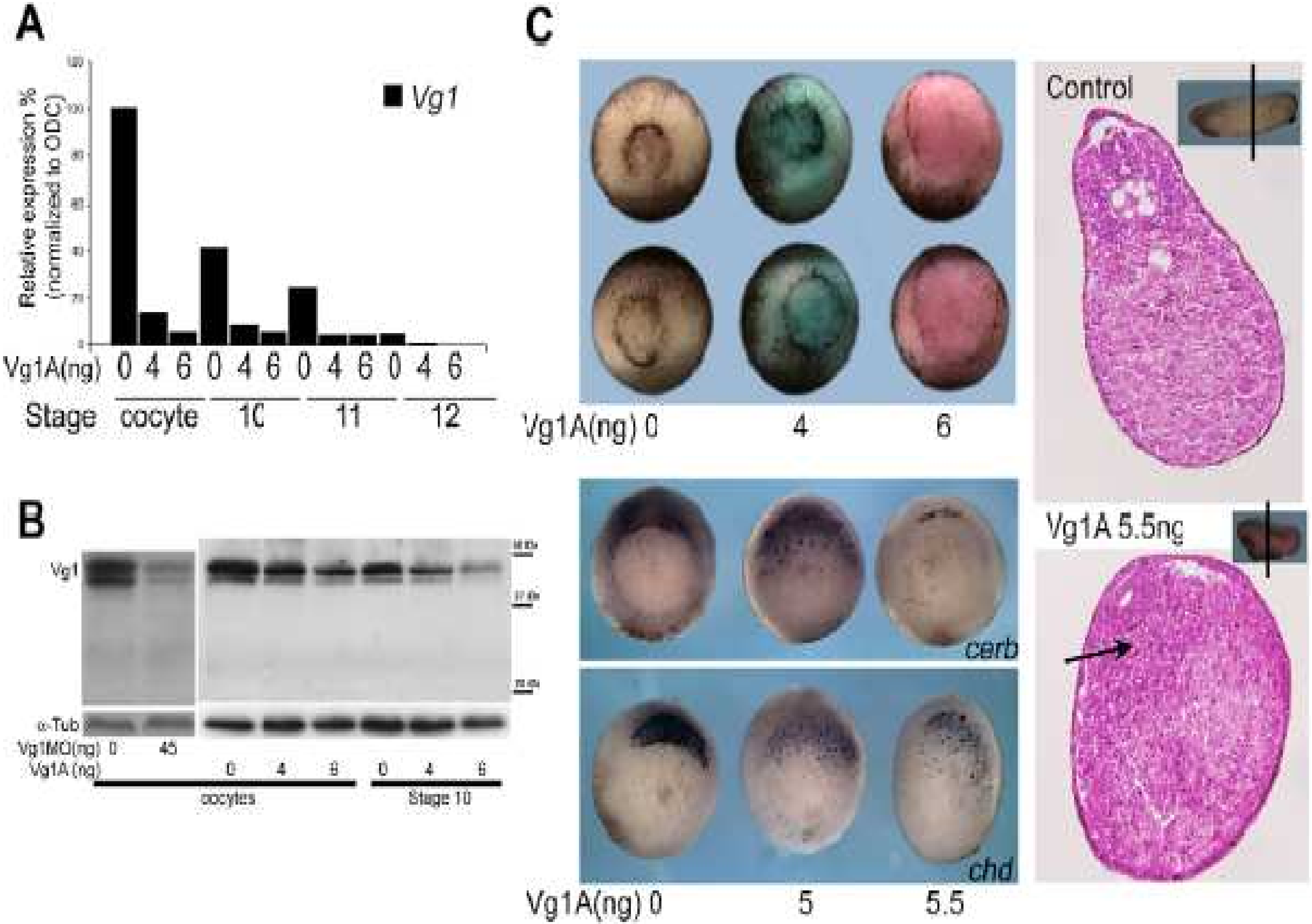


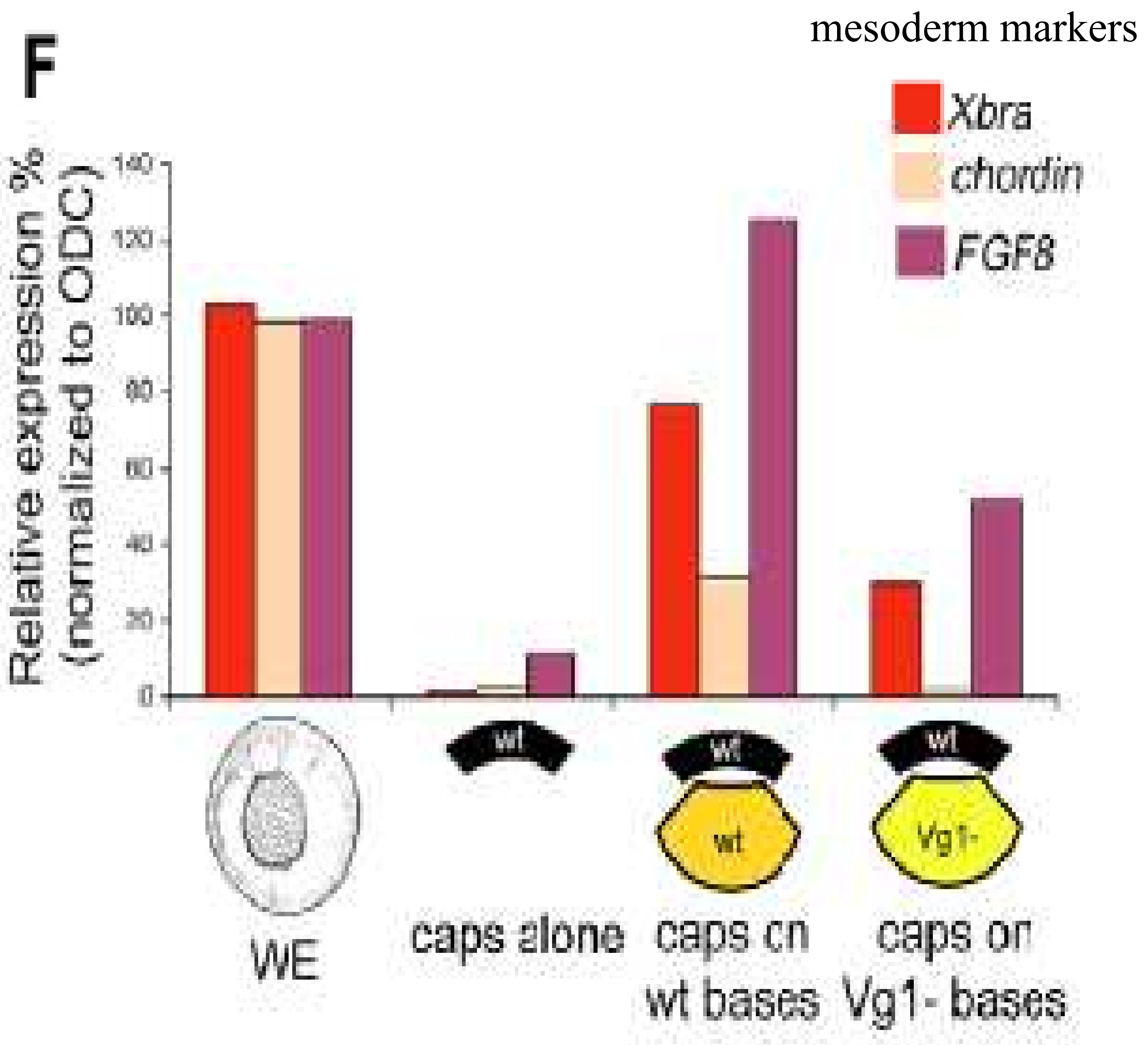
Fig. 3.2 Distribution of mRNA for the growth factor Vg-1 in the amphibian egg. *In situ* hybridization with a radioactive probe for maternal Vg-1 mRNA shows its localization (yellow) in the vegetal region. Scale bar = 1 mm.

