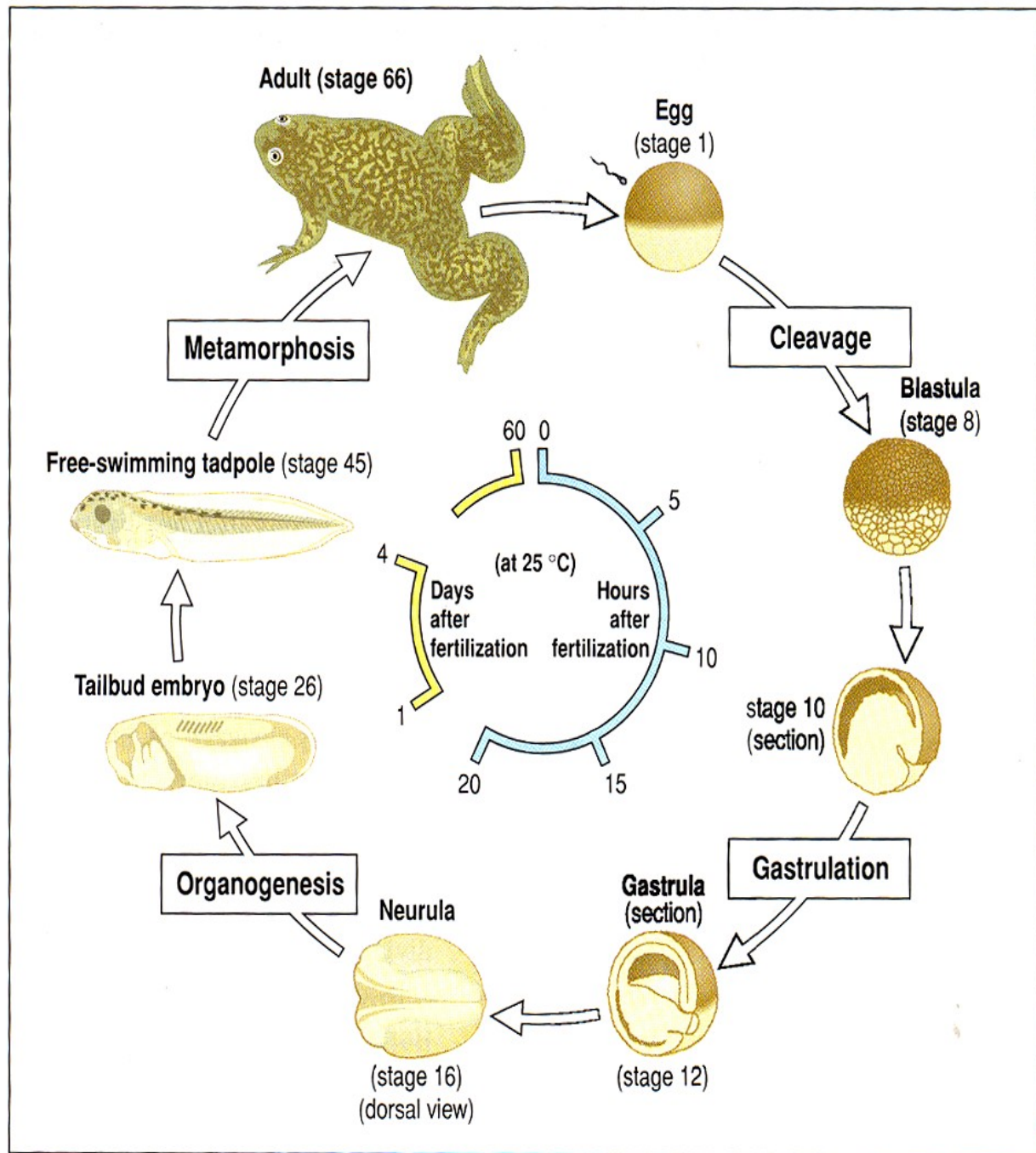
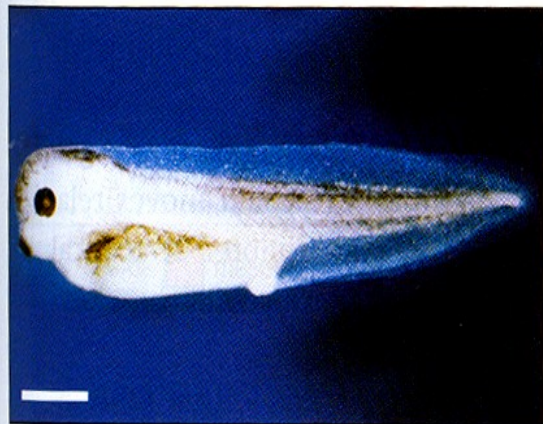
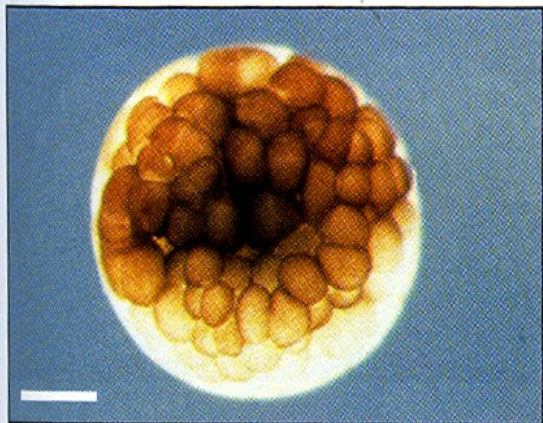


# MATERNAL mRNA LOCALIZATION IN THE FROG DEVELOPMENT

Putting RNAs at the right place in the right time



# GASTRULATION/NEURULATION

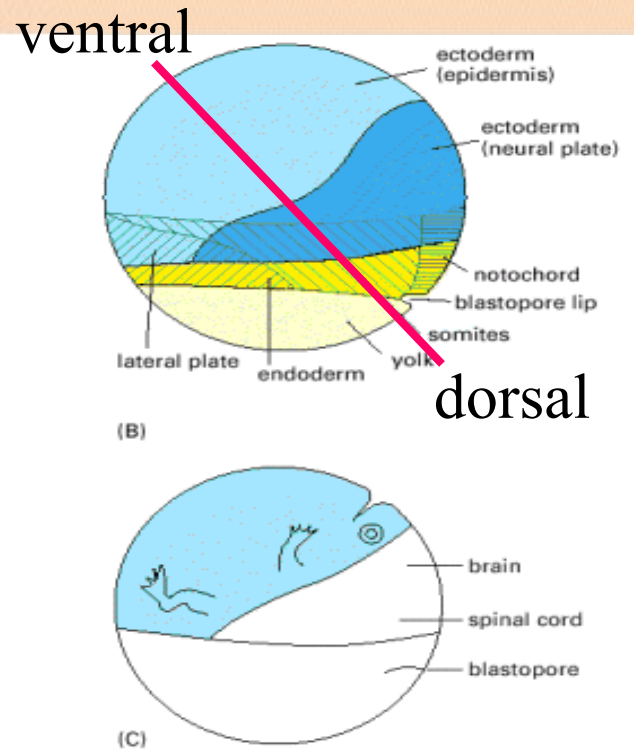
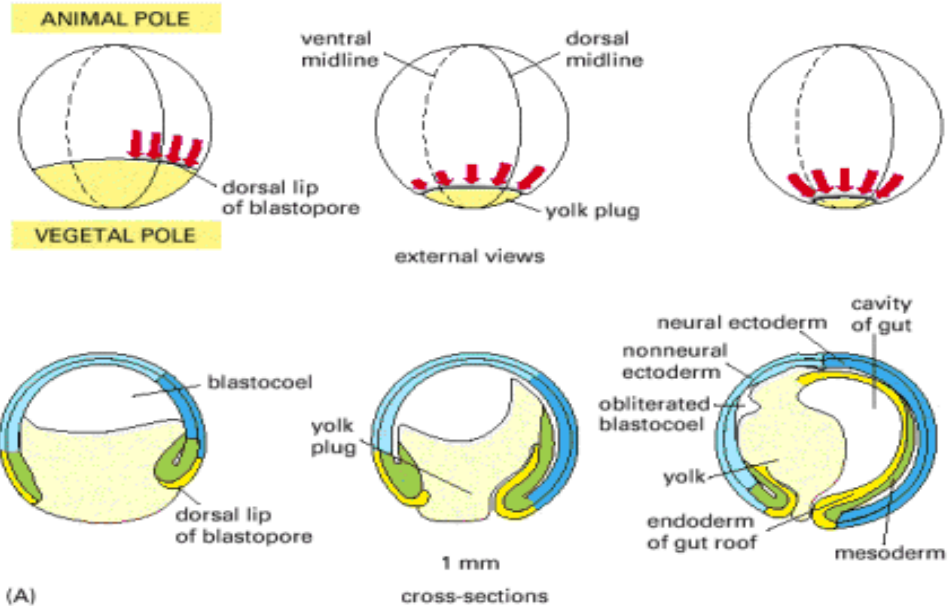
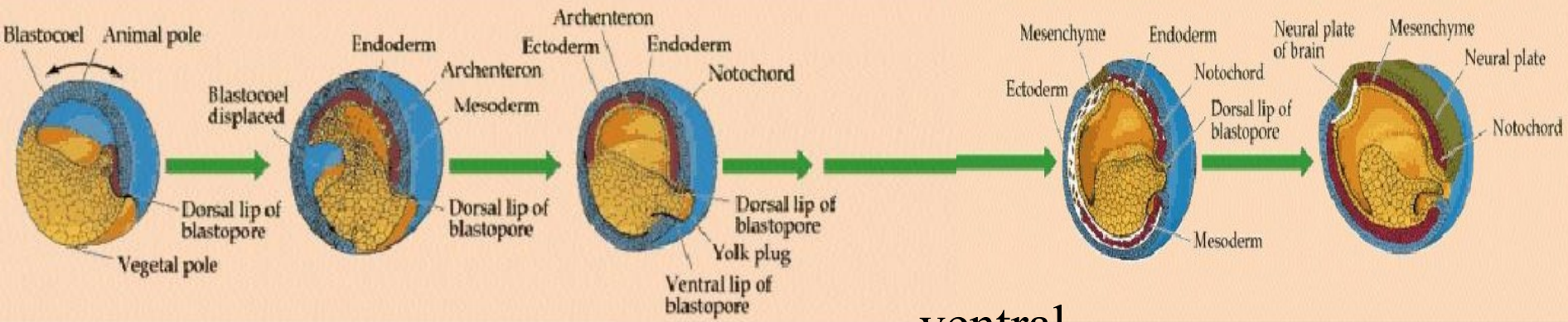
**1** Gastrulation begins when cells just below the center of the gray crescent invaginate to form the dorsal lip of the future blastopore.

