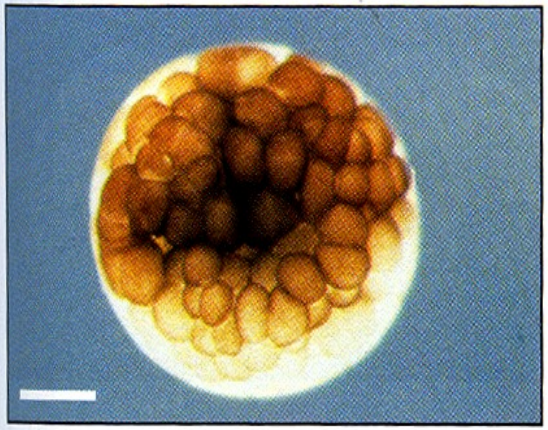
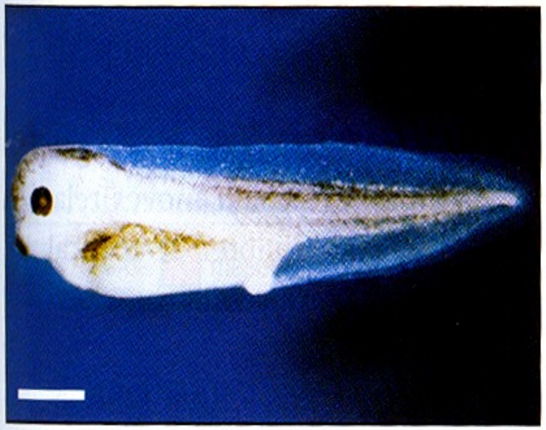
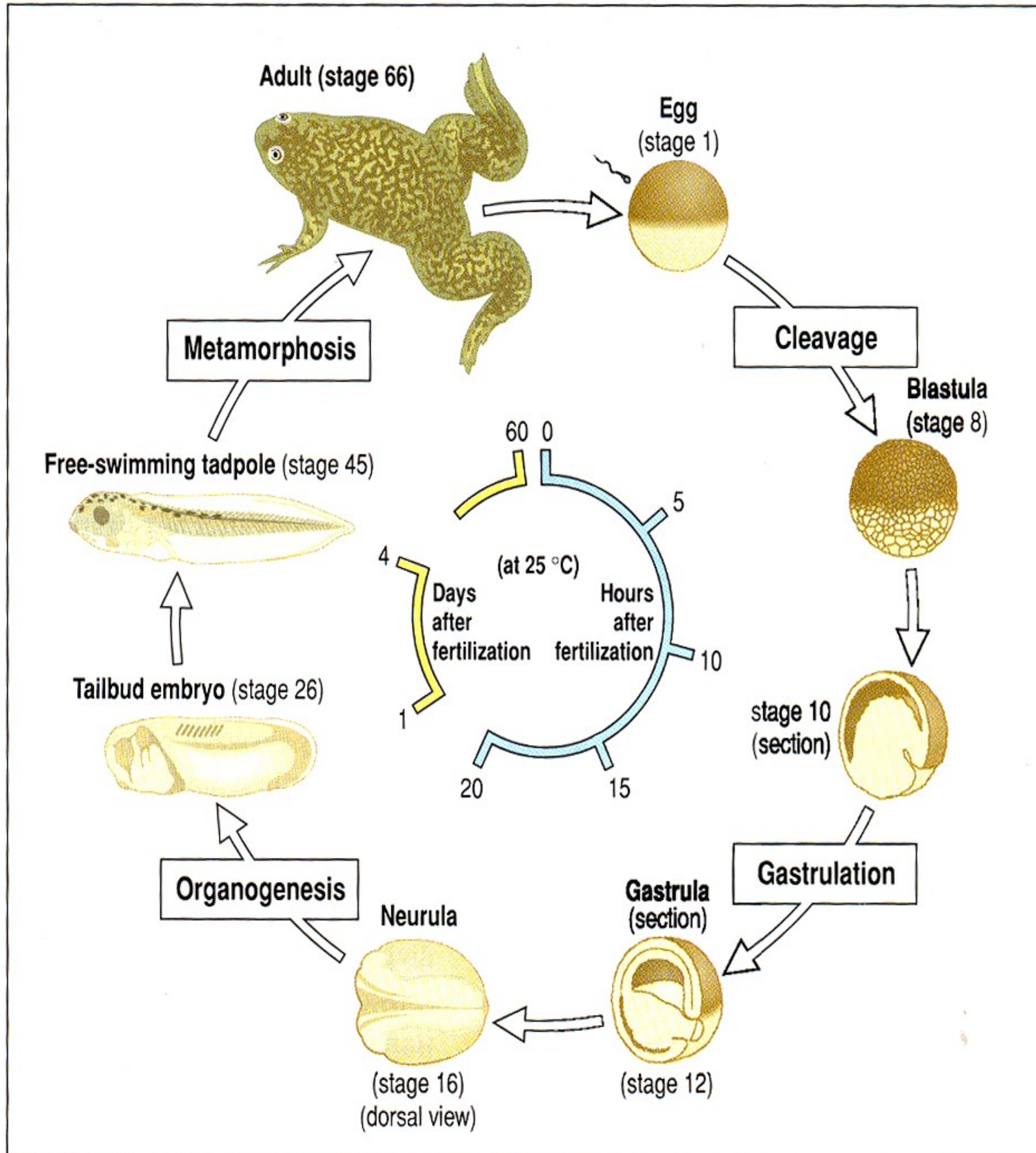
MATERNAL mRNA LOCALIZATION IN THE FROG DEVELOPMENT

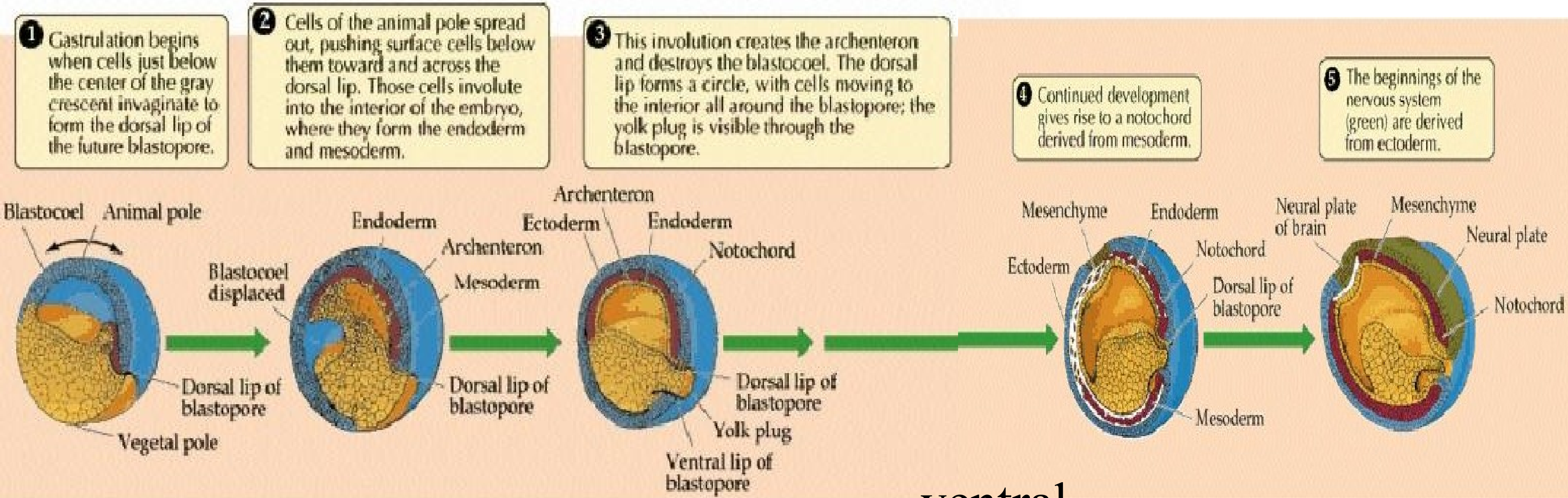
Putting RNAs at the right place in the right time

