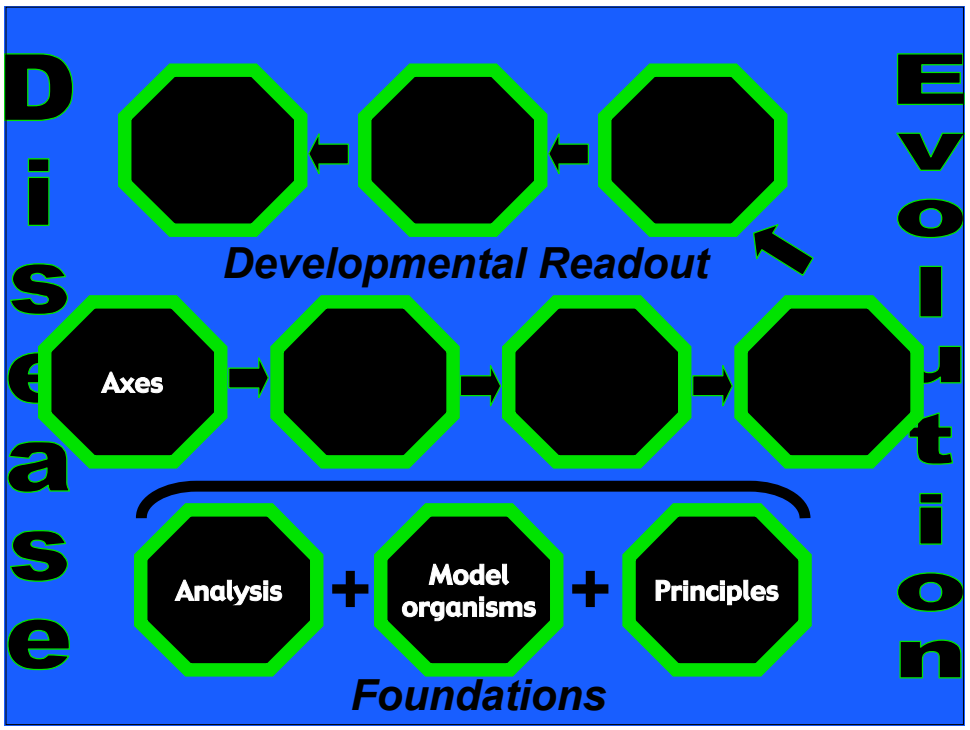
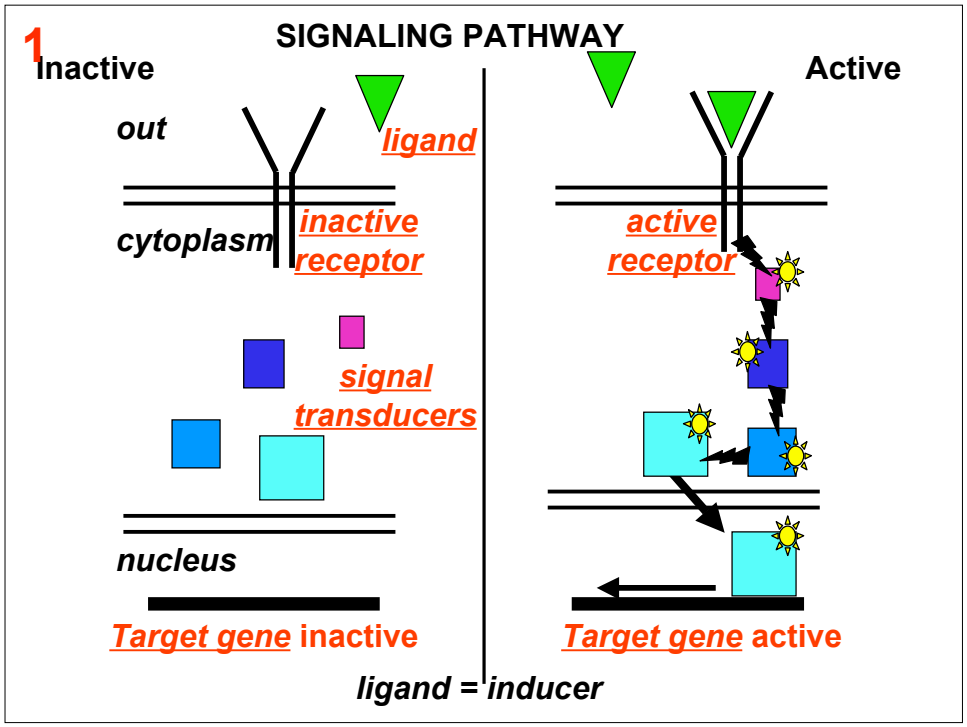
Development and Evolution
7.72
9.11.06