**2** Cells of the animal pole spread out, pushing surface cells below them toward and across the dorsal lip. Those cells involute into the interior of the embryo, where they form the endoderm and mesoderm.

**3** This involution creates the archenteron and destroys the blastocoel. The dorsal lip forms a circle, with cells moving to the interior all around the blastopore; the yolk plug is visible through the blastopore.

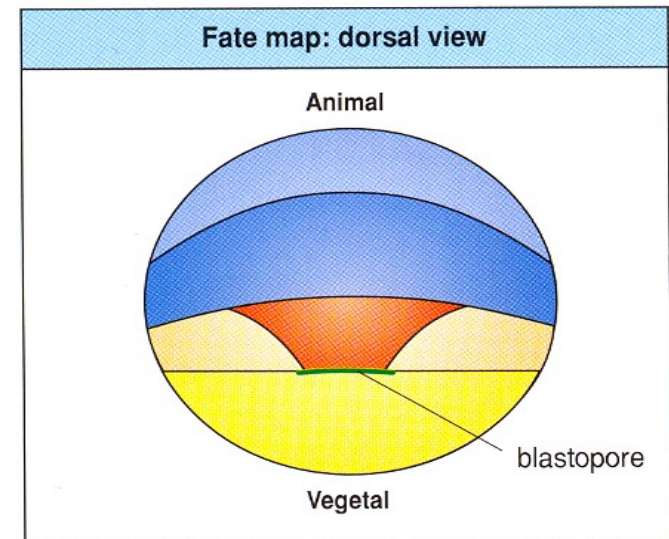
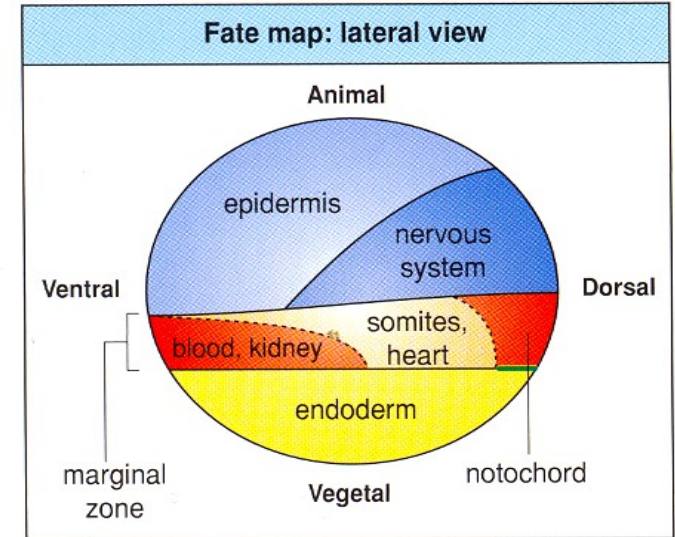
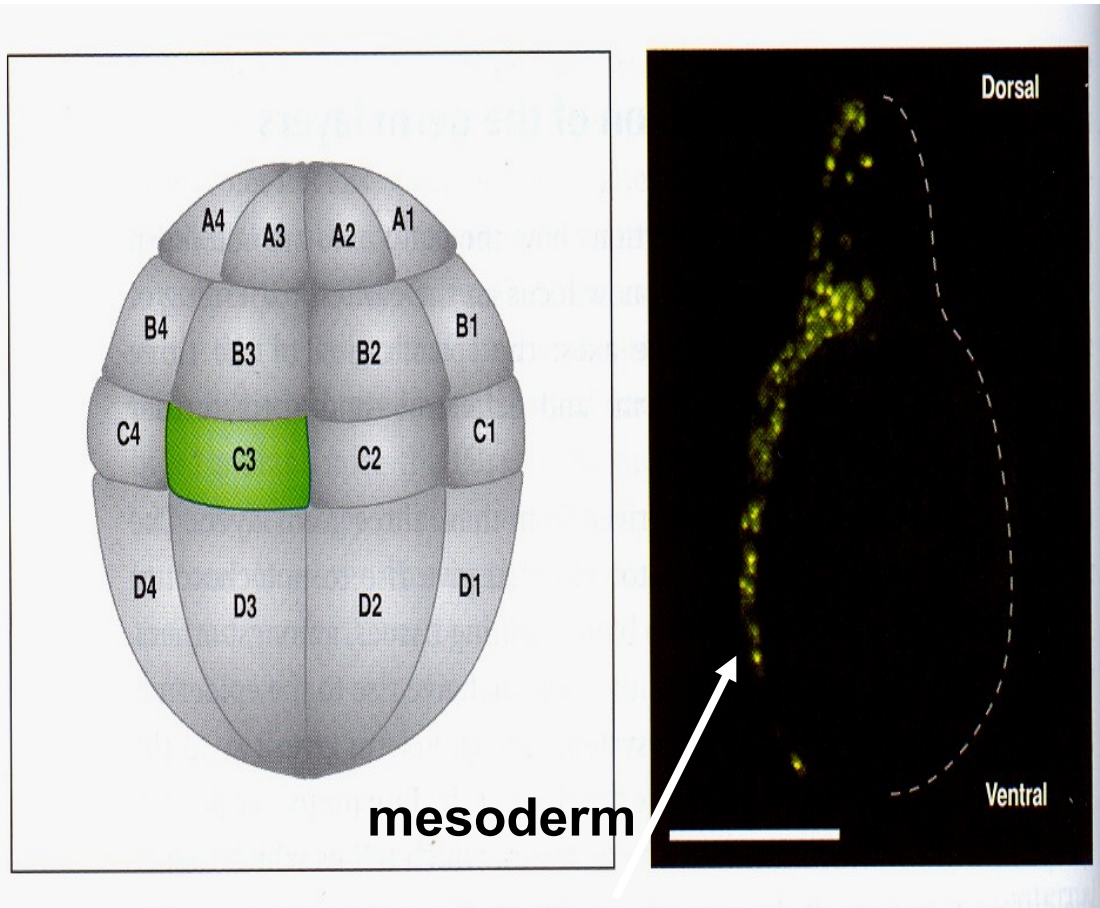
**4** Continued development gives rise to a notochord derived from mesoderm.

**5** The beginnings of the nervous system (green) are derived from ectoderm.





# ACCURACY AND REPRODUCIBILITY IN ACQUIRING THE CELLULAR FATE WITHIN THE EMBRYO



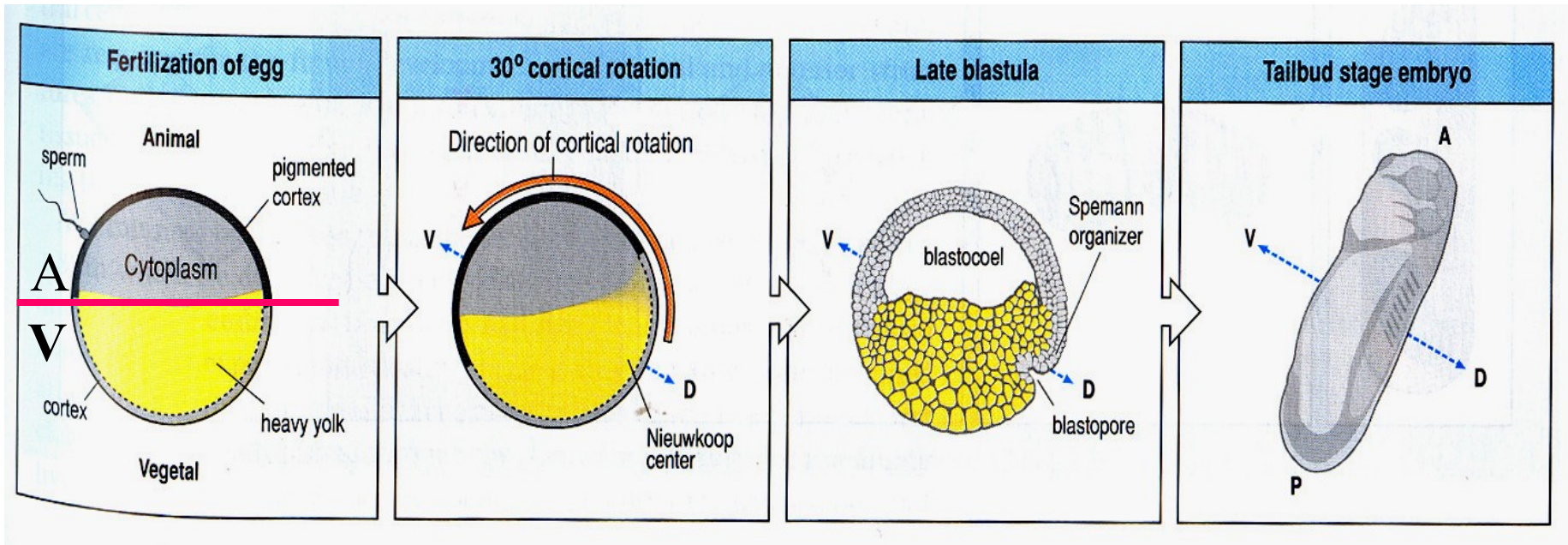
**Fig. 3.18** Fate map of a late *Xenopus* blastula.