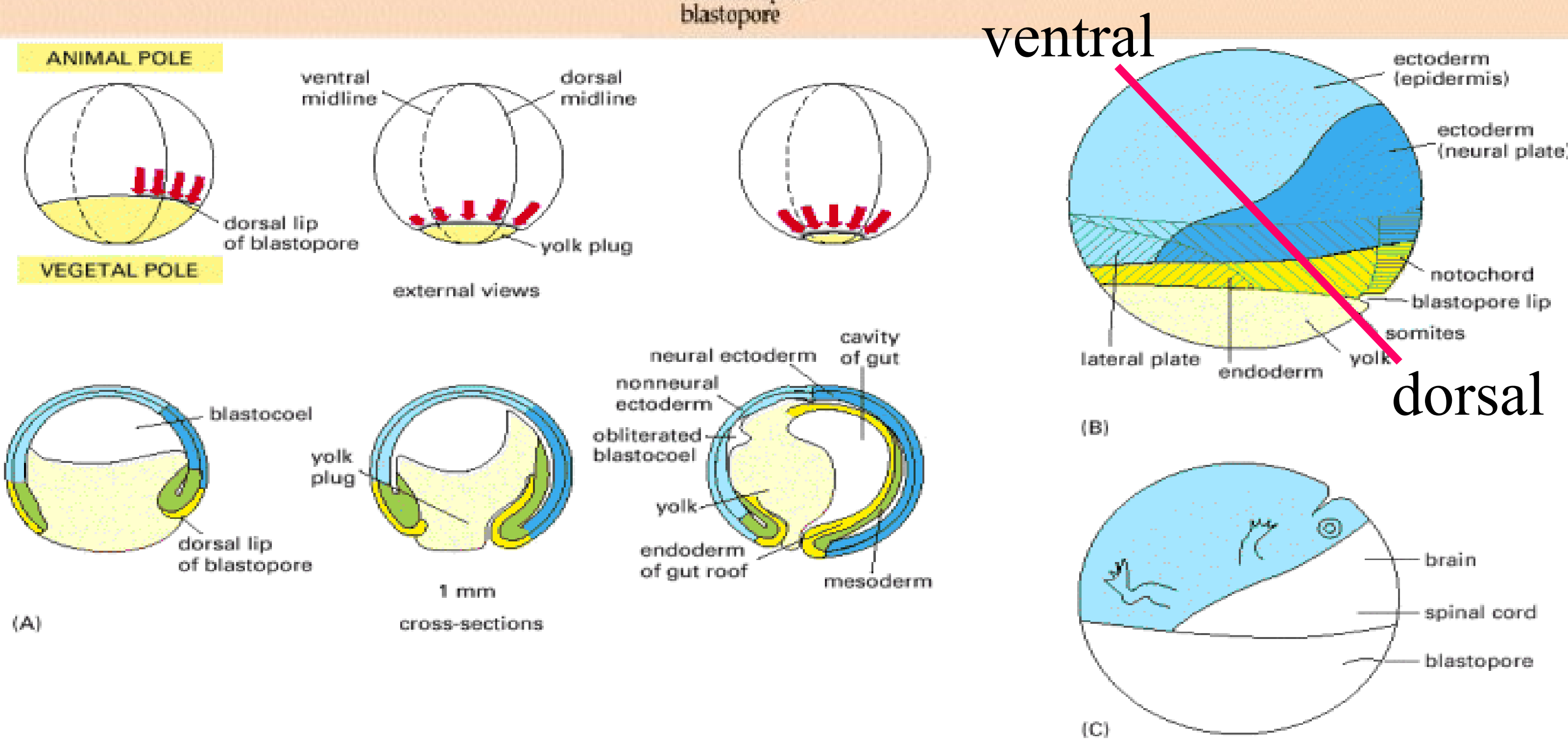




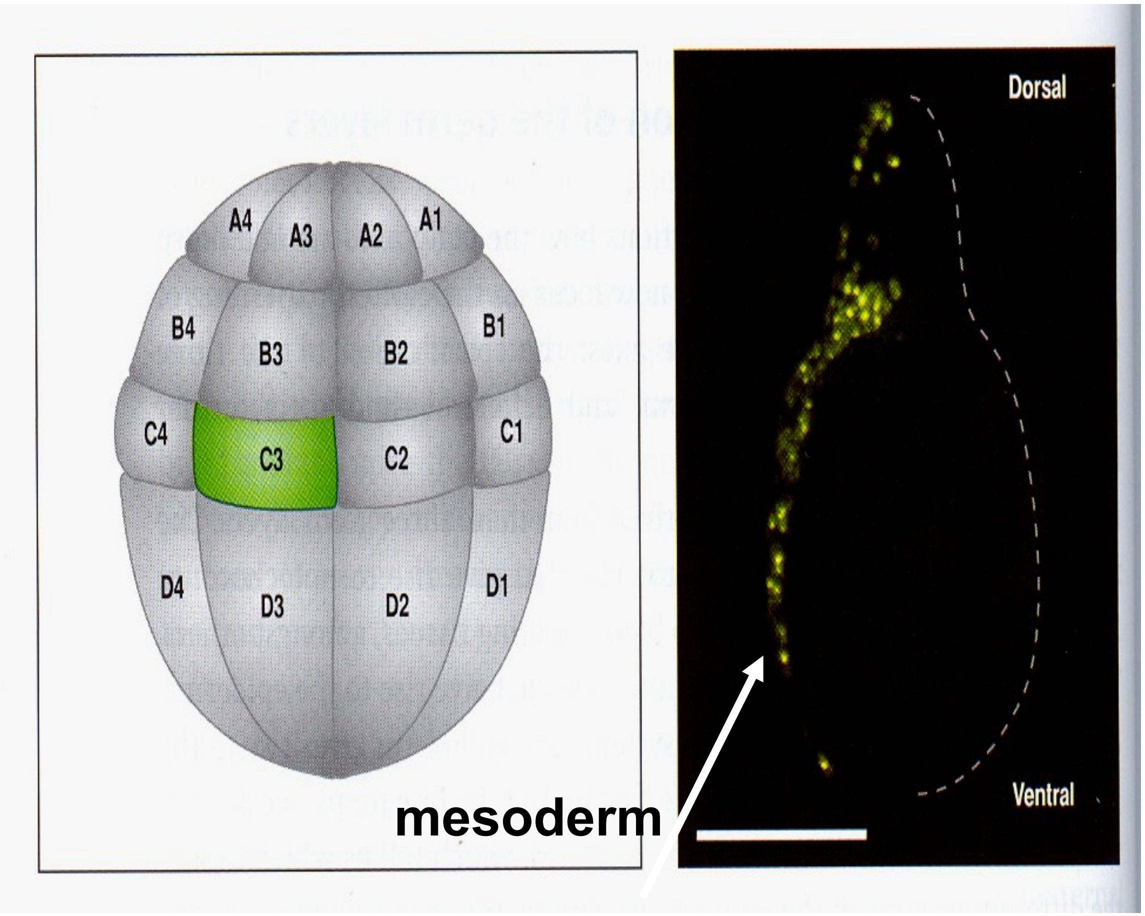

Adult (stage 66)
Egg
(stage 1)
Cleavage
Blastula
(stage 8)
stage 10
(section)
Gastrulation
Gastrula
(section)
(stage 12)
Neurula
(stage 16)
(dorsal view)
Organogenesis
Tailbud embryo (stage 26)
Free-swimming tadpole (stage 45)
Metamorphosis
60 0
5
10
15
20
1
4
(at 25 °C)
Days
after
fertilization
Hours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

GASTRULATION/NEURULATION





ACCURACY AND REPRODUCIBILITY IN ACQUIRING THE CELLULAR FATE WITHIN THE EMBRYO



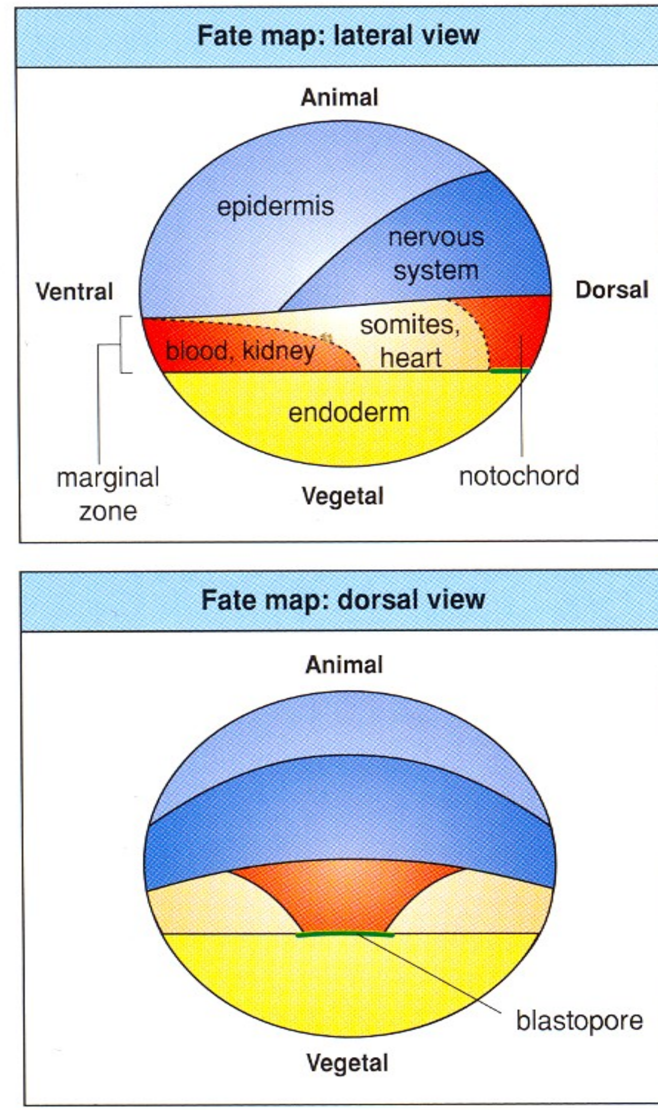
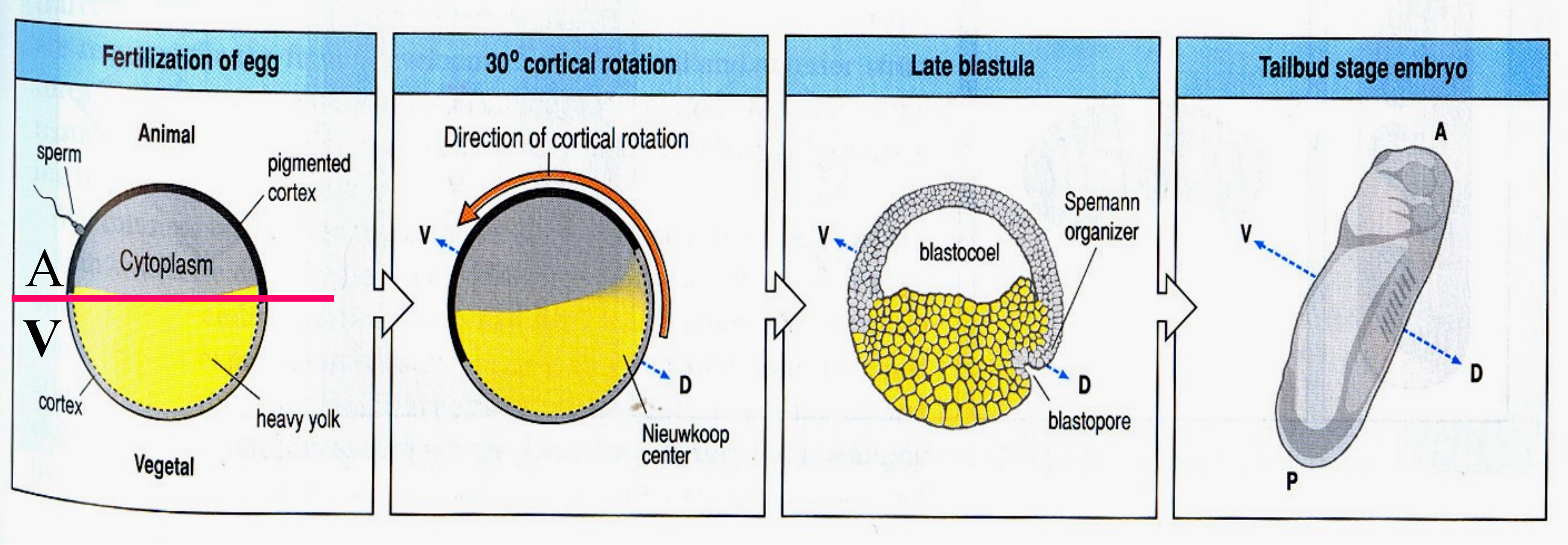


Fig. 3.18 Fate map of a late *Xenopus* blastula.

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 subcellular 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.

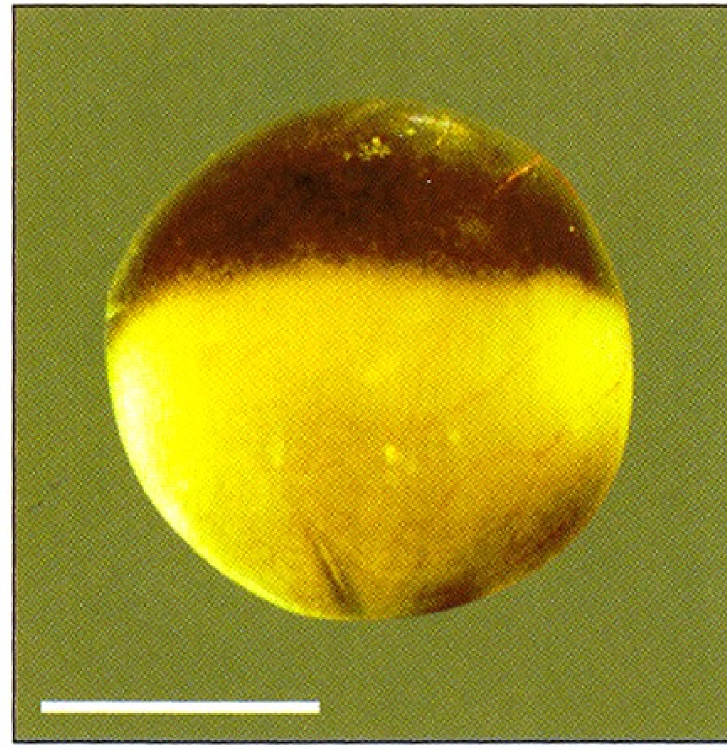


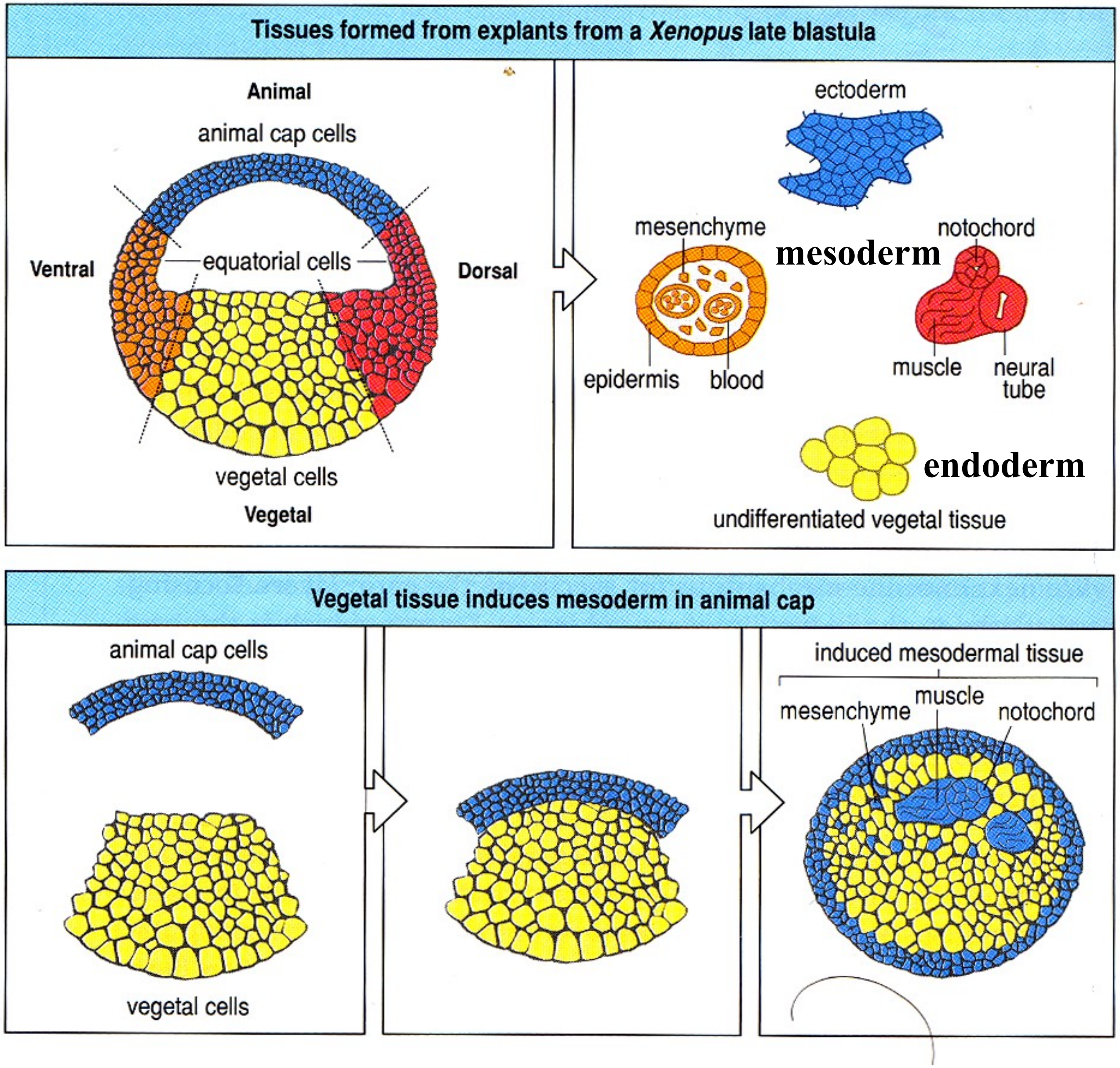
Fig. 2.4 The unfertilized egg of *Xenopus*. The surface of the animal half (top) is pigmented and the paler, vegetal half of the egg is heavy with yolk. Scale bar = 1 mm.