Dorsoventral axis

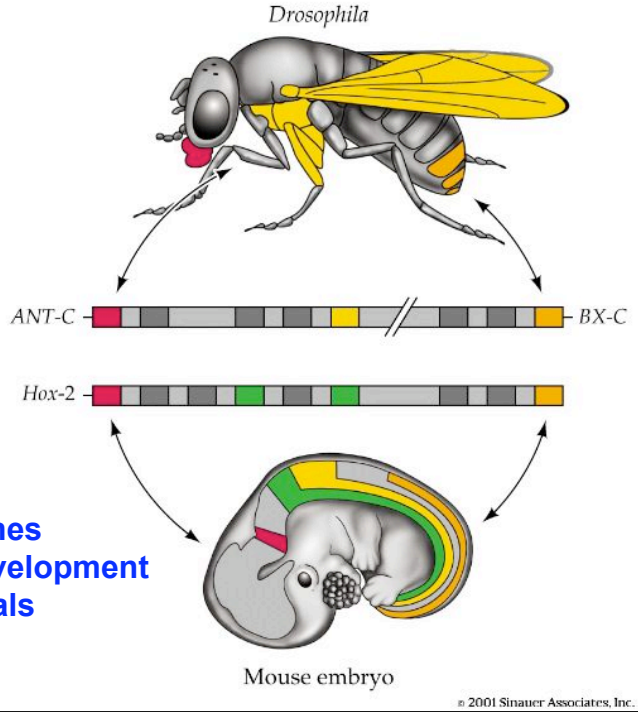


1. Signaling

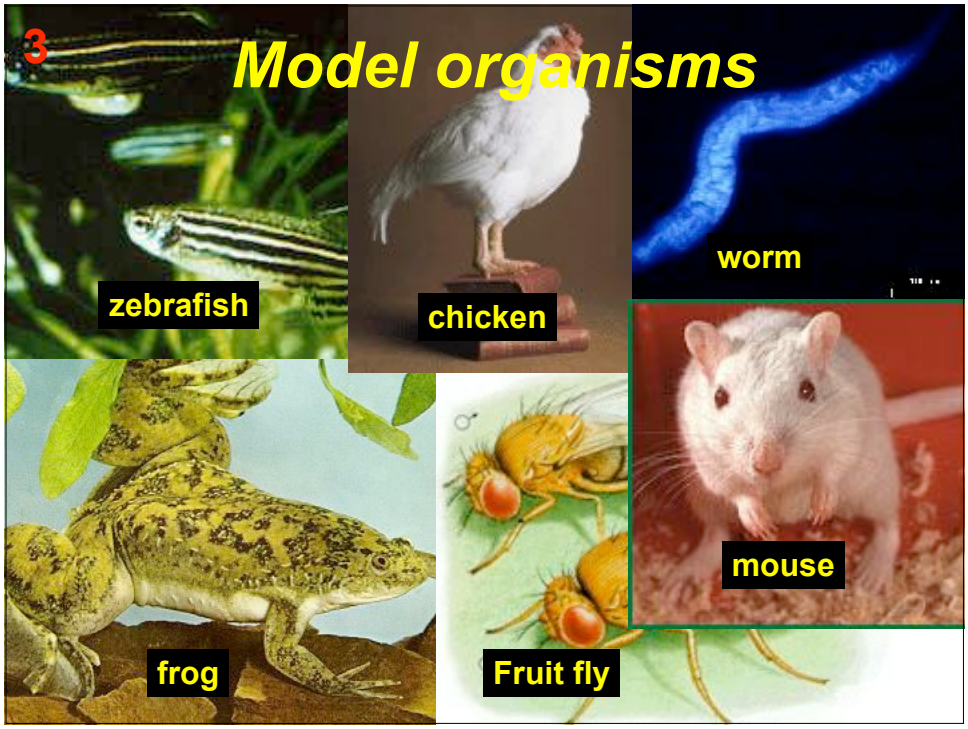

1
SIGNALING PATHWAY
Inactive
Active
out
ligand
cytoplasm
inactive receptor
active receptor
signal transducers
nucleus
Target gene inactive
Target gene active
ligand = inducer

2. Model organisms

2

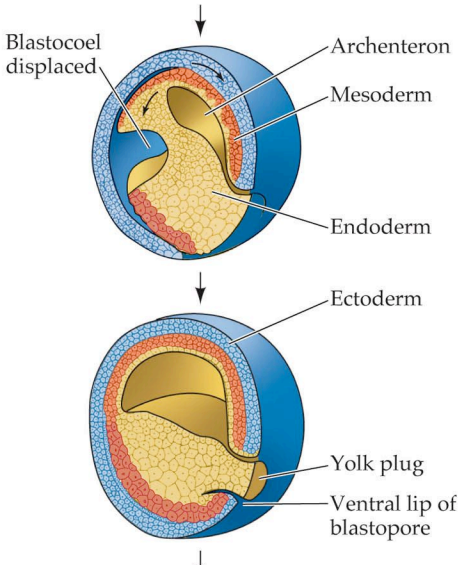