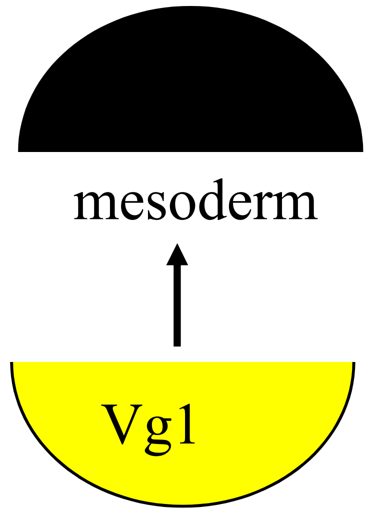
Photograph courtesy of D. Melton.

Vg1 depletion by morpholinos delayes gastrulation and mesoderm induction with loss of head structures, absence of notochord and fusion of somites (arrow)



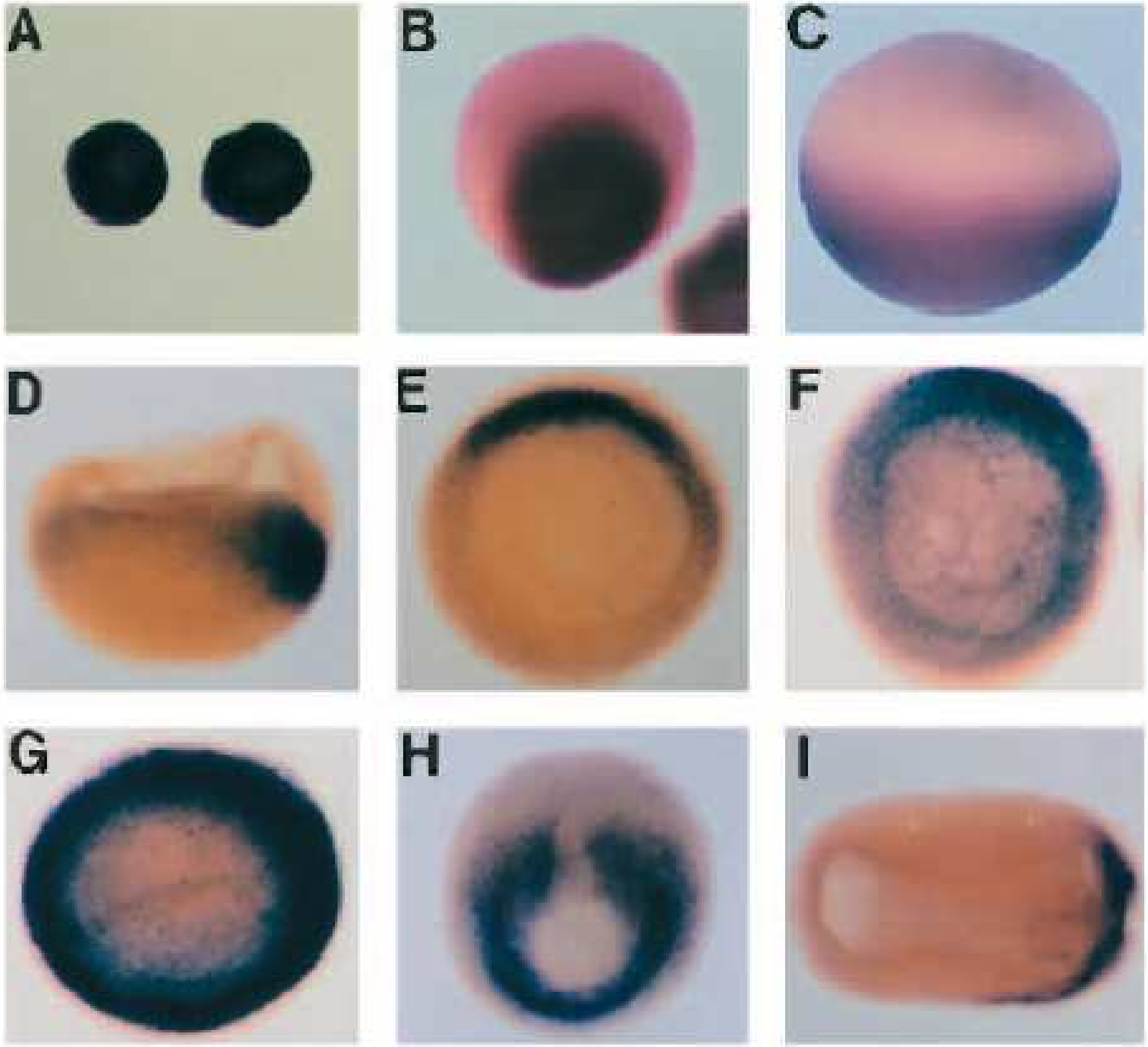
via loss of the induction of the mesodermal markers