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

Mesoderm is induced by vegetal tissue



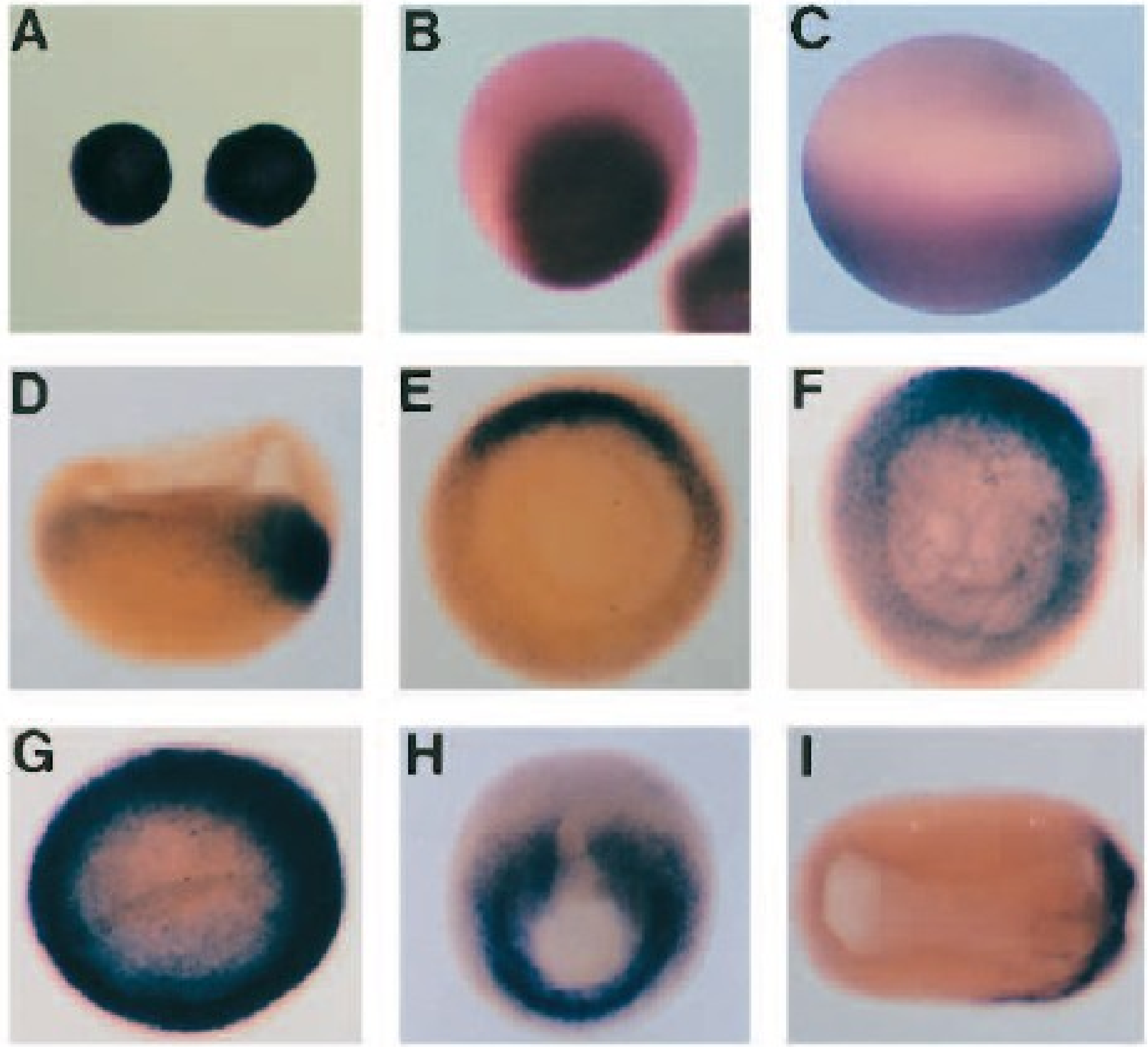
MATERNAL vs. ZYGOTIC REGULATORS

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

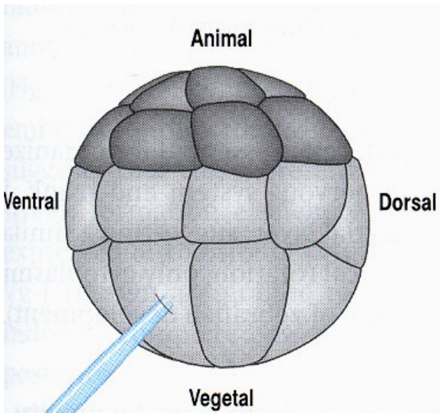
VegT (T-box family transcription factor)

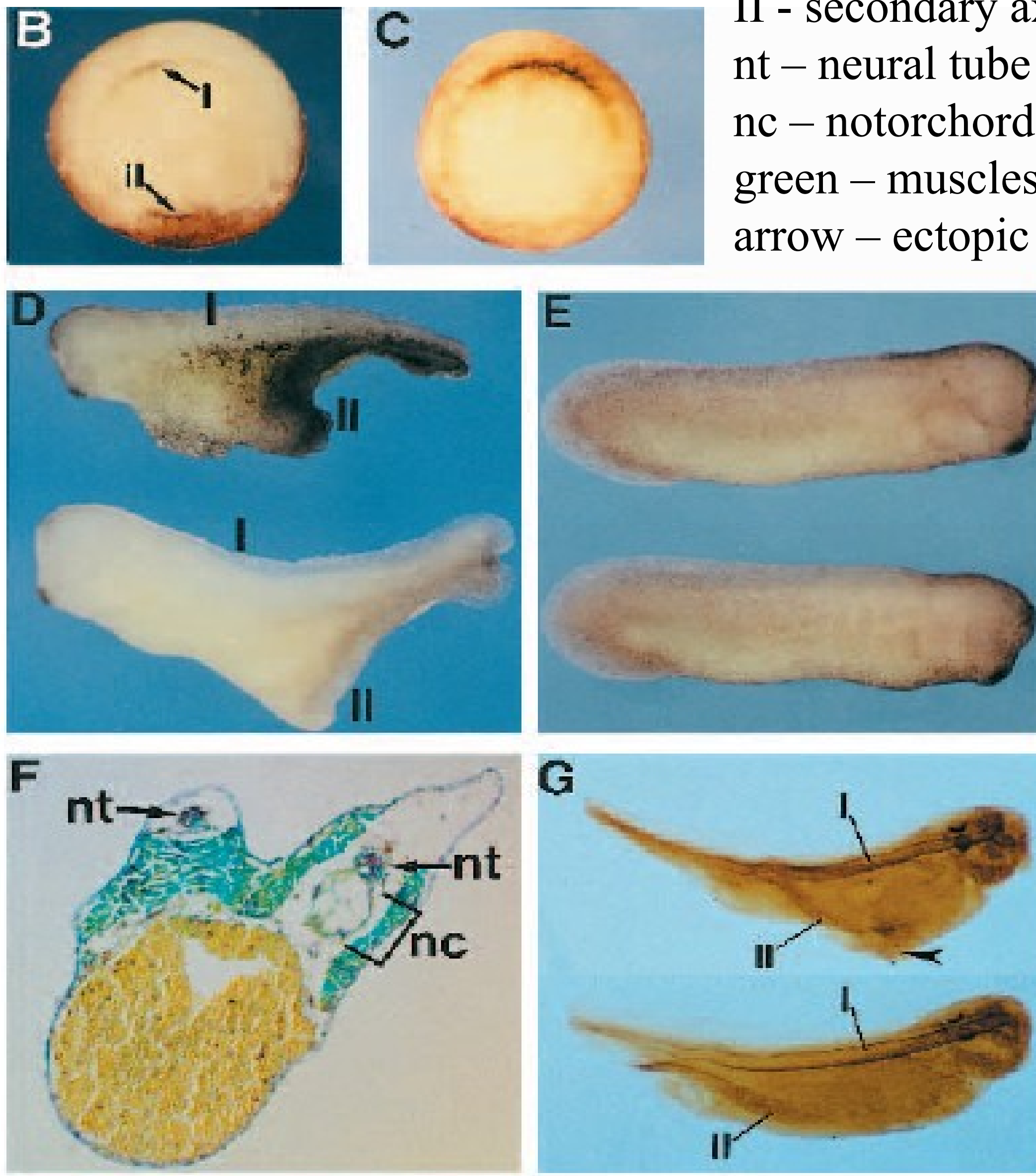
A – stage I oocytes
B – stage IV oocytes
C – ovulated egg
D – stage 9.5 embryo
E – stage 9.5 embryo (vegetal pole view)
F – stage 10.25 embryo (vegetal pole view)
G – stage 10.5 embryo (vegetal view)
H – stage 12.5 embryo (posterior view)
I- mid neural fold embryo (stage 16)



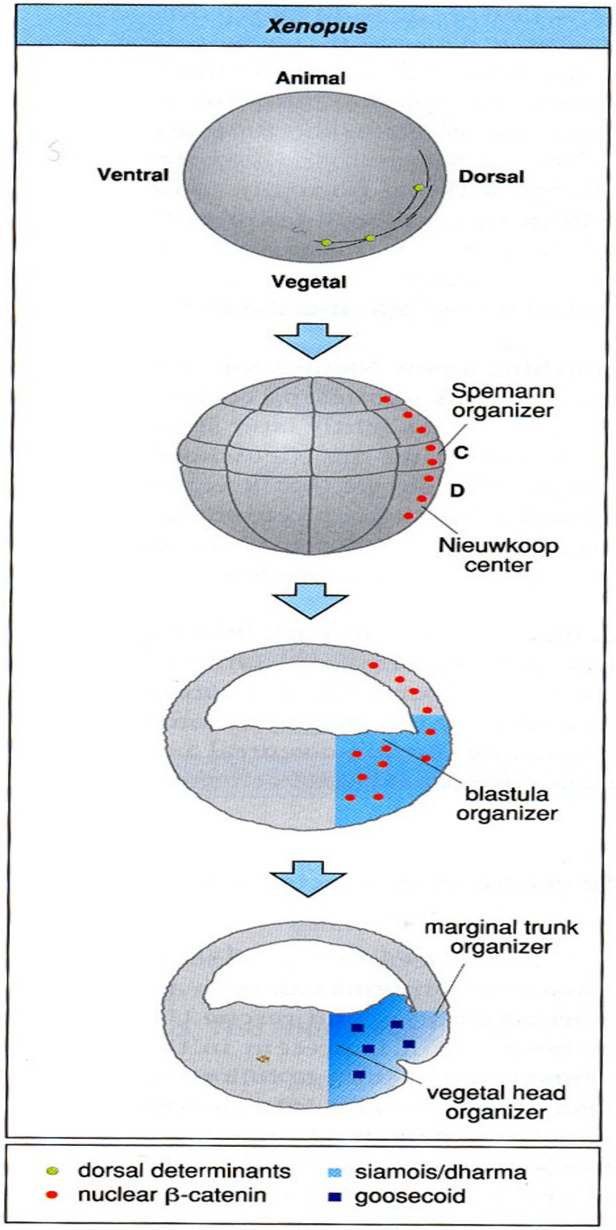
VegT RNA injection into vegetal/ventral blastomeres can induce secondary exis via induction of dorsal fate…….