Similar genes control development in all animals



3. Landmarks in development
Germ layers

4



Ectoderm
epidermis
nervous system

Mesoderm
muscle
blood
kidney
heart

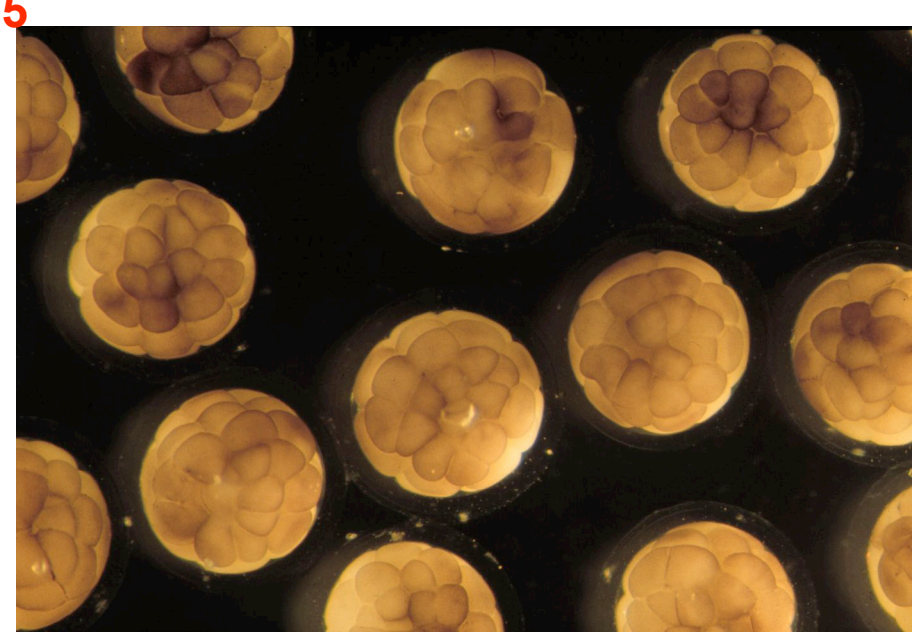
Endoderm
gut
lung

Embryonic "Germ Layers"
the first cell types

Y, Seventh Edition, Figure 20.9 Gastrulation in the Frog Embryo (Part 2)