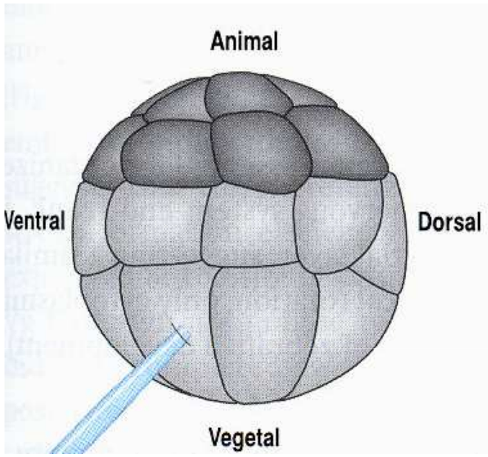


VegT (T-box family transcription factor)

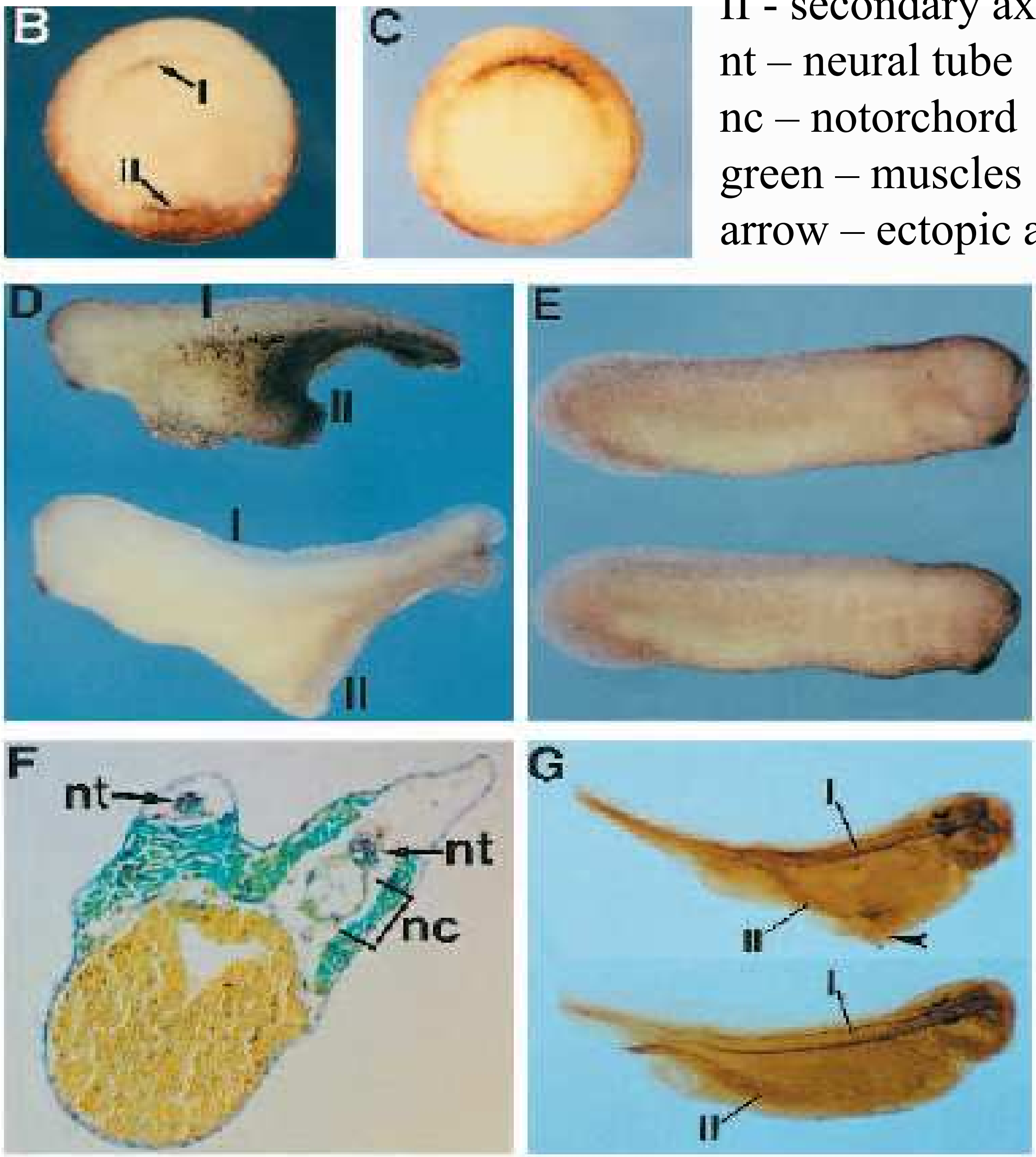
A – stage I oocytes
B – stage IV oocytes
C – ovulated egg
D – stage 9.5 embryo
E – stage 9.5 embryo (vegetal pole view)
F – stage 10.25 embryo (vegetal pole view)
G – stage 10.5 embryo (vegetal view)
H – stage 12.5 embryo (posterior view)
I- mid neural fold embryo (stage 16)



VegT RNA injection into vegetal/ventral blastomeres can induce secondary exis via induction of dorsal fate…….