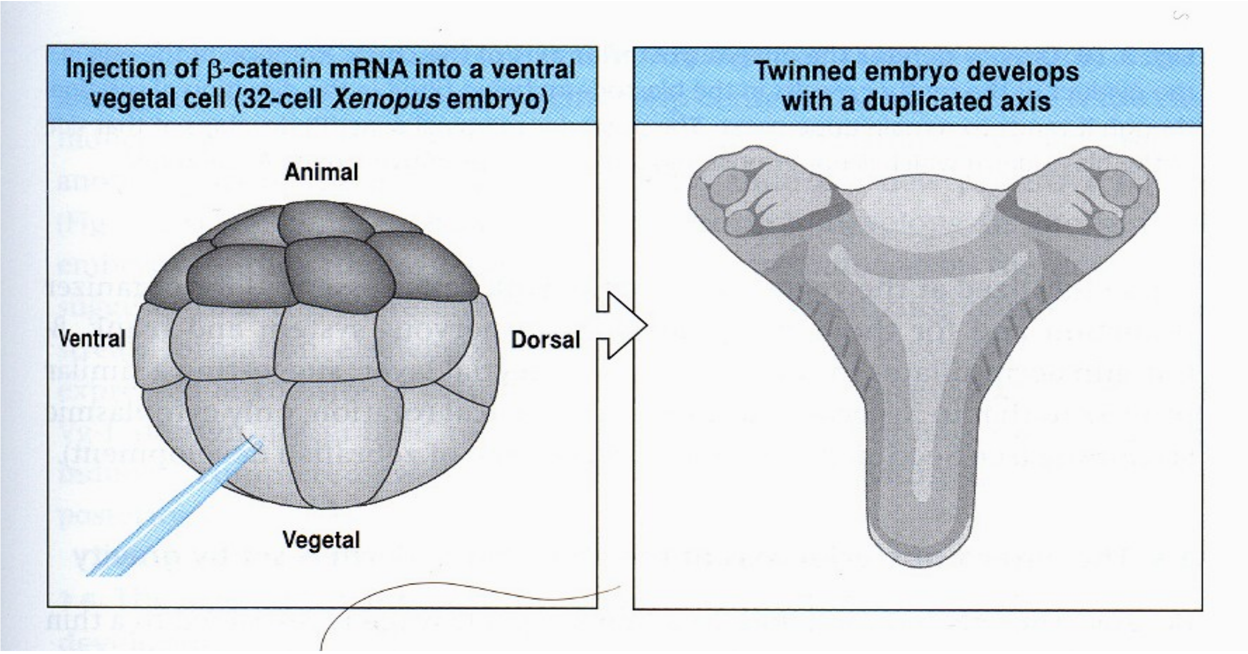
I – primary axis
II - secondary axis
nt – neural tube
nc – notorchord
green – muscles
arrow – ectopic auditory vesicles

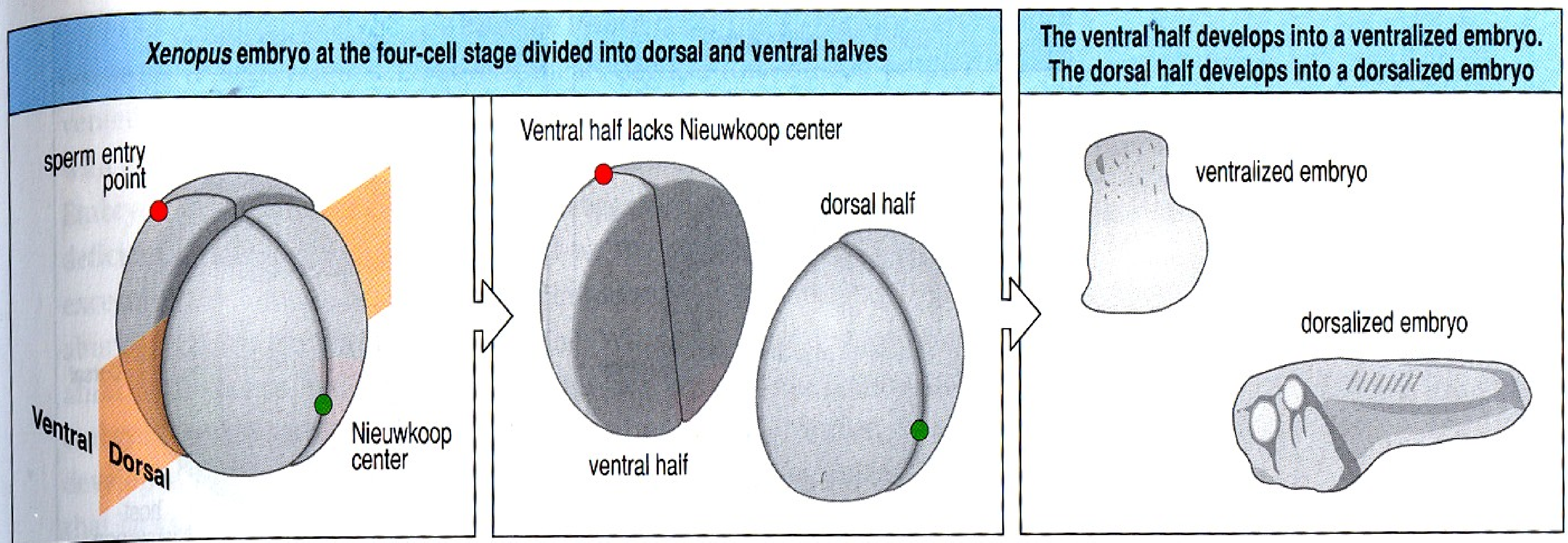




………by activation of Xwnt8/β-catenin pathway







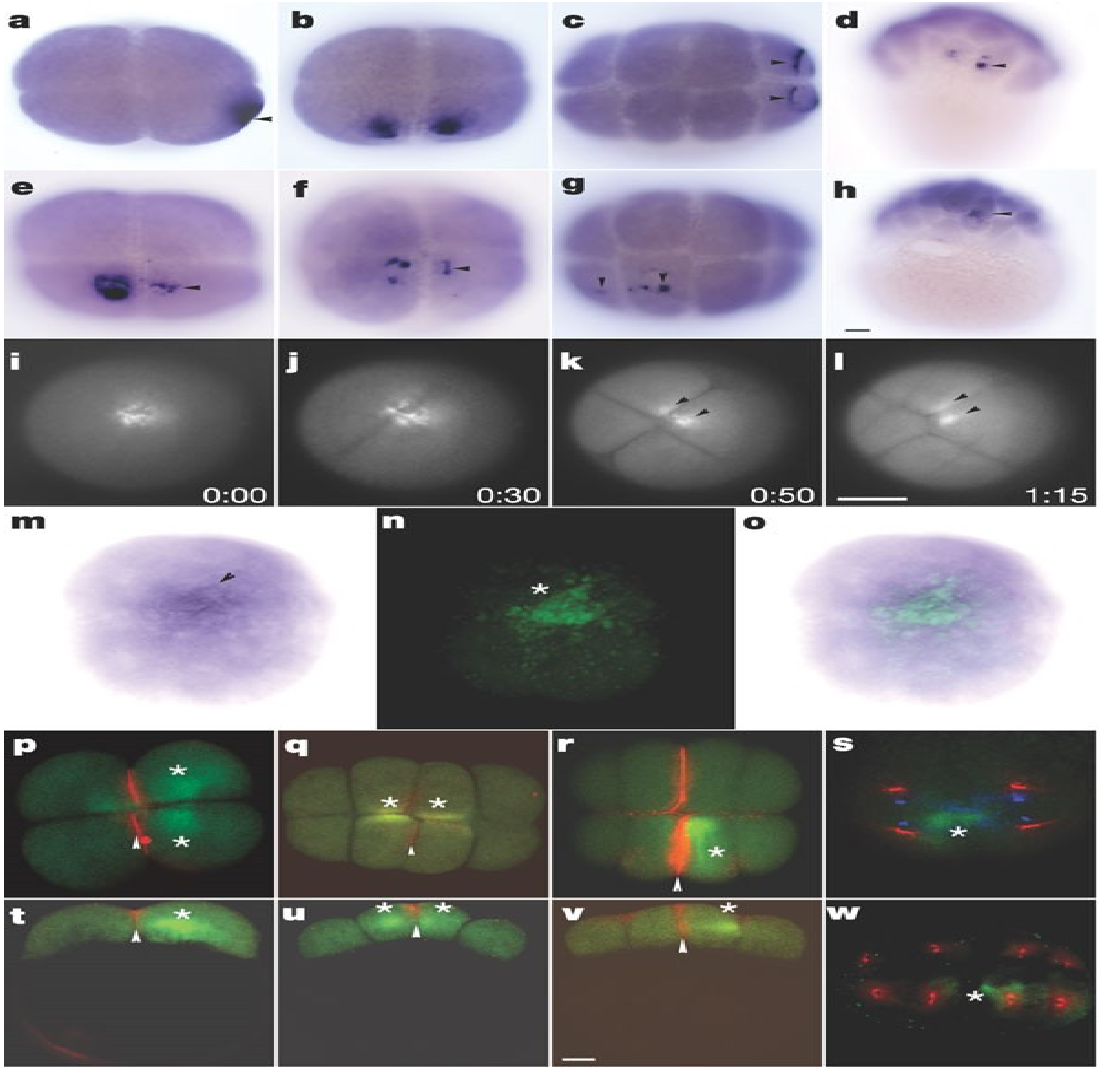
Xenopus embryo at the four-cell stage divided into dorsal and ventral halves
The ventral half develops into a ventralized embryo.
The dorsal half develops into a dorsalized embryo
sperm entry point
Nieuwkoop center
Ventral
Dorsal
Ventral half lacks Nieuwkoop center
dorsal half
ventral half
ventralized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

nature Vol 438|15 December 2005|doi:10.1038/nature04184

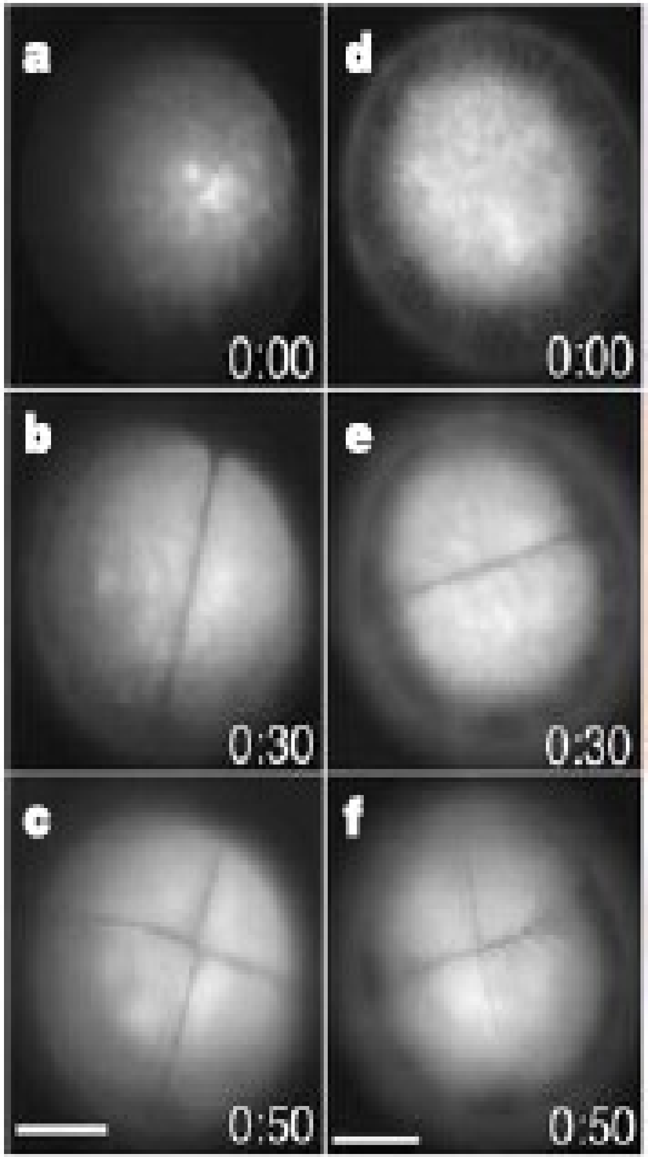
The zebrafish dorsal axis is apparent at the four-cell stage

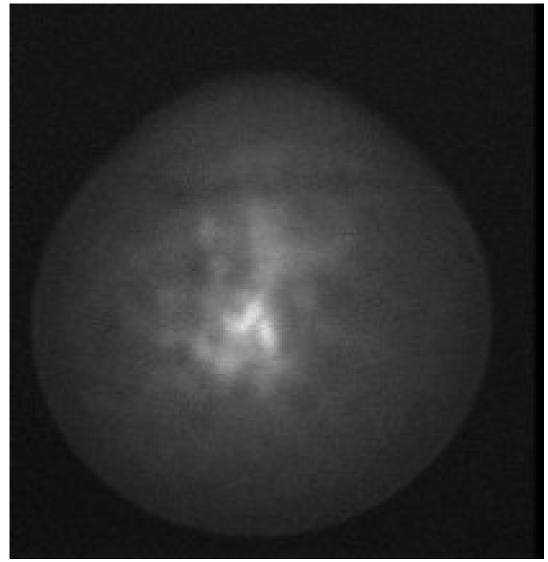
Aniket V. Gore[1,2], Shingo Maegawa[3], Albert Cheong[1], Patrick C. Gilligan[1], Eric S. Weinberg[3] & Karuna Sampath[1,2]

Squint – nodal-related morphogen important for establishment of dorsoventral axis