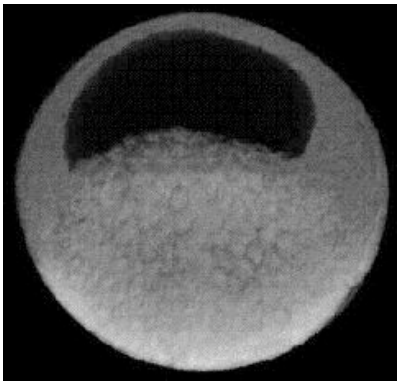
3. Landmarks in development
Stages

5



Cleavage stage frog embryos = cell division

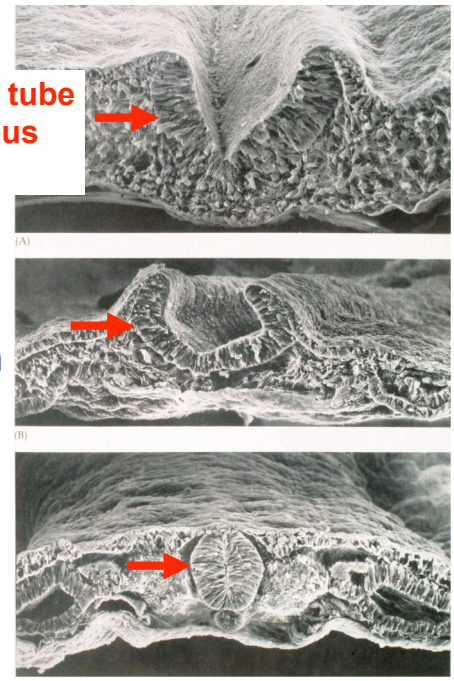
6



Gastrulation in the frog
= cell movement

7

Closing neural tube (future nervous system)

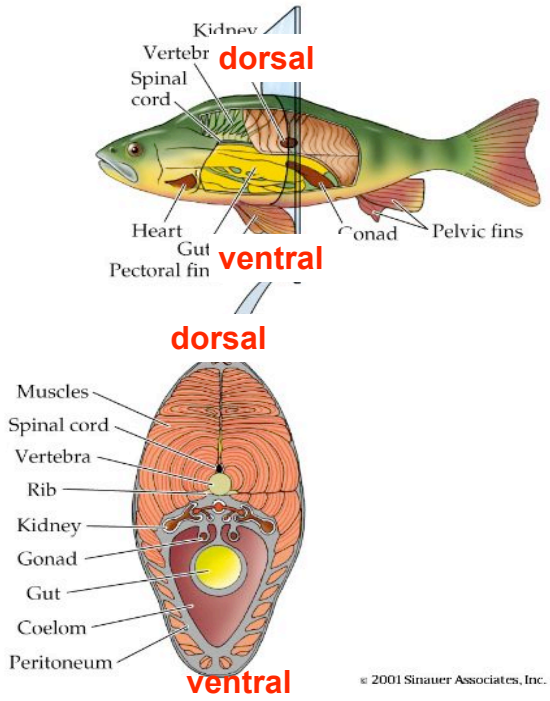


Neurulation in the chicken

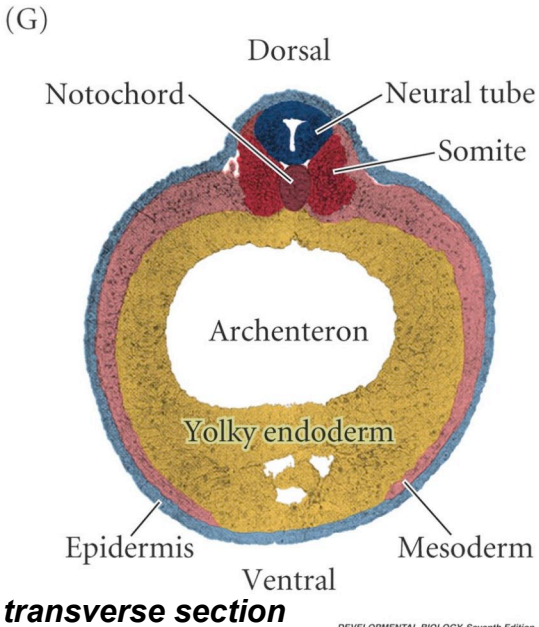
4. Dorsoventral axis

What is this?