The mesoderm gives rise to the epidermis and

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

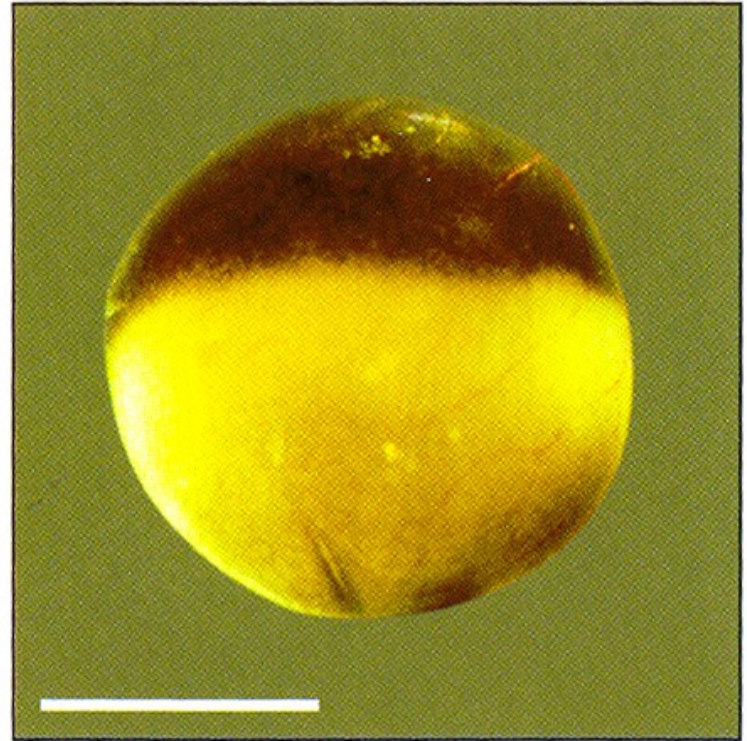


GASTRULATION/NEURULATION – dorsal surface view



# 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.

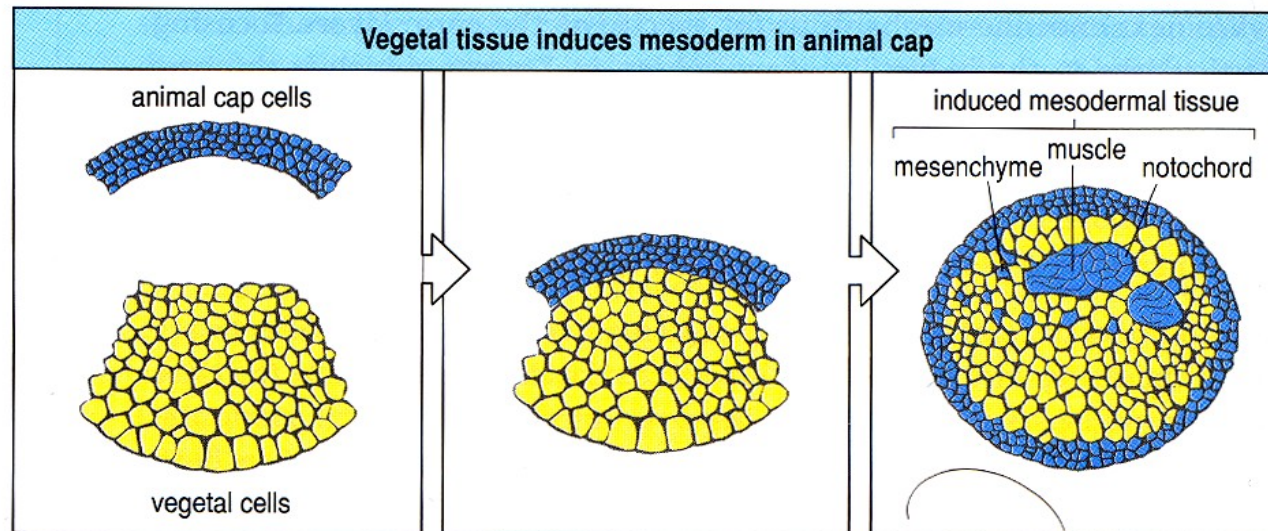
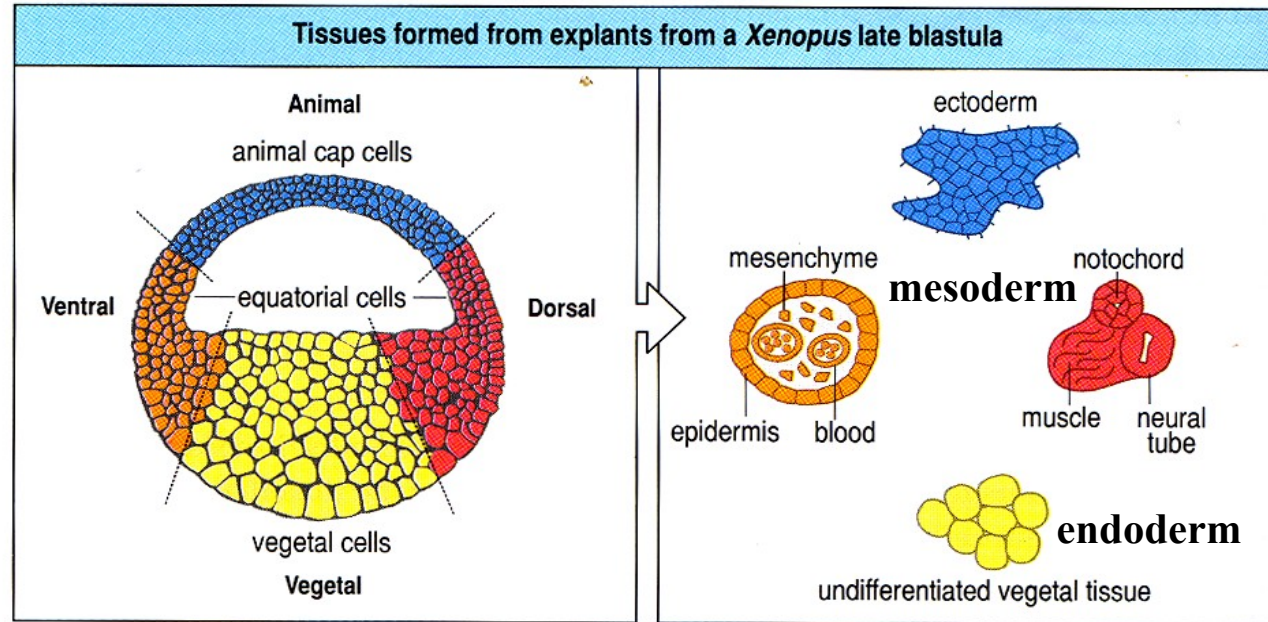


**Fig. 2.4** The unfertilized egg of *Xenopus*. The surface of the animal half (top) is pigmented and the paler, vegetal half of the egg is heavy with yolk. Scale bar = 1 mm.

Photograph courtesy of J. Smith.