3’UTR is necessary and sufficient for *Squint* localization






```
Barbodes UGCCACUGAGUC-UCCAAACCCCAAAU-GAACUCAACACUAGCA----CUUUGGAUAUGU
Danio    UGCCACUGAUUC-UUCAAACCCCAAAG-GAACUCAACUCUAGCA----CUUUGGAUAUGC
Rasbora  UGCCACUGAGCC-UCCAG-UCCCAAAC-GAACGCAACGCUAGCA----CUUUGGACACGC
Botia    UGCCACUGACCCCUCCAAAUCCCAAACUGAACUCAACAUUAGCACUUUCUUUGGAUAUGC
         *********   *  *  **    ******  ****  ****  *****     ******** *  *

Barbodes UCCUUAAACU--ACAAAUACAUAUUUAAGCACGAAUGAAGAUAAAUUGCUUUAAUUUGUA
Danio    UCCUUGACCCC-AAAAAUAUGUAUUUAAGAA-----------AAACUGCUGUCAAUU---
Rasbora  UCCUCAAACCCAGAAAAUAUGUAUUUAAGUU-------------------AAAGUU---
Botia    UCCUCAAAACC-AAAAAUAUAUACGAGCACA------A--AUGA--CGAUAAUACUCGU-
         ****  *        *****  **                              *  *

Human    UGAUGACAUCCUG-GAGGGAGACUGCAUUUGCCUGCACUCUGGAAGGCUGGGAAACUCCUG
Chimp    UGAUGACAUCCUG-GAGGGAGACUGCAUUUGCCUGCACUCUGGAAGGCUGGGAAACUCCUG
Bos      UGAGGAGCUCCUG-CUGGUGUGUUGUAUUUGCCUGUAC-CUGGAAGGUUGGGAAGCUCCUG
Dog      UGAGGCAAUCCUGGCGGGGGGGCUGCAUUUGCCUAUACCCUGGGAAGUUGGGAAGCUCCUA
         ***  *   *****    **      **  *********  **  ****  *  *  ******   ****
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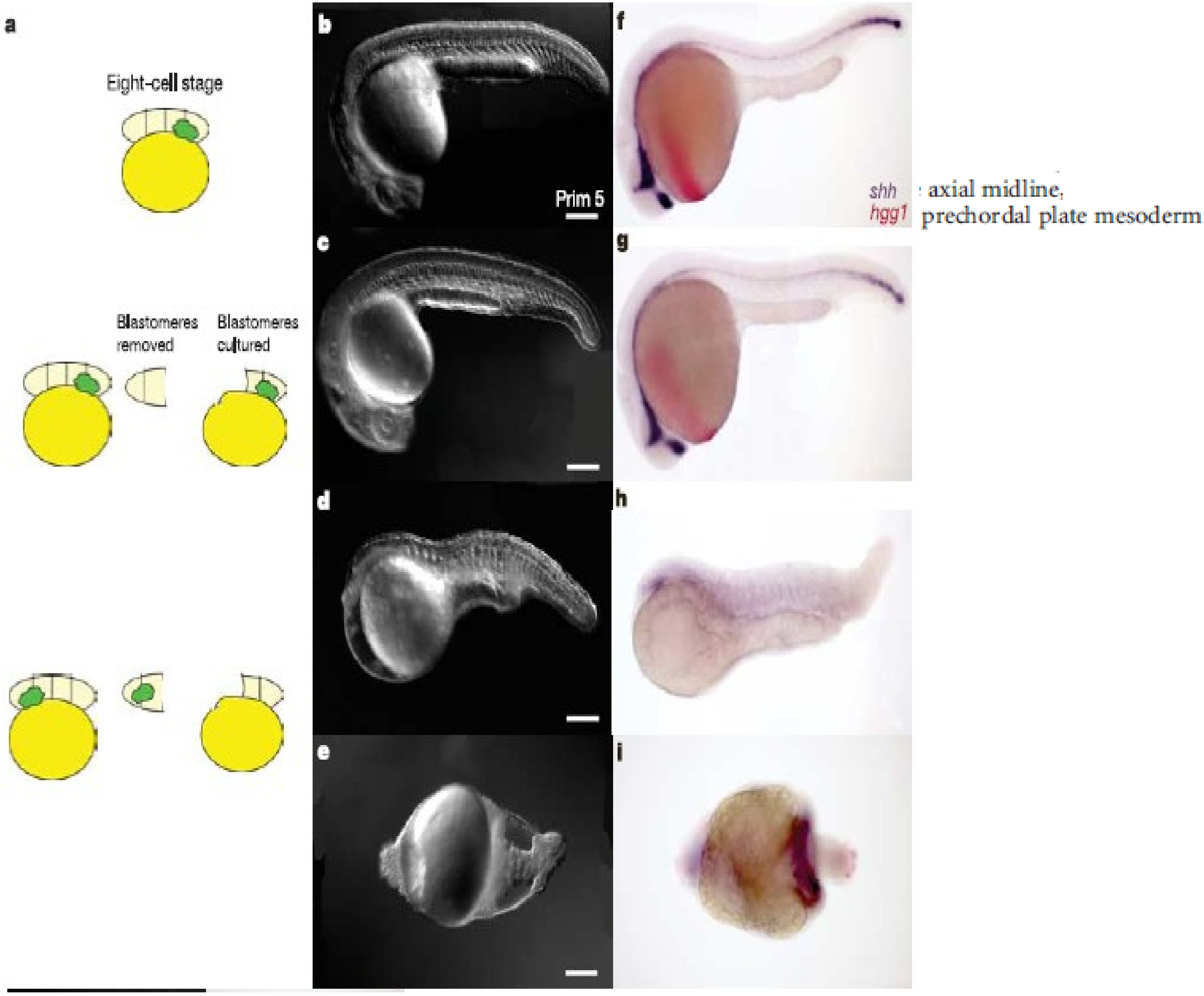