8



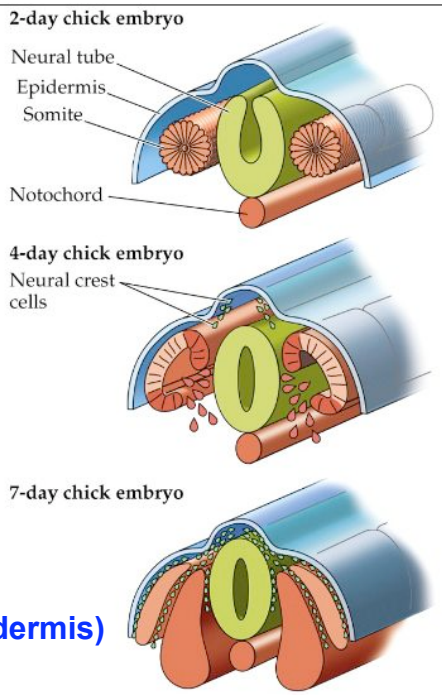
9



Xenopus
D/V axis, transverse section

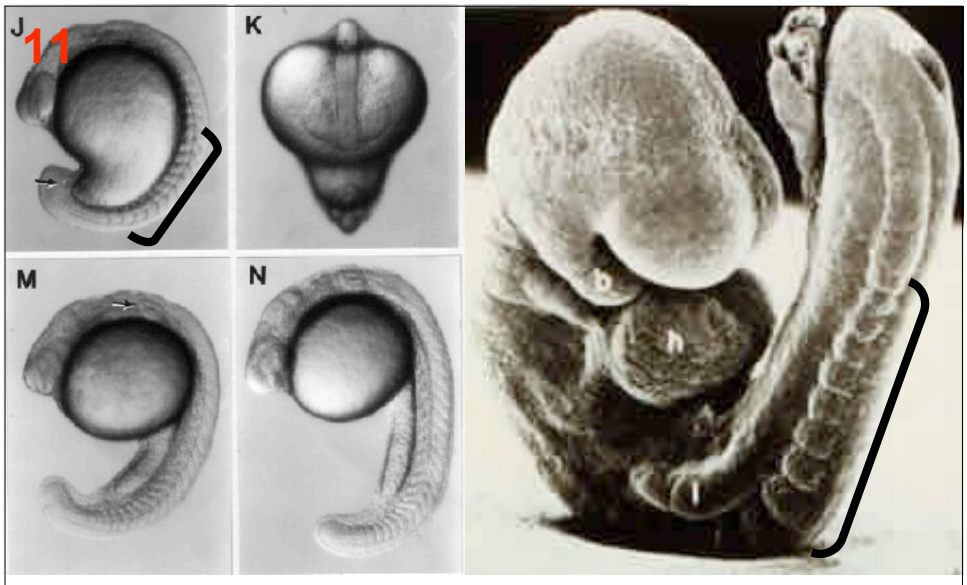
DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 2.3 (Part 2) Sinauer Associates, Inc.

10



Somites will form skeletal muscle, skeleton and skin (dermis)

11



Somites:

zebrafish **chick**

Segments that will form muscle, skeleton and skin

4. Dorsoventral axis

Which cells give rise to different regions?