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

Ectoderm and endoderm are specified by maternal factors in the egg  
Mesoderm is induced by vegetal tissue





# MATERNAL vs. ZYGOTIC REGULATORS

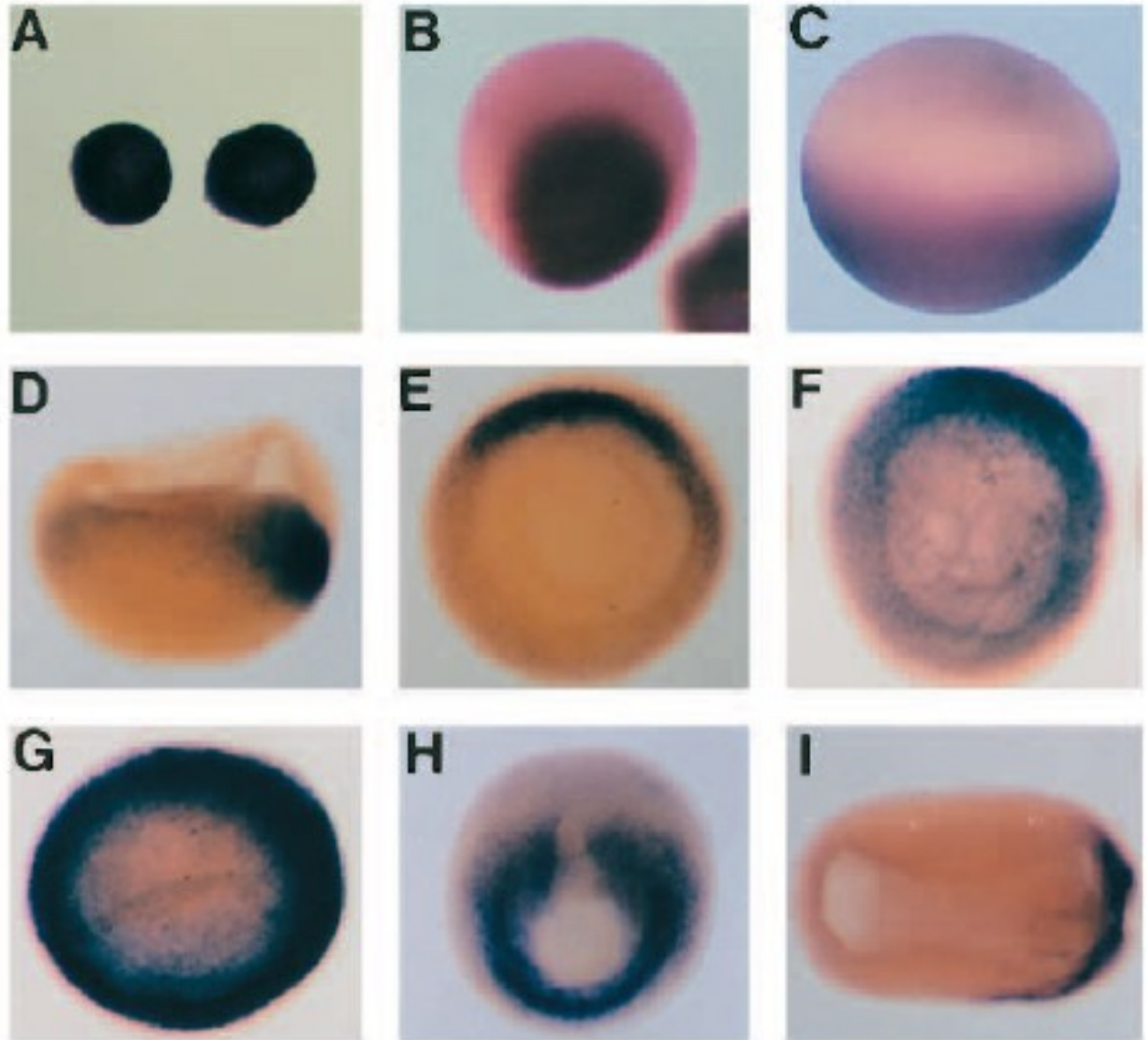
## Summary: genes involved in patterning of axes and germ layers

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

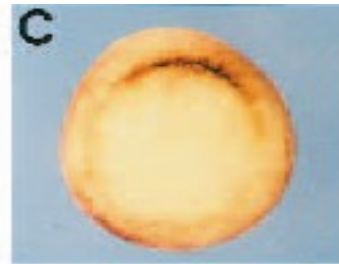
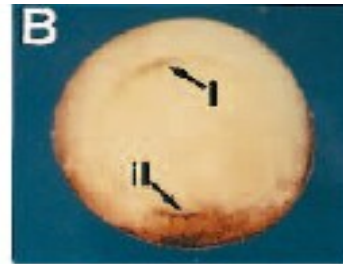
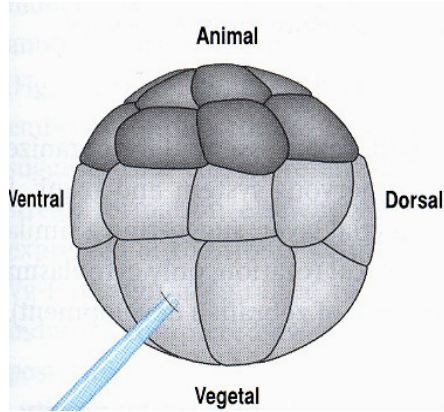


# VegT (T-box family transcription factor)

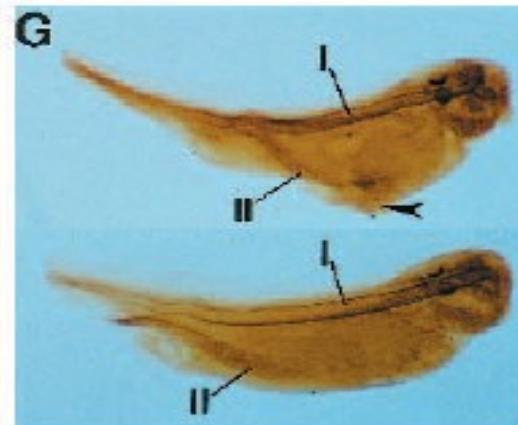
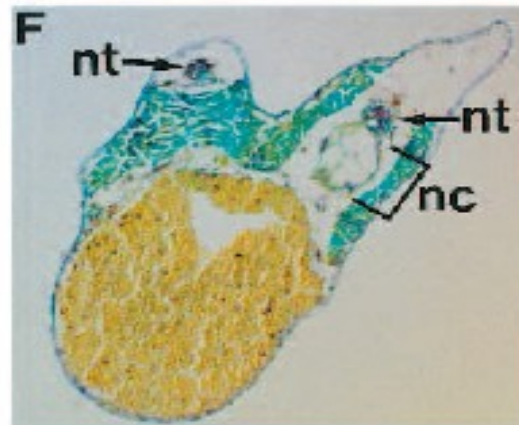
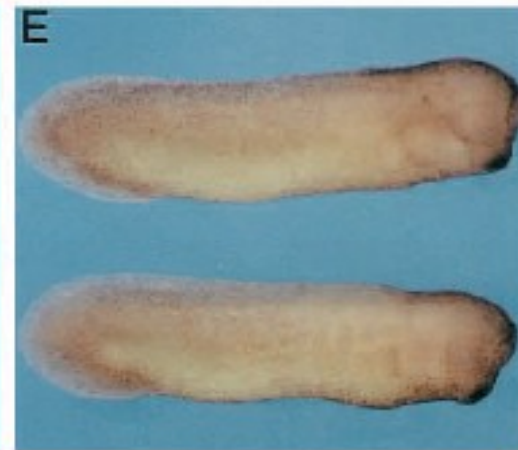
- A** – stage I oocytes
- B** – stage IV oocytes
- C** – ovulated egg
- D** – stage 9.5 embryo
- E** – stage 9.5 embryo  
(vegetal pole view)
- F** – stage 10.25 embryo  
(vegetal pole view)
- G** – stage 10.5 embryo  
(vegetal view)
- H** – stage 12.5 embryo  
(posterior view)
- I** – mid neural fold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