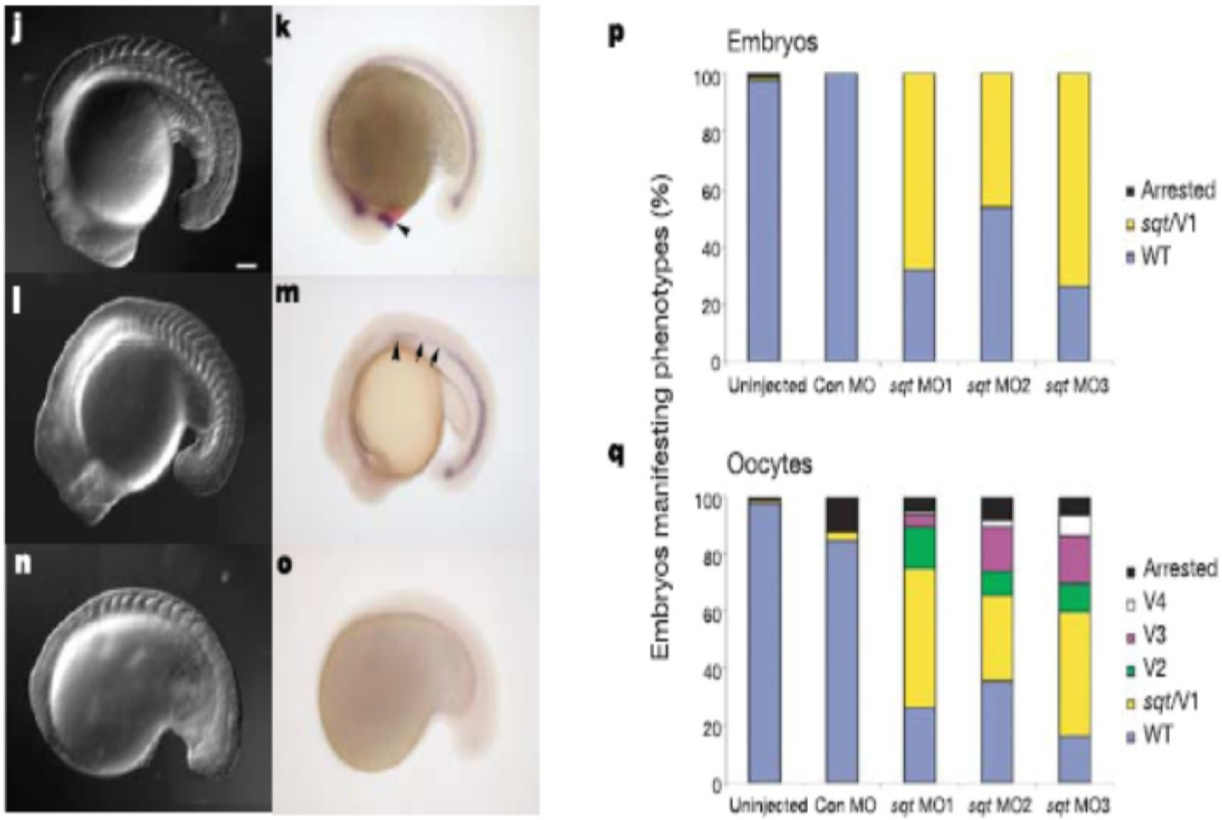
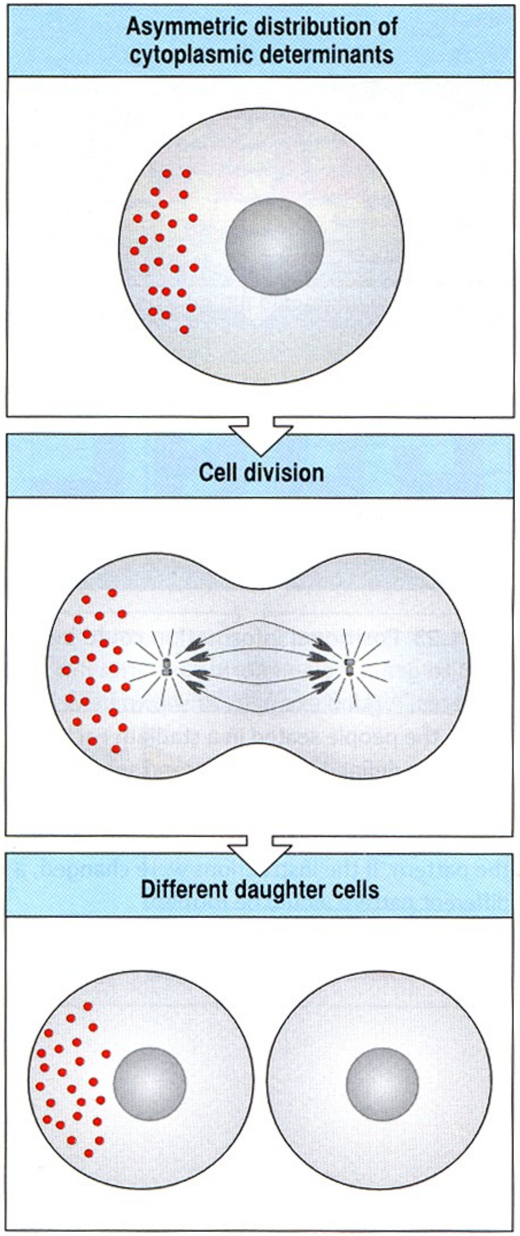


a
Eight-cell stage
Blastomeres removed
Blastomeres cultured
b
Prim 5
c
d
e
f
g
h
i
shh : axial midline,
hgg1 prechordal plate mesoderm

Table 2). Dorsal specification is therefore initiated as early as cleavage stages in zebrafish, with the cells containing localized maternal *sqt* RNA required for the formation of dorsal structures.



igure 3 | Removal of cells with *sqt* RNA from embryos or injection of *sqt* norpholinos into oocytes leads to loss of dorsal structures. a, Schematic epresentation of eight-cell embryos to show removal of blastomeres with lexa-488-labelled *lacZ:sqt* 3′ UTR RNA (green, sqt^+) or control removal of ells without *sqt* (sqt^-). **b–e**, Live embryos at 24 h showing wild-type (WT) henotypes in unmanipulated control (**b**), sqt^- cell removed (**c**), or sqt^+ emoved (**d**, **e**) embryos. **f–i**, Expression of *shh* (purple) and *hgg* (red) in nmanipulated (**f**), control sqt^- removed (**g**) or sqt^+ removed (**h**, **i**) mbryos. Phenotypes range from mild ventralization, V2 (not shown), and loss of anterior structures, V3 (**d**, **h**), to complete ventralization, V4 (**e**, **i**), on sqt^+ cell removal. Injection of