Xenopus laevis

12

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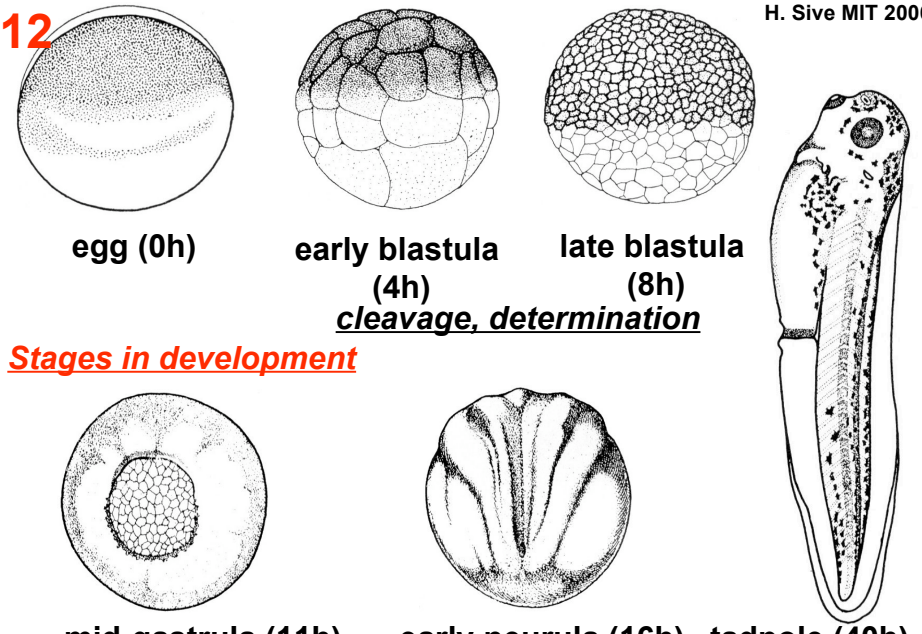

egg (0h)

early blastula (4h)

late blastula (8h)

cleavage, determination

Stages in development

mid-gastrula (11h)

early neurula (16h)

tadpole (40h)

movement, determination

differentiation

13

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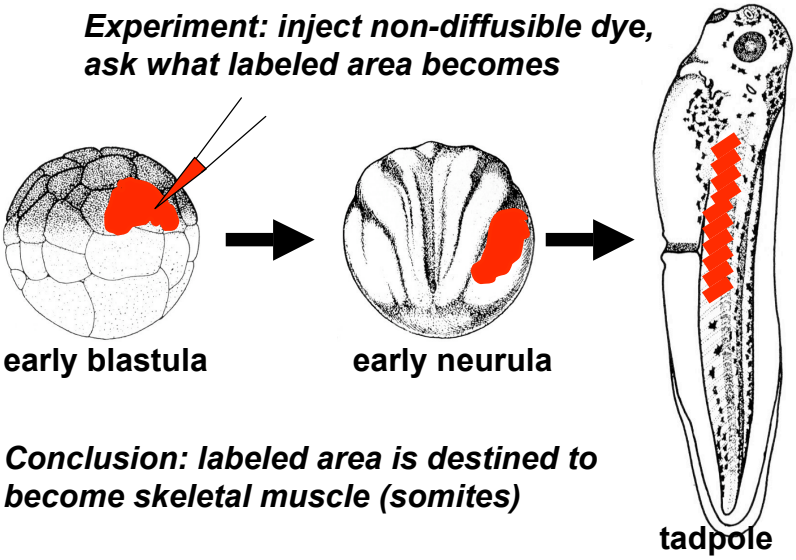
Fate mapping shows what cells will become

Experiment: inject non-diffusible dye, ask what labeled area becomes



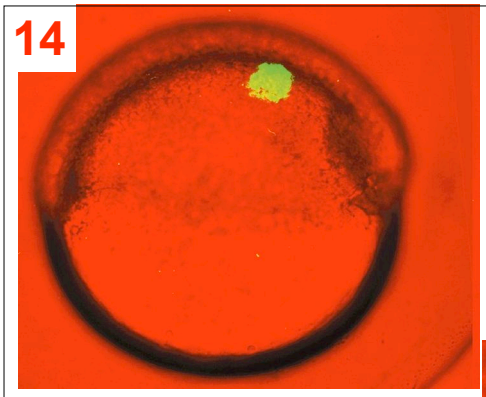
early blastula