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

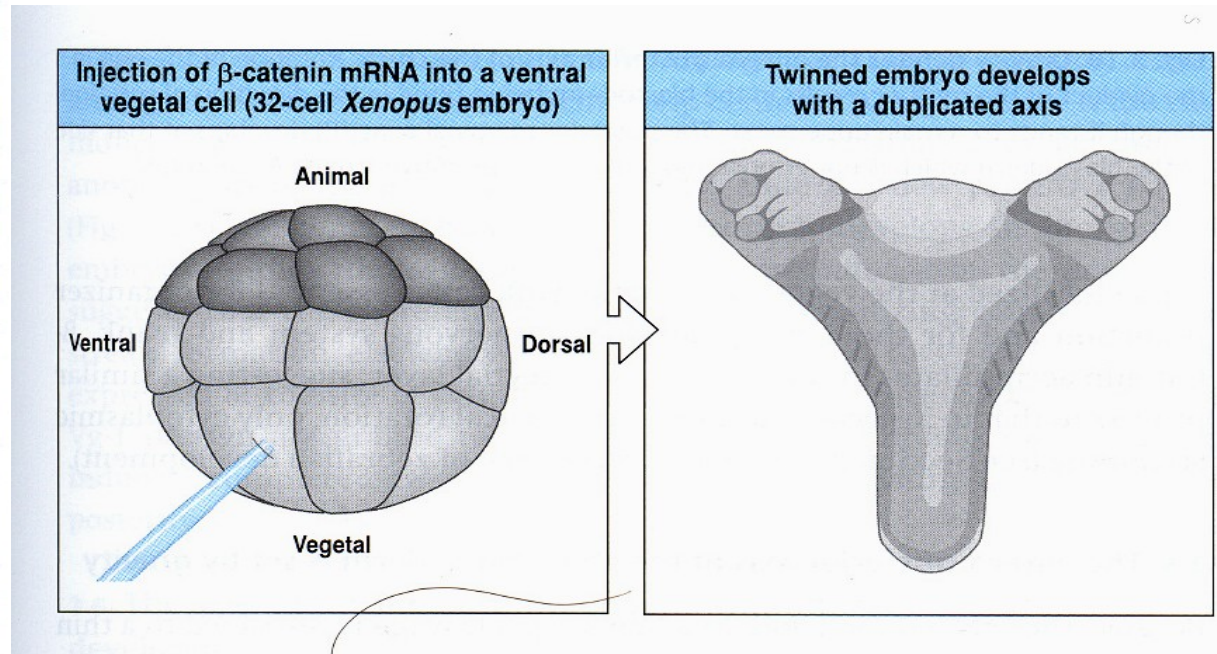
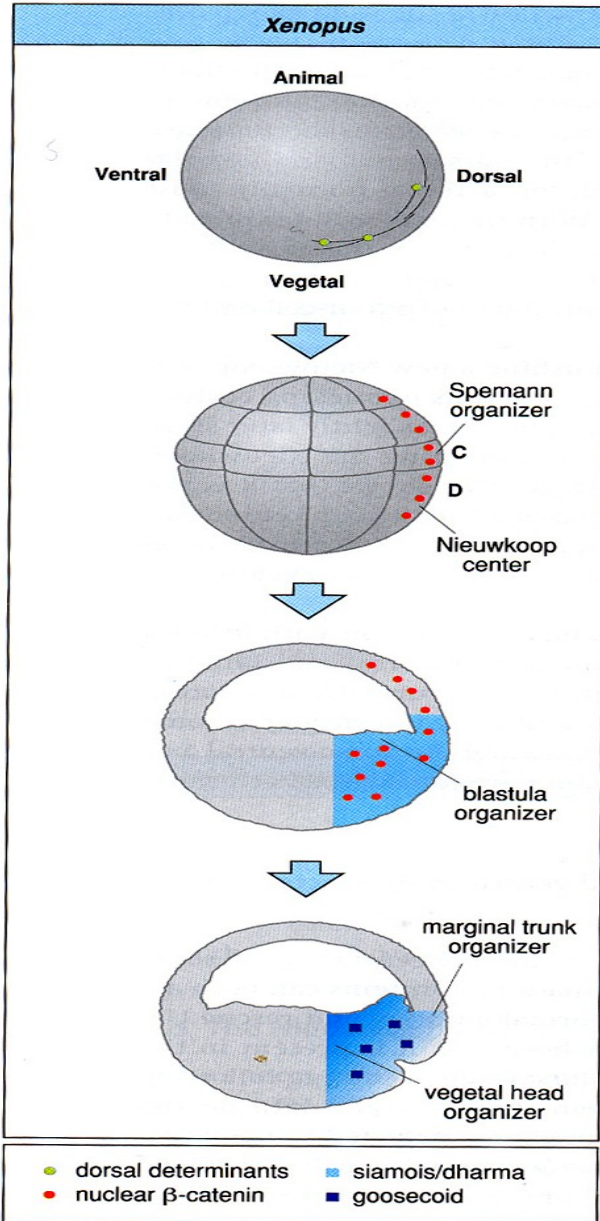


I – primary axis  
II - secondary axis  
nt – neural tube  
nc – notochord  
green – muscles  
arrow – ectopic auditory vesicles

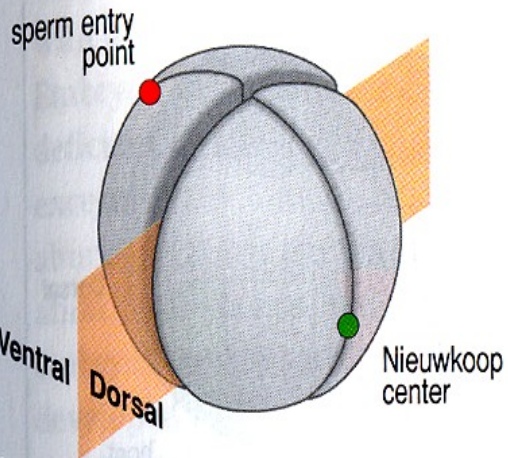




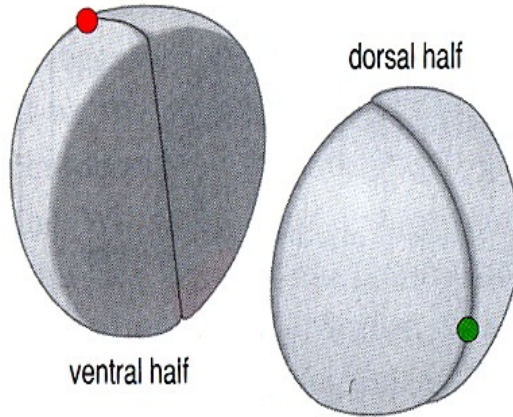
# .....by activation of Xwnt8/ $\beta$ -catenin pathway



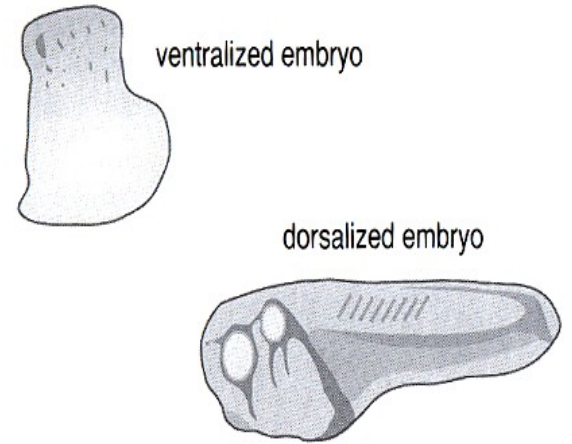
**Xenopus embryo at the four-cell stage divided into dorsal and ventral halves**