morpholinos (MOs) against *sqt* into oocytes (**n**, **o**, **q**) also results in loss of dorsal and anterior structures, in comparison with oocytes injected with control *sqt* mismatch morpholinos (**j**, **k**, **q**) or fertilized embryos injected with *sqt* morpholinos (**l**, **m**, **p**). **j**, **l**, **n**, Live embryos at 18 h; **k**, **m**, **o**, expression of *shh* and *hgg* in the same embryos. Arrowheads in **k** and **m** mark the anterior limit of *shh* expression, and arrows in **m** indicate gaps in *shh* expression. Lateral views with anterior to the left. Scale bars, 100 μm (**b**, **j**) and 50 μm (**c–e**).



Vg1 (TGFβ family ligand)

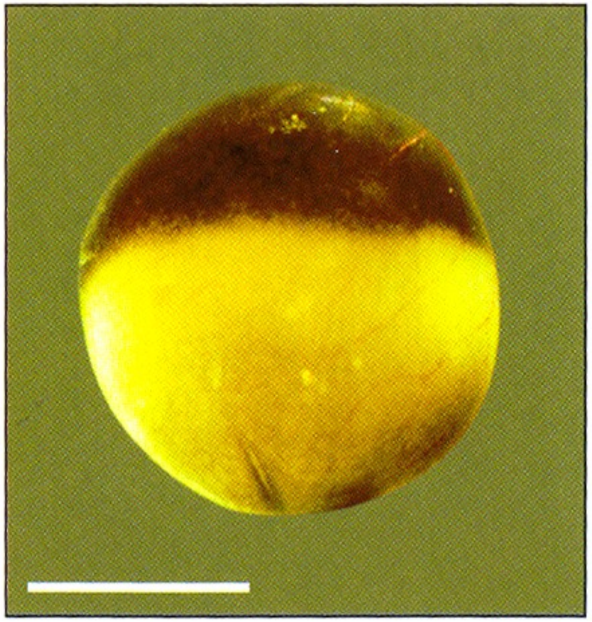


Fig. 2.4 The unfertilized egg of *Xenopus*. The surface of the animal half (top) is pigmented and the paler, vegetal half of the egg is heavy with yolk. Scale bar = 1 mm.

Photograph courtesy of J. Smith.

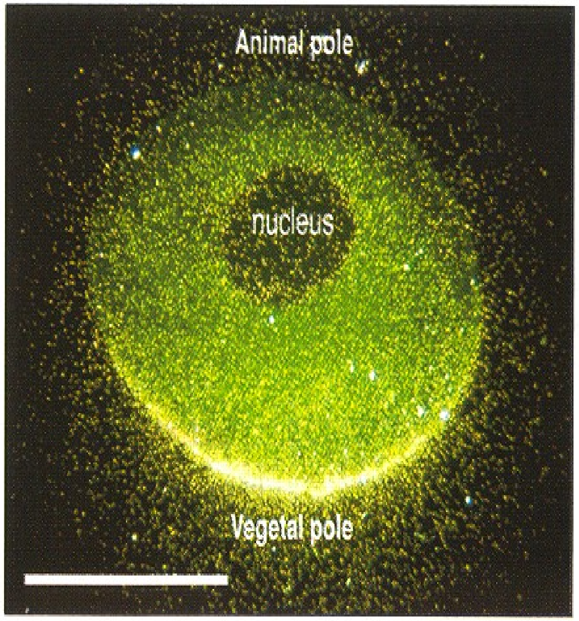
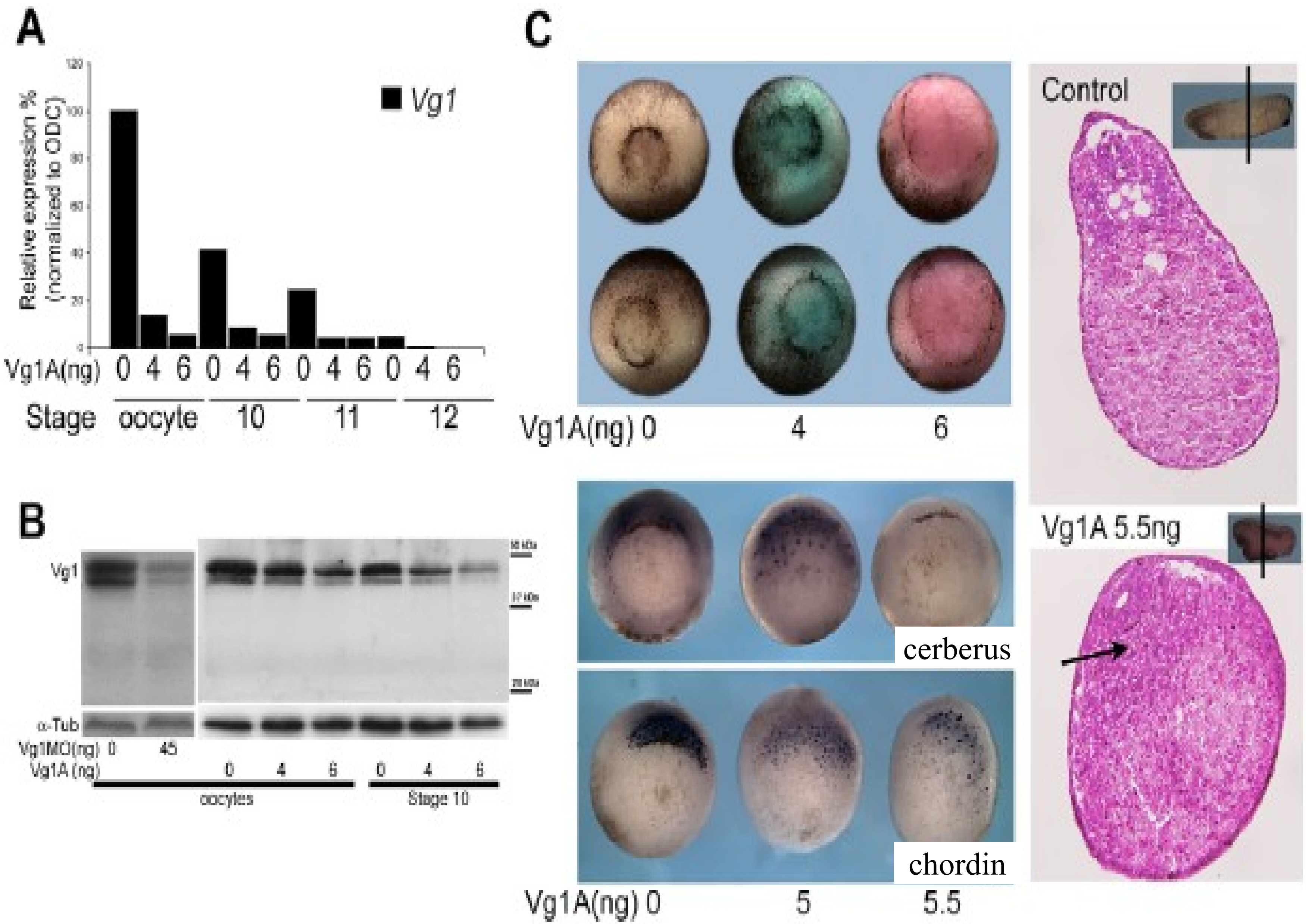


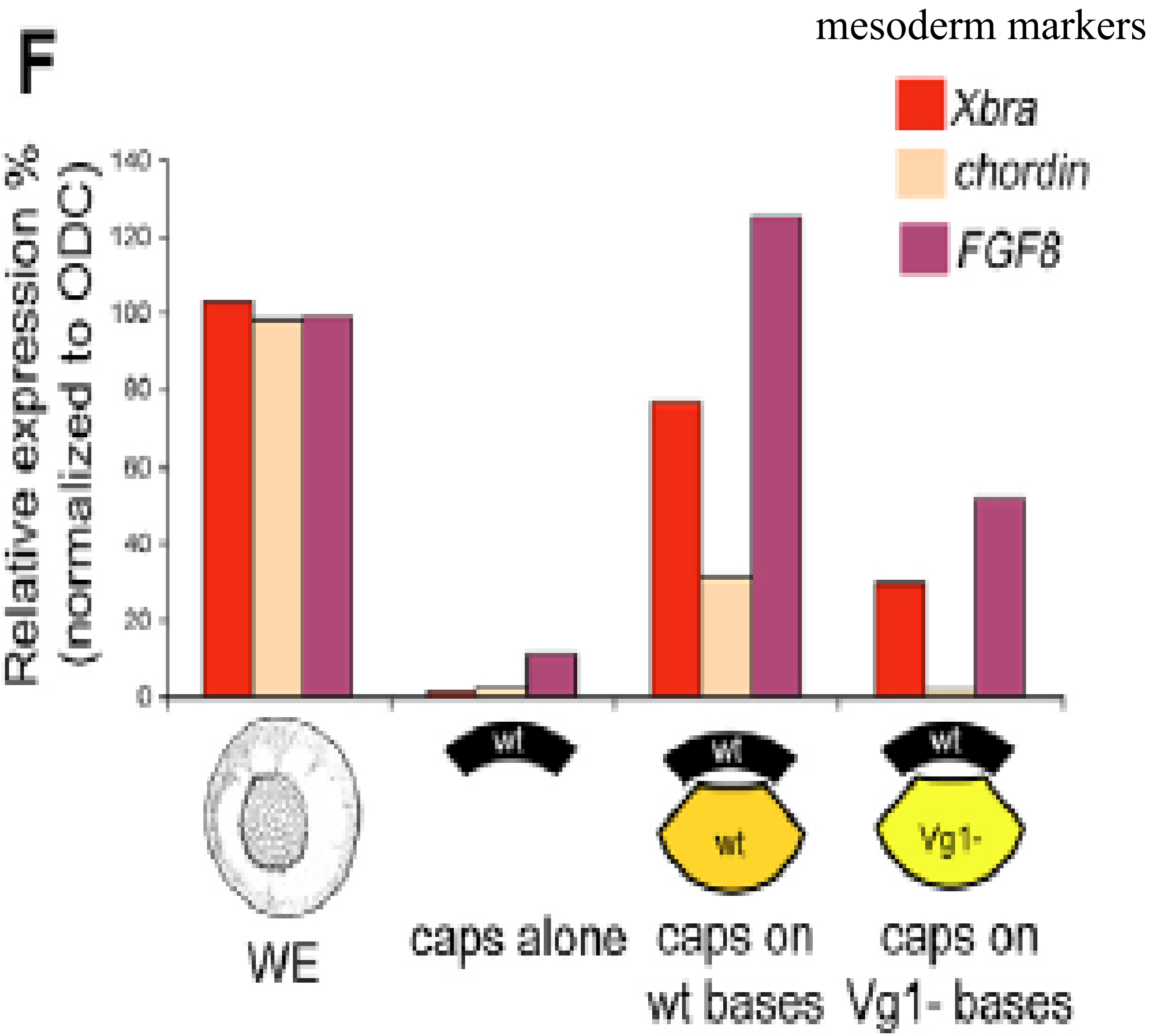
Fig. 3.2 Distribution of mRNA for the growth factor Vg-1 in the amphibian egg. *In situ* hybridization with a radioactive probe for maternal Vg-1 mRNA shows its localization (yellow) in the vegetal region. Scale bar = 1 mm.