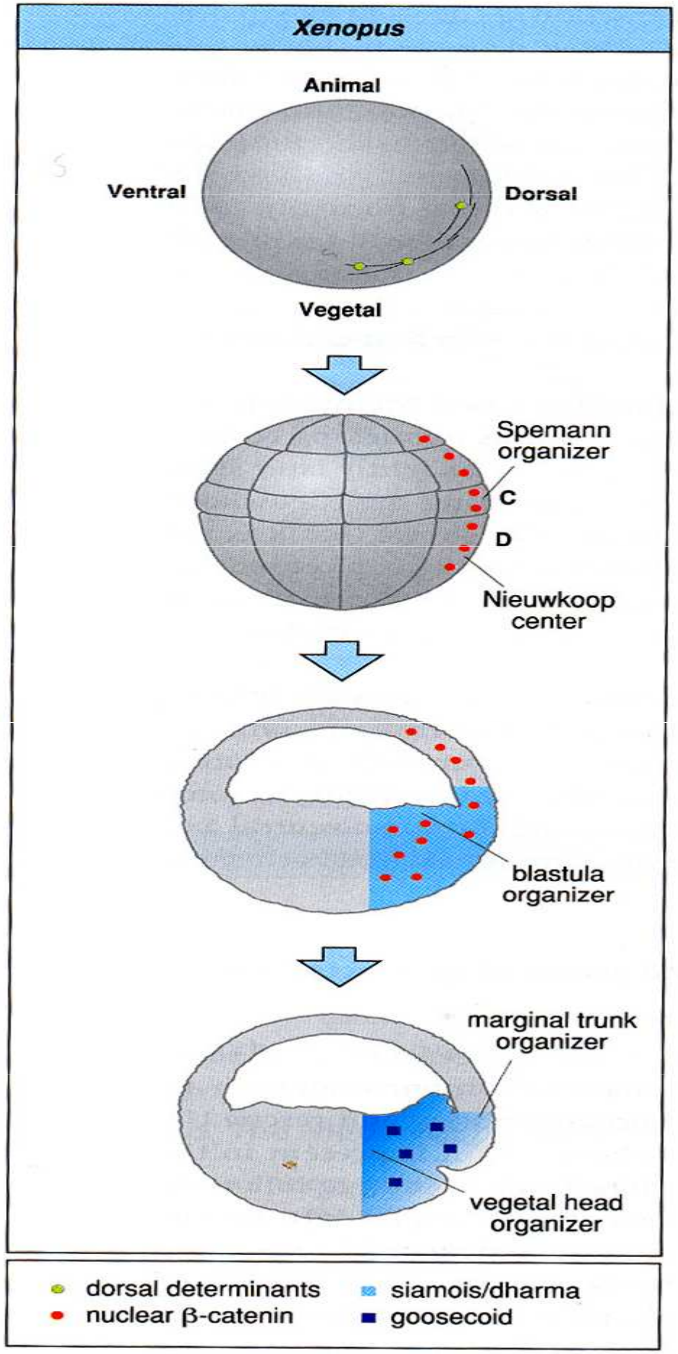


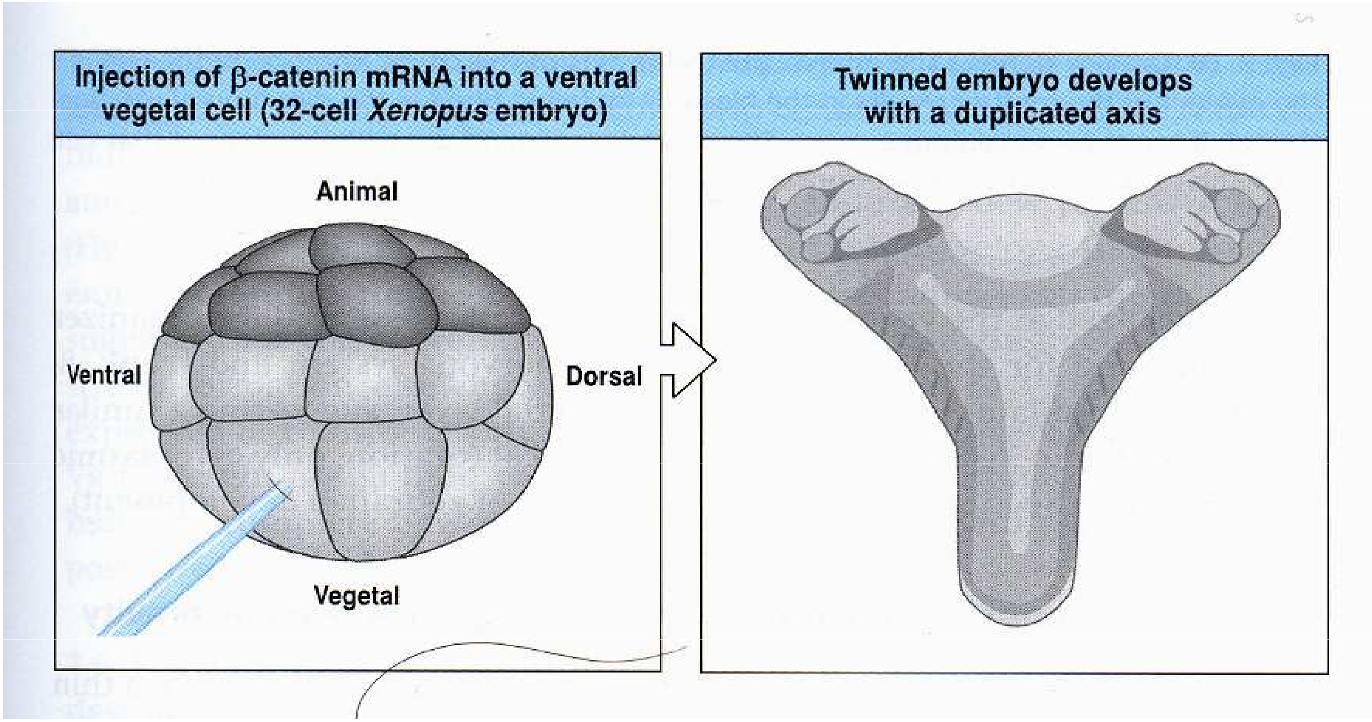
VegT RNA

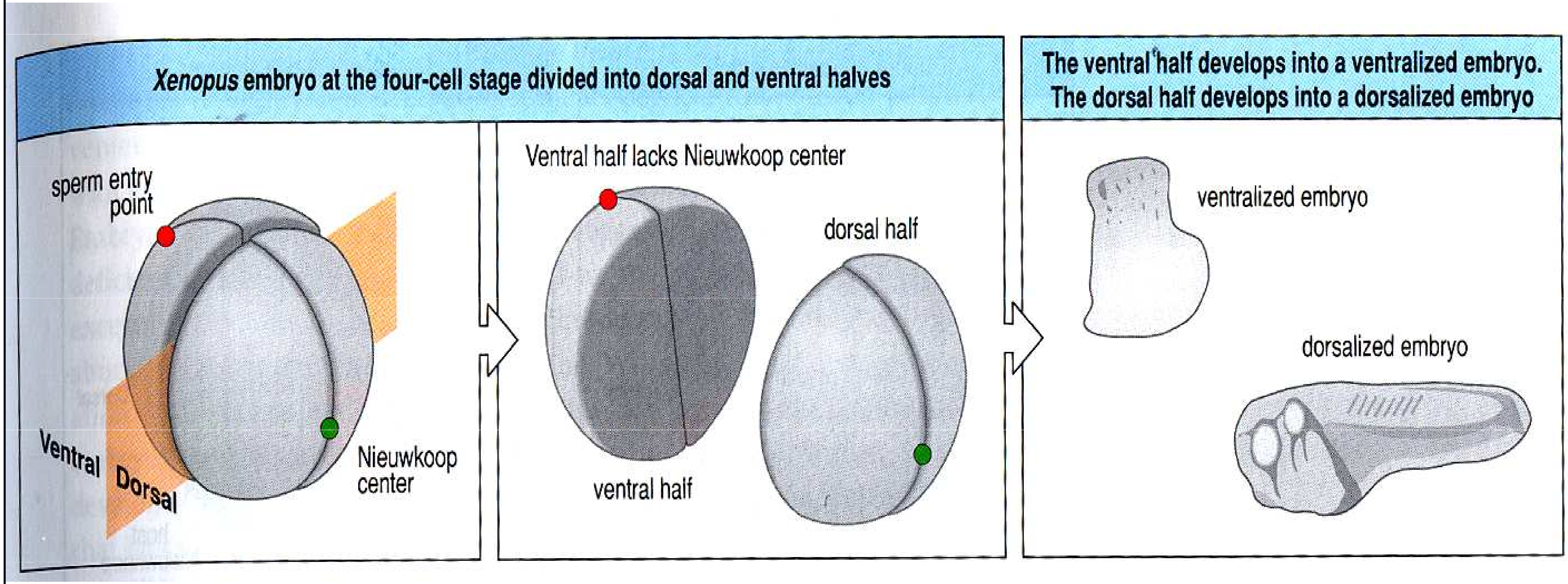


I – primary axis
II - secondary axis
nt – neural tube
nc – notorchord
green – muscles
arrow – ectopic auditory vesicles