Ventral half lacks Nieuwkoop center

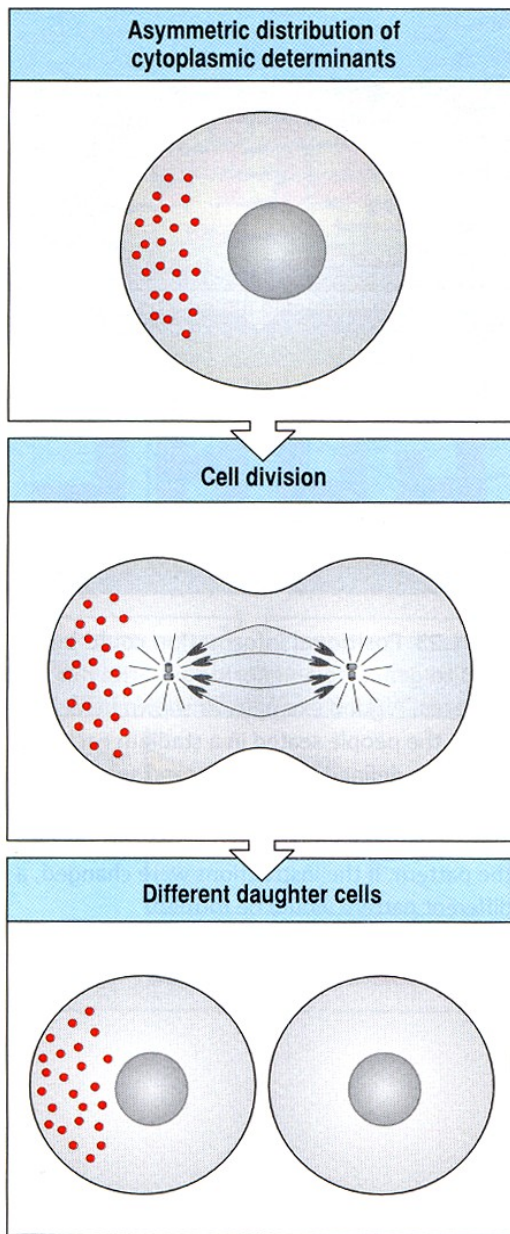


**The ventral half develops into a ventralized embryo. The dorsal half develops into a dorsalized embryo**



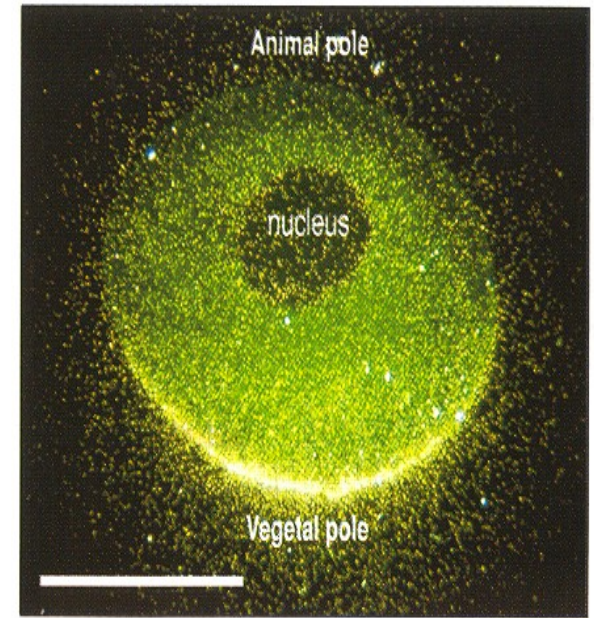


# Vg1 (TGFβ family ligand)



**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.

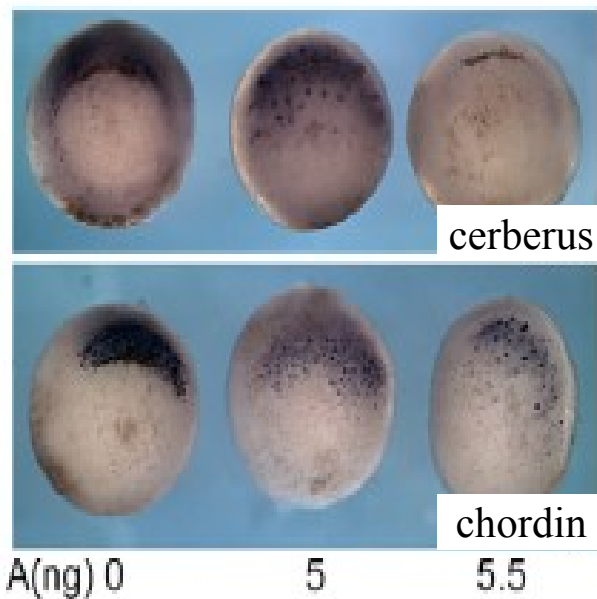
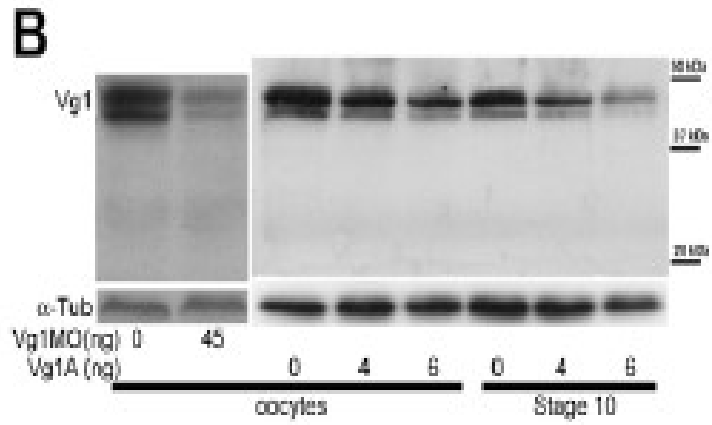
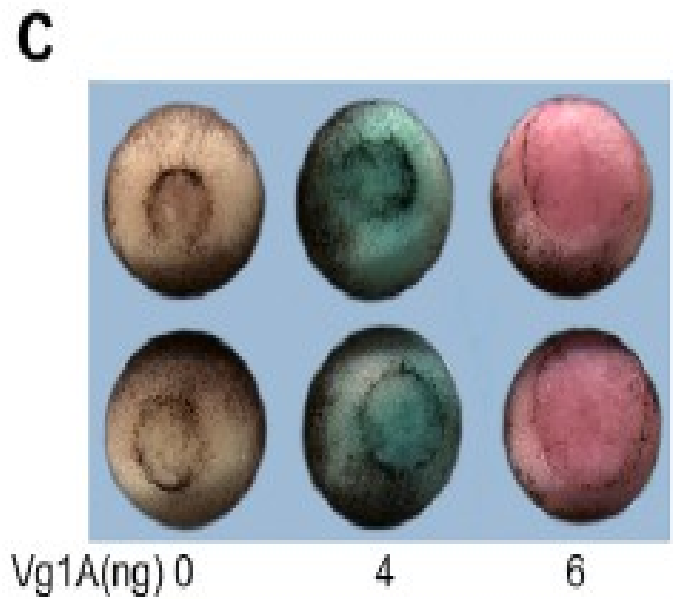
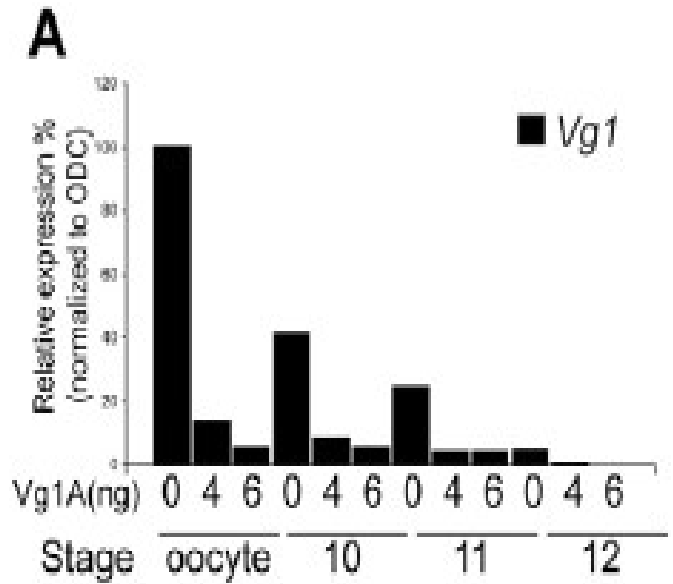
Photograph courtesy of J. Smith.



**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.

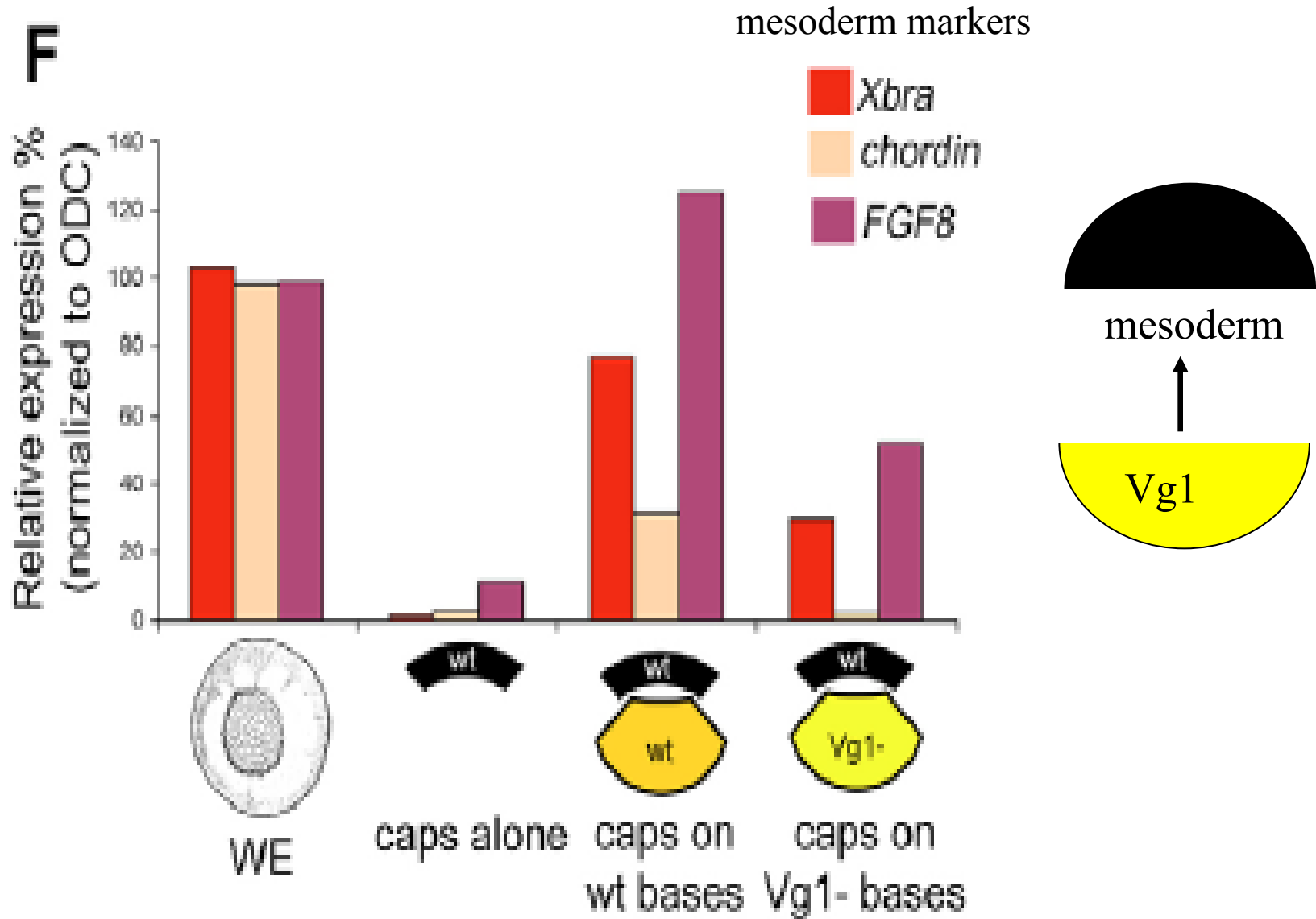
Photograph courtesy of D. Melton.