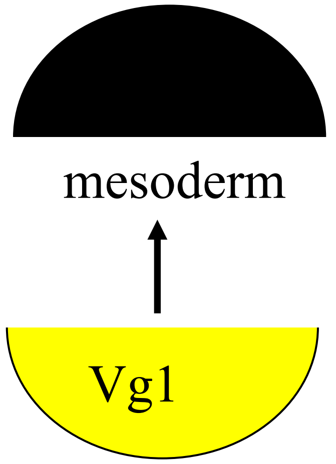
Photograph courtesy of D. Melton.

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

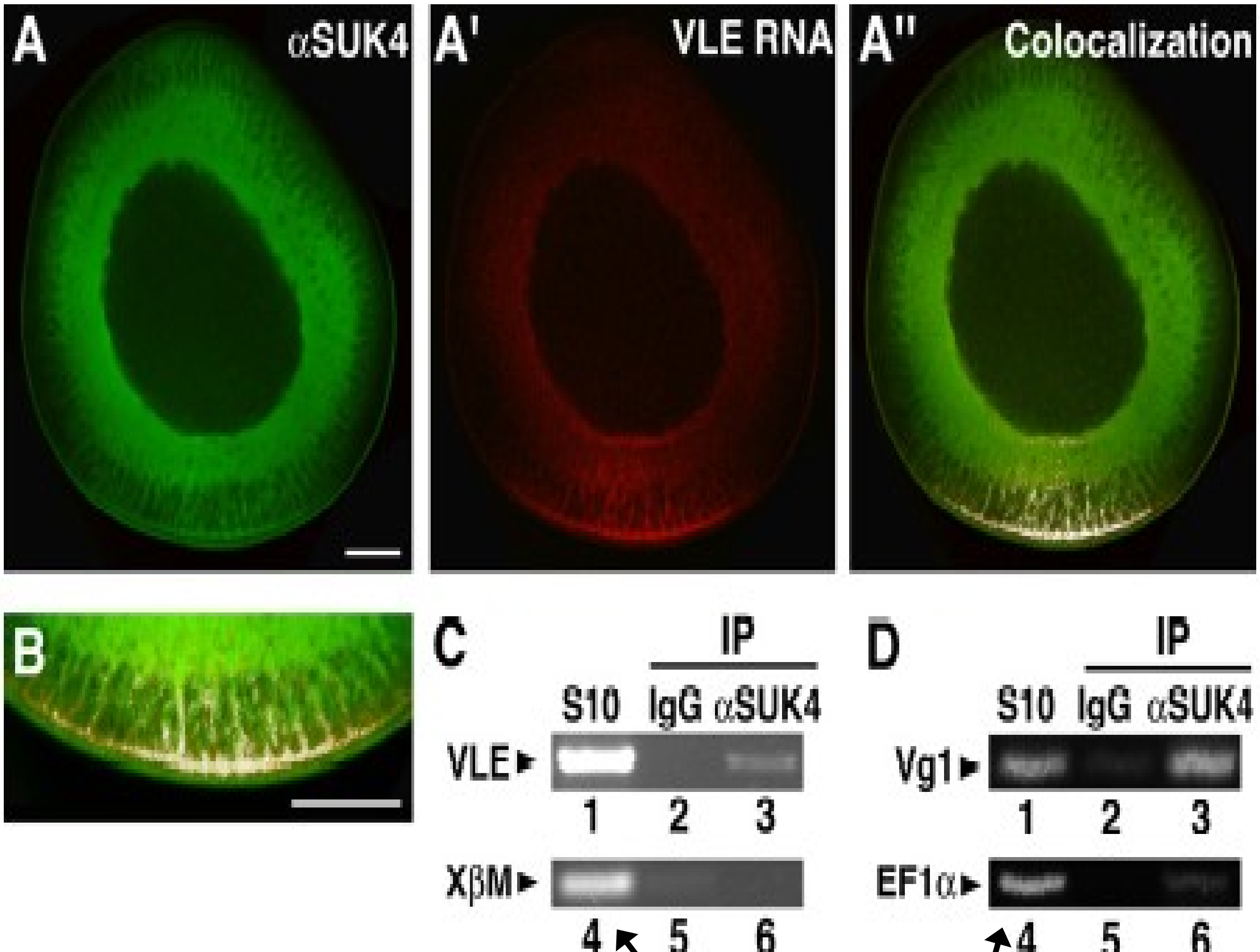


…..via loss of the induction of the mesodermal markers



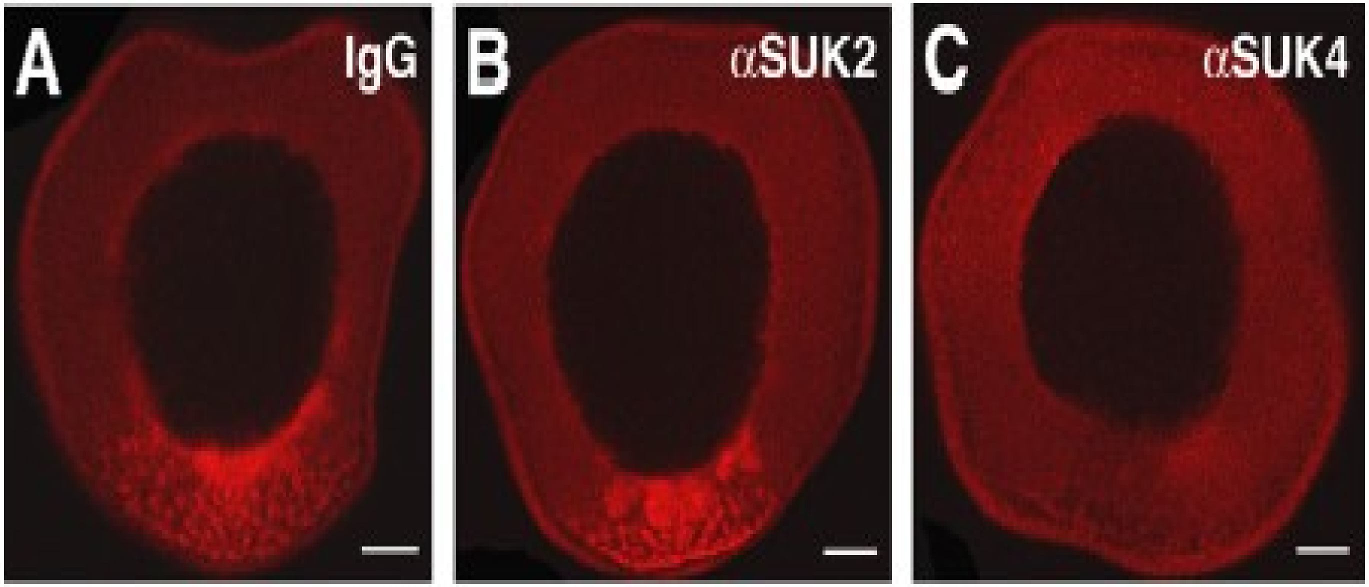


MECHANISMS OF INTRACELLULAR mRNA SORTING



total oocyte lysate

VLE - Vg1
SUK4- kinesin-1 heavy chain



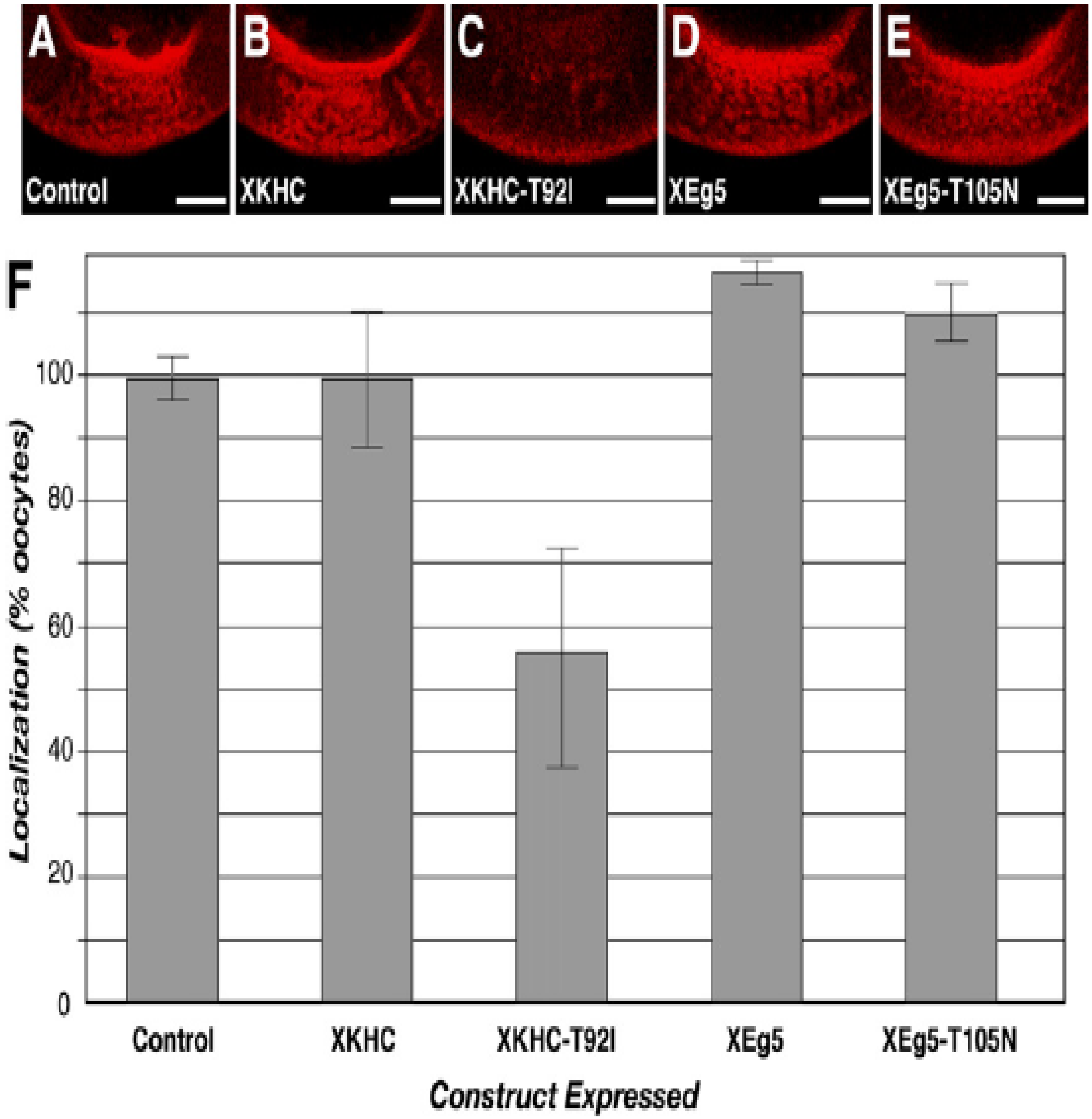
D

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



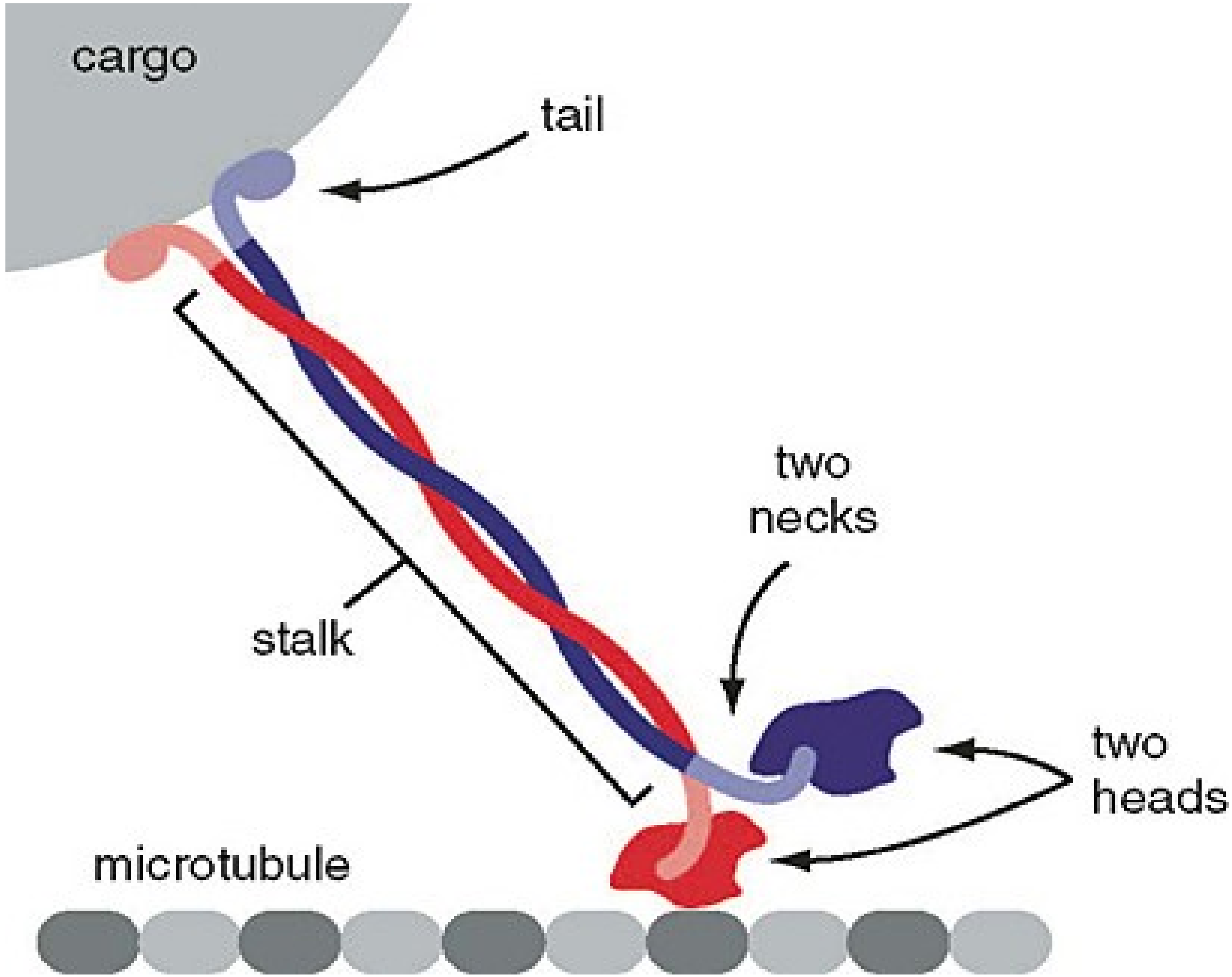
XKHC - kinesin 1

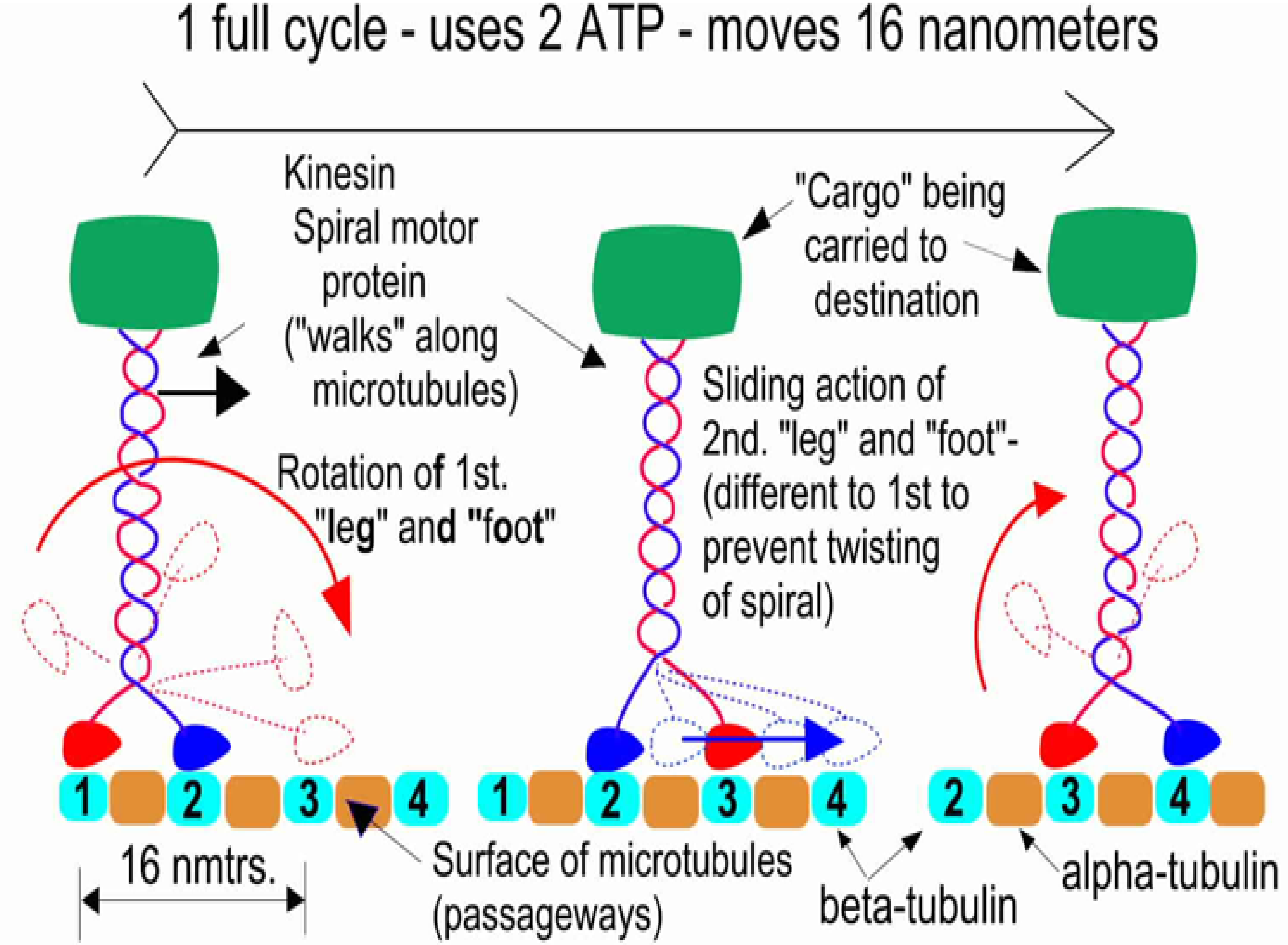
Red - injected Vg1 RNA

XKHC-T591 – rigor mutant (can not move – binds tight to microtubules)

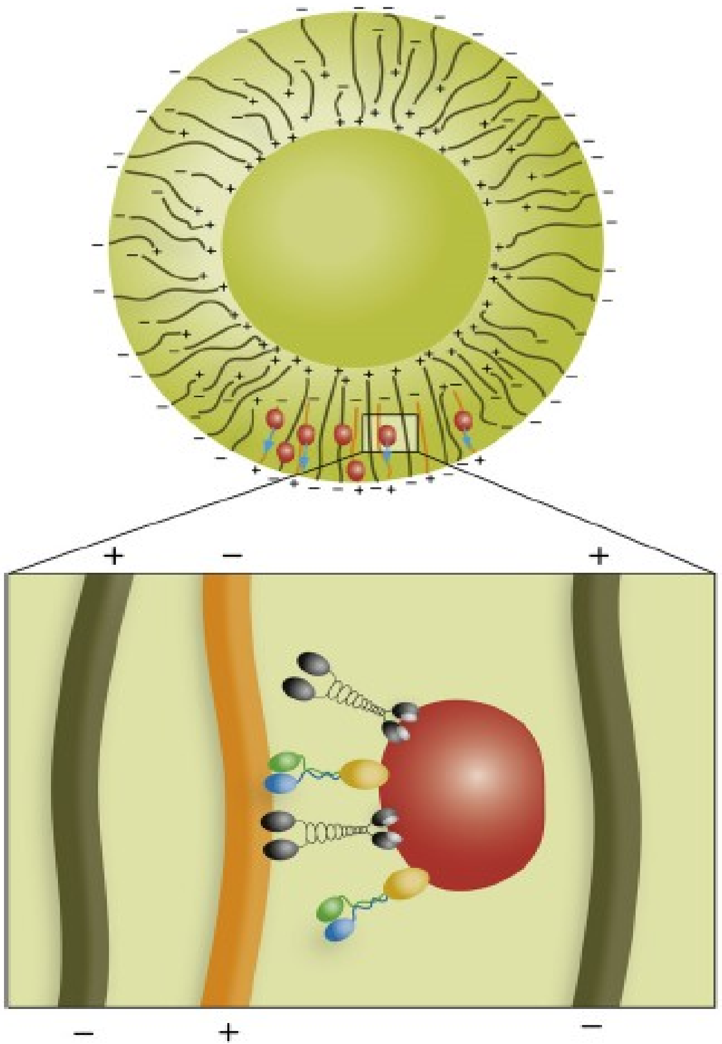
XEg5-T105N – rigor mutant of kinesin unrelated to XKHC

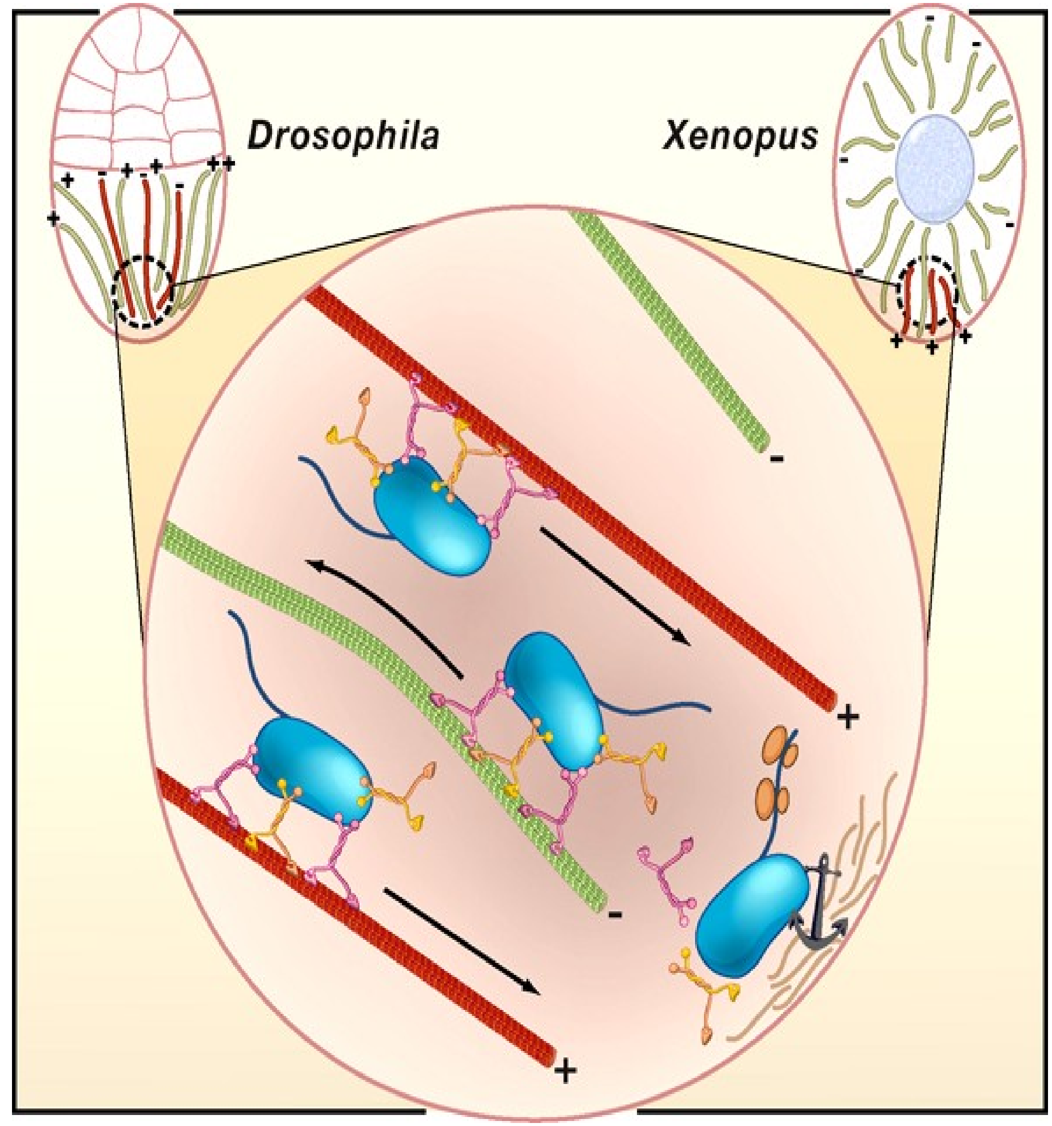
KINESIN TRANSPORT

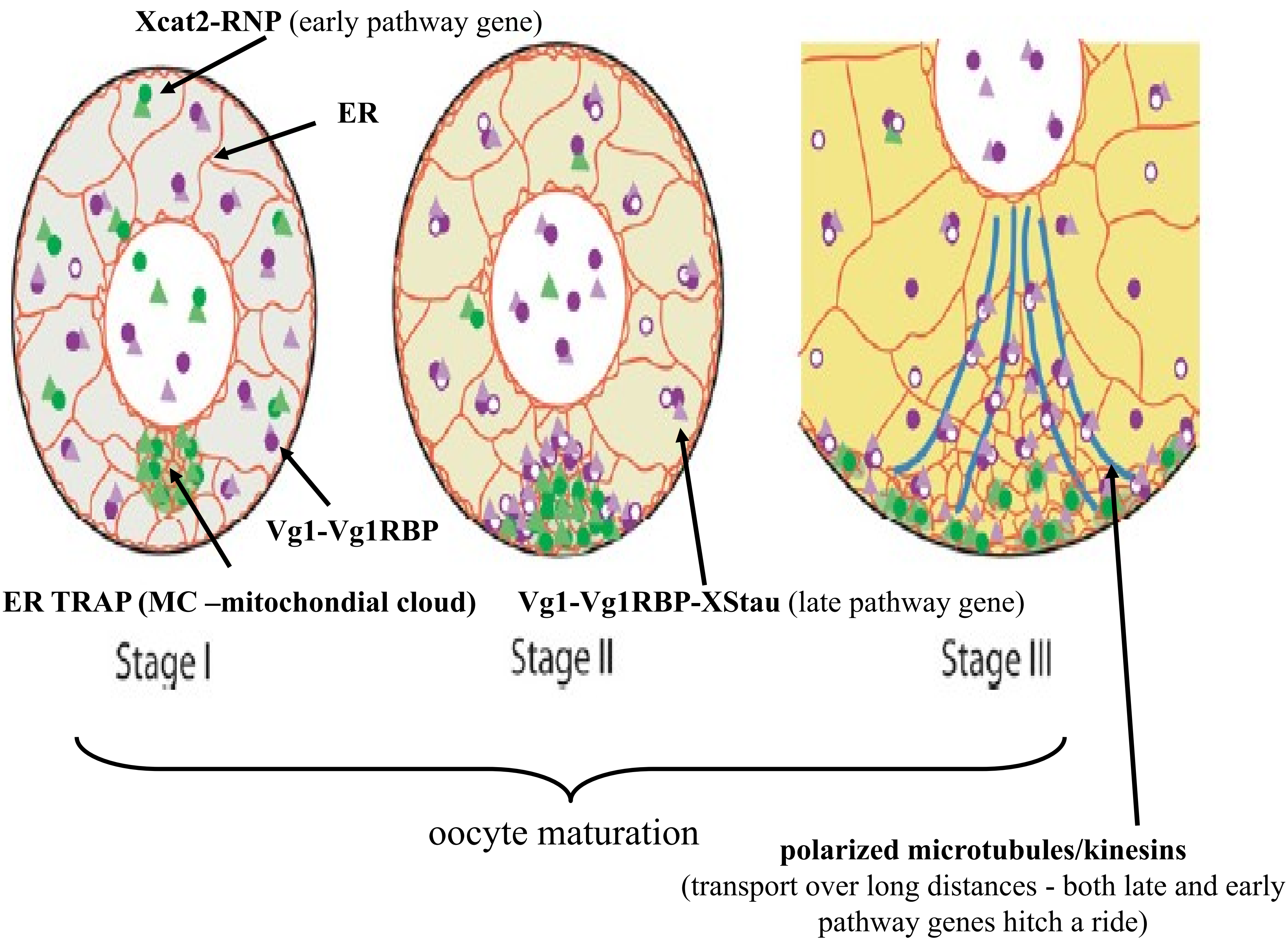




The (Ncr) Kinesin "walking" transportation of cell chemicals




Drosophila
Xenopus



Xcat2-RNP (early pathway gene)
ER
Vg1-Vg1RBP
ER TRAP (MC –mitochondial cloud)
Vg1-Vg1RBP-XStau (late pathway gene)
Stage I
Stage II
Stage III
oocyte maturation
polarized microtubules/kinesins
(transport over long distances - both late and early pathway genes hitch a ride)