………by activation of Xwnt8/β-catenin pathway

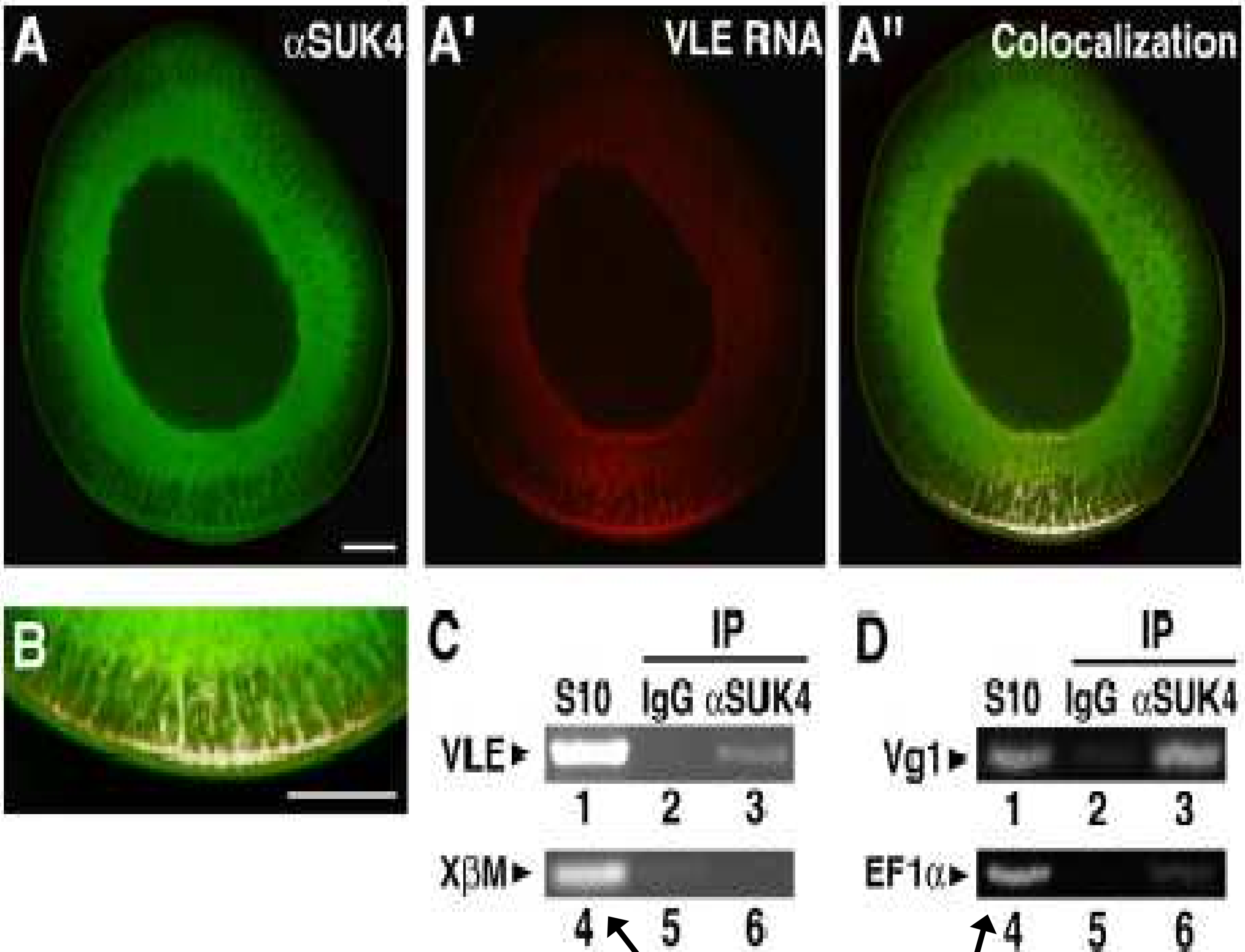






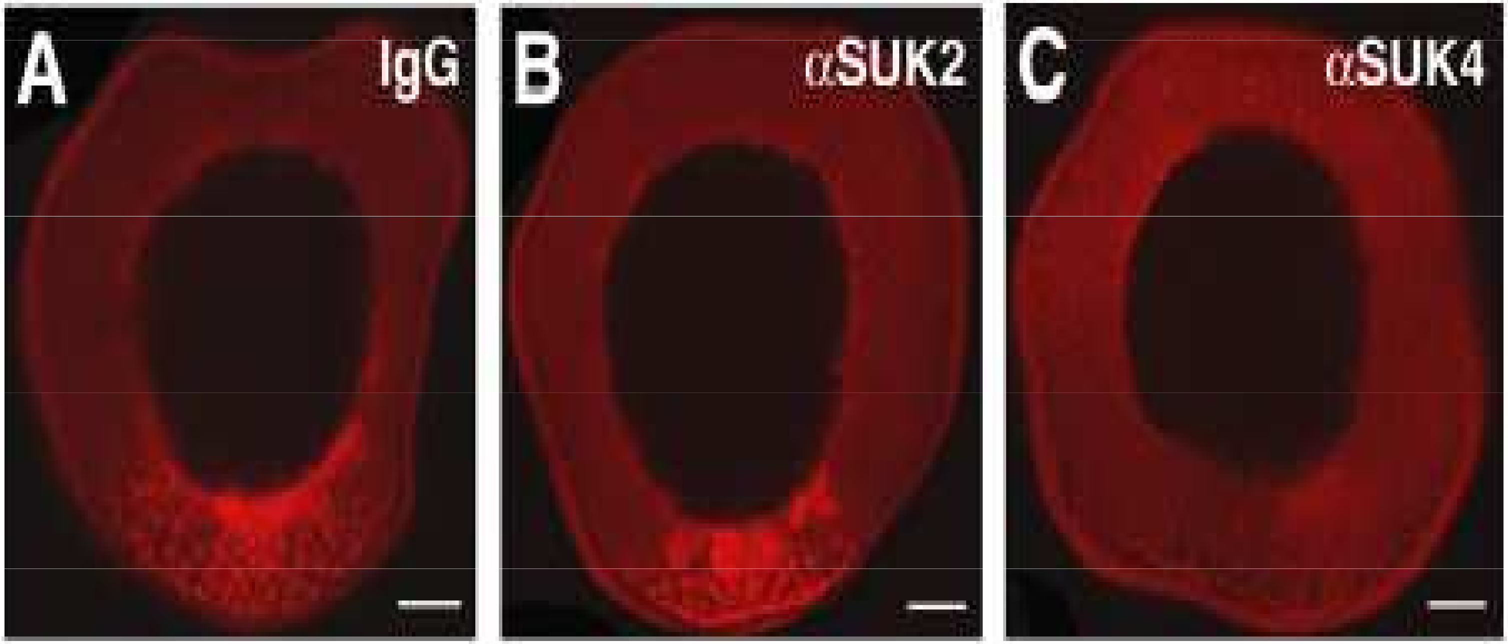
Xenopus embryo at the four-cell stage divided into dorsal and ventral halves
sperm entry point
Ventral
Dorsal
Nieuwkoop center
Ventral half lacks Nieuwkoop center
dorsal half
ventral half
The ventral half develops into a ventralized embryo.
The dorsal half develops into a dorsalized embryo
ventralized embryo
dorsalized embryo