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

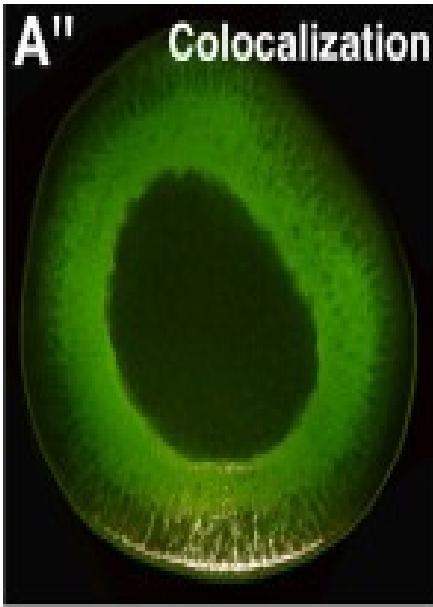
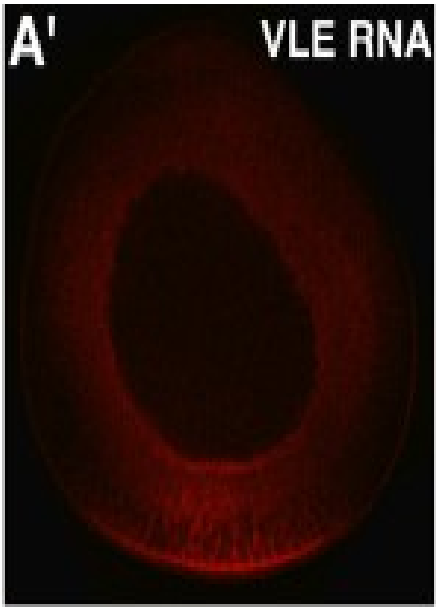
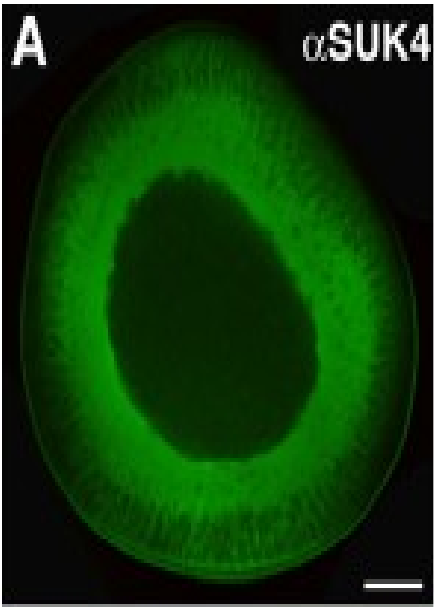




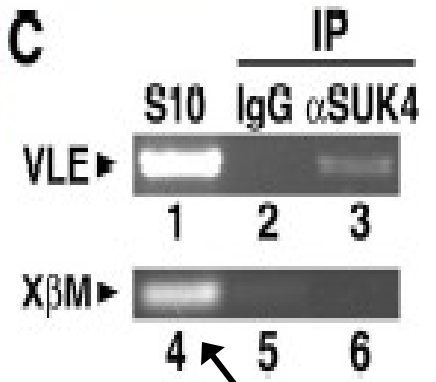
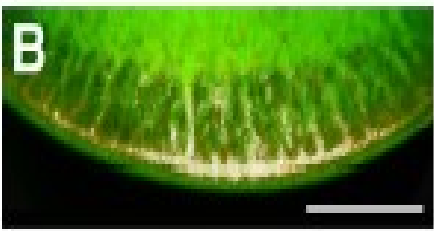
.....via loss of the induction of the mesodermal markers



# MECHANISMS OF INTRACELLULAR mRNA SORTING

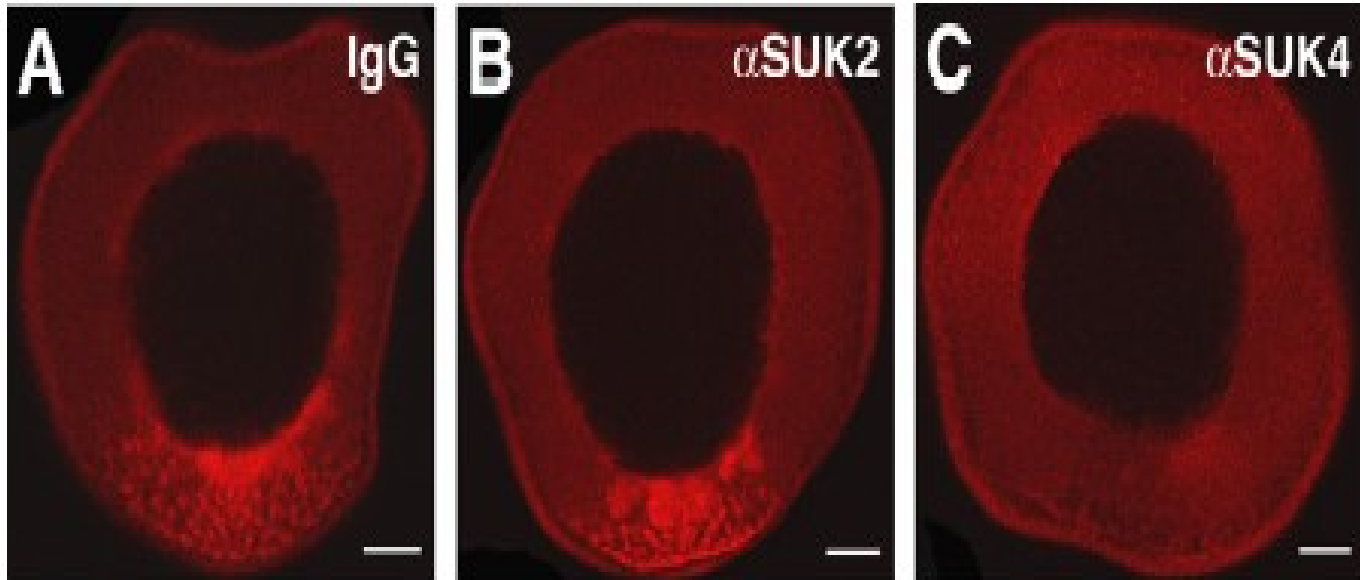


VLE - Vg1  
 SUK4- kinesin-1 heavy chain



total oocyte lysate





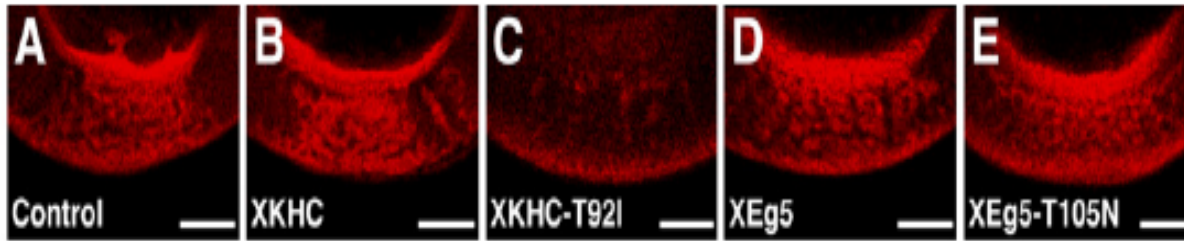
**D**

Injection	Localization (%)	<i>n</i>
IgG	100	62
$\alpha$ SUK2	102	36
$\alpha$ SUK4	52	53

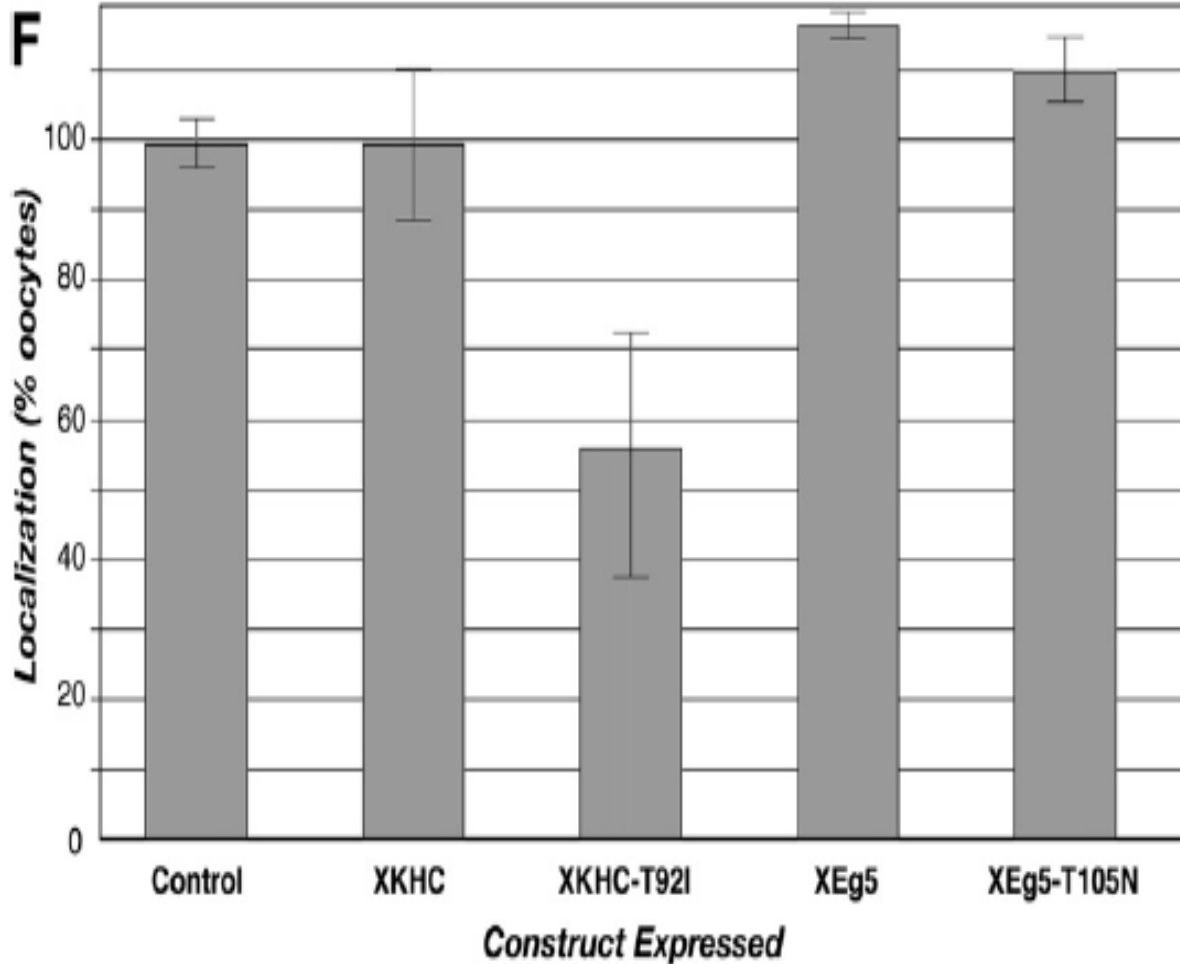
**IgG** – isotypic control  
**SUK2** –non-neutralizing Ab  
**SUK4** –neutralizing Ab