MECHANISMS OF INTRACELLULAR mRNA SORTING



total oocyte lysate

VLE - Vg1
SUK4- kinesin-1 heavy chain



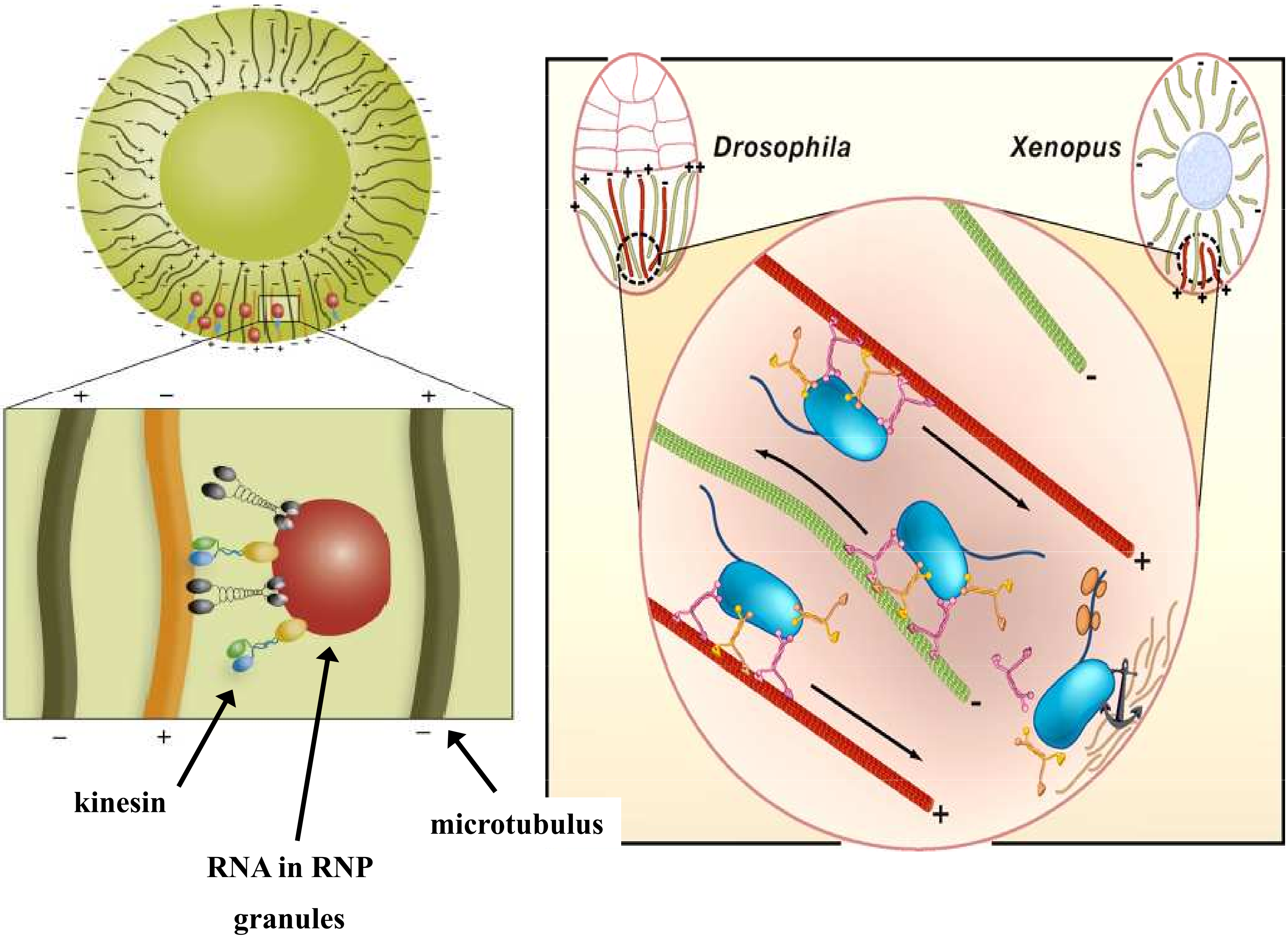
D

Injection	Localization (%)	*n*
IgG	100	62
αSUK2	102	36
αSUK4	52	53

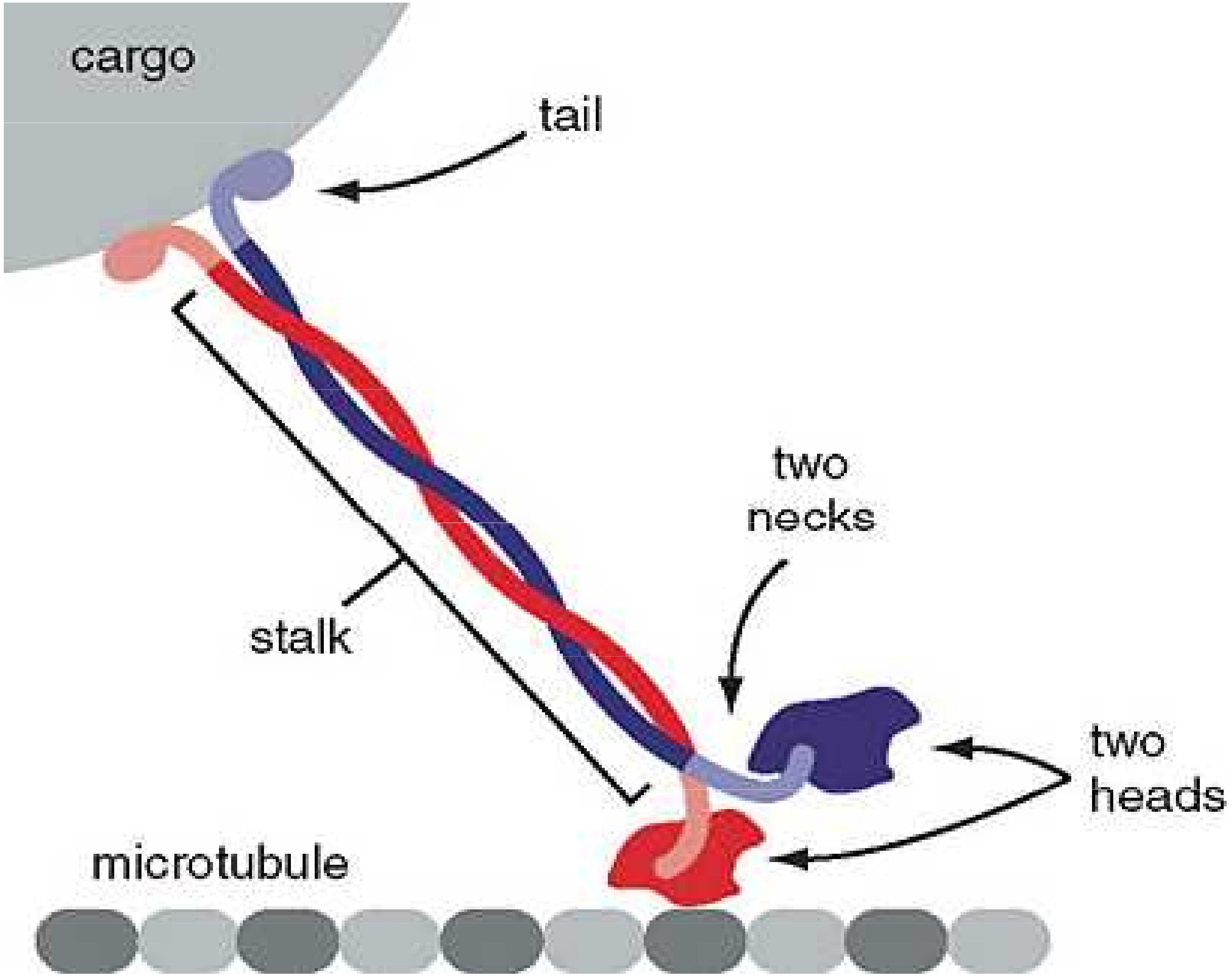
↑

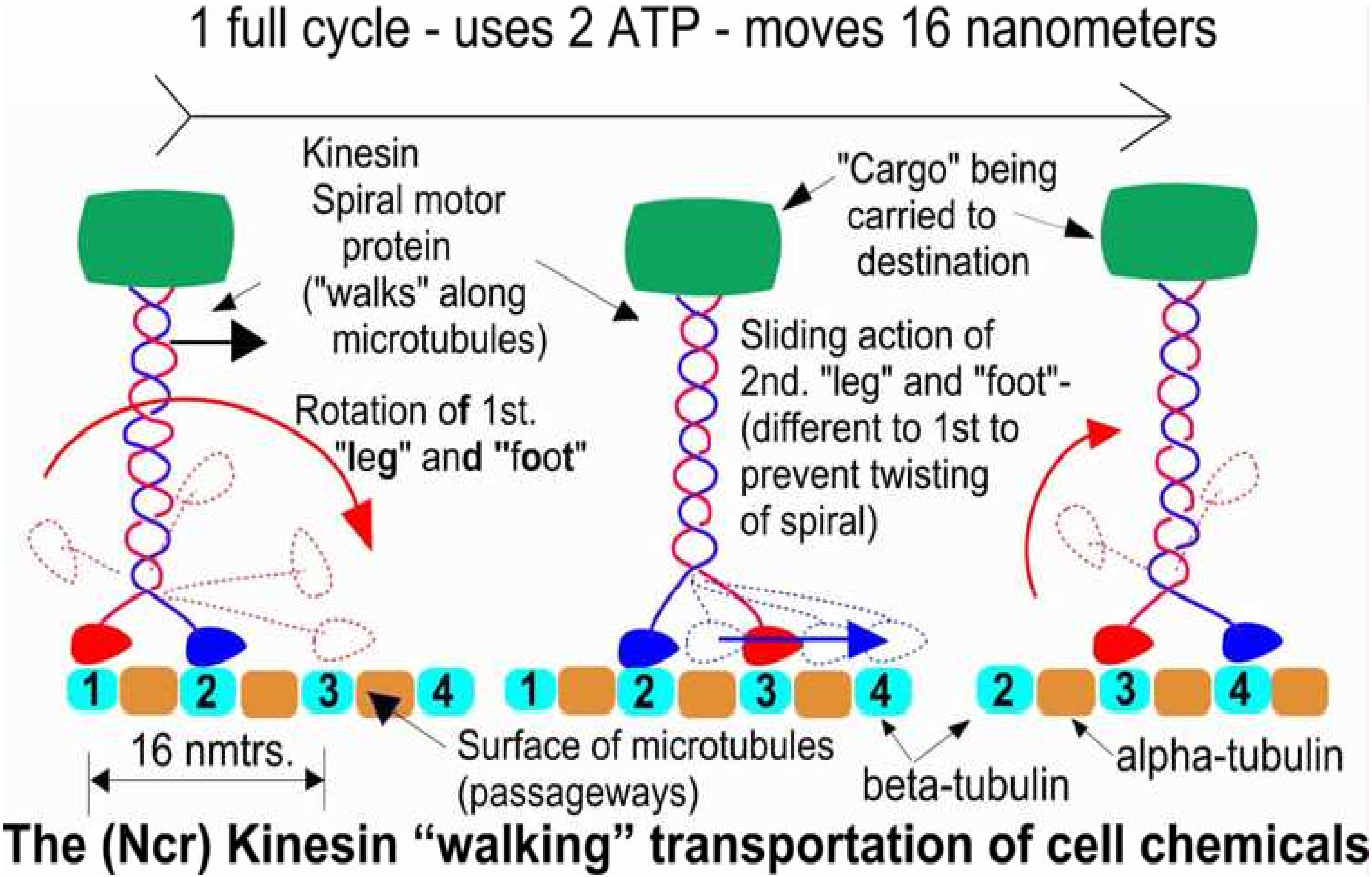
Vg1 mRNA localization

IgG – isotypic control
SUK2 –non-neutralizing Ab
SUK4 –neutralizing Ab


kinesin
RNA in RNP
granules
microtubulus
Drosophila
Xenopus

KINESIN TRANSPORT





KINESIN WALK MOVIE