Vg1 mRNA localization

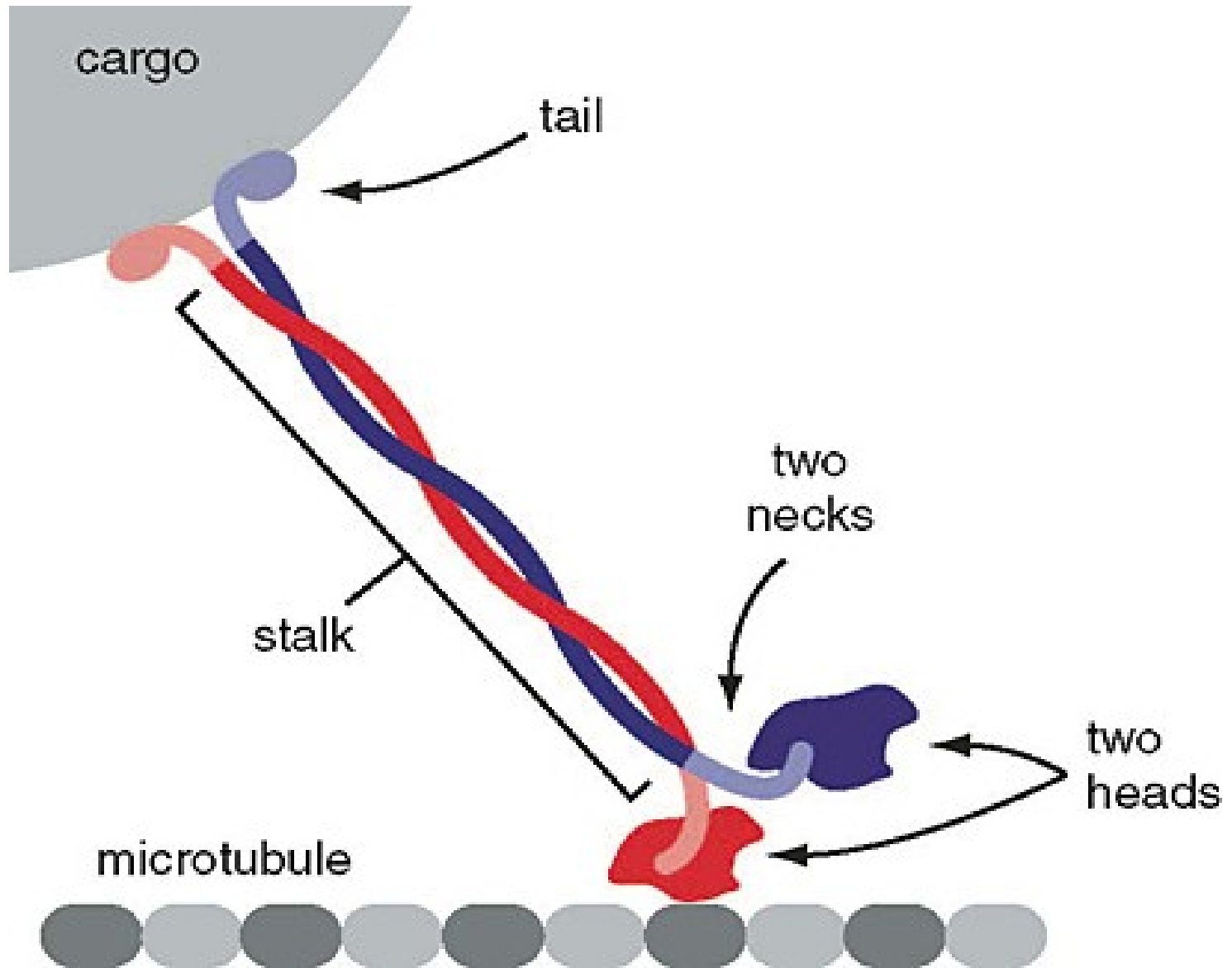


**XKHC** - kinesin 1  
**Red** - injected Vg1 RNA  
**XKHC-T591** – rigor mutant  
 (can not move – binds tight to microtubules)  
**XEg5-T105N** – rigor mutant  
 of kinesin unrelated to XKHC

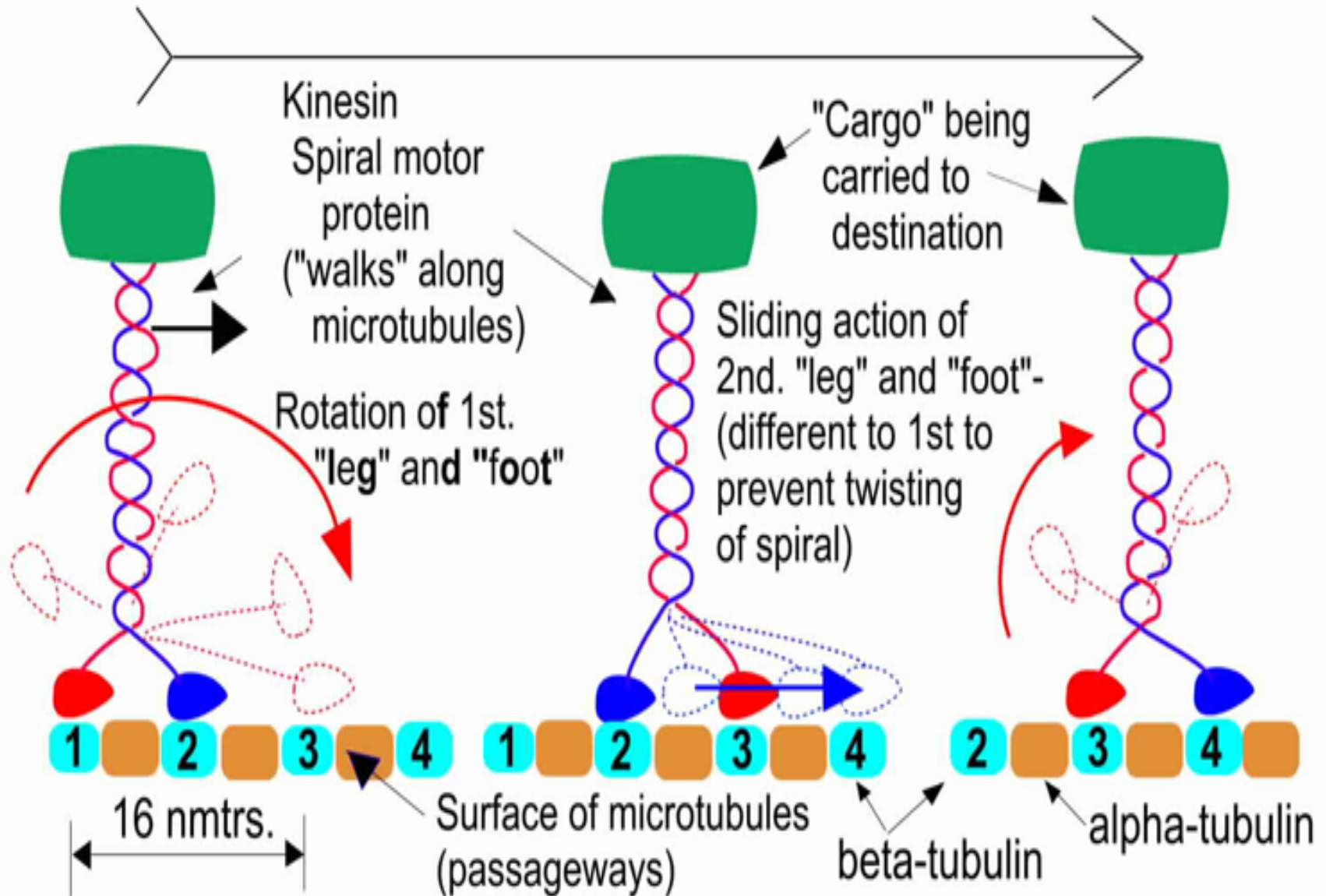




# KINESIN TRANSPORT



1 full cycle - uses 2 ATP - moves 16 nanometers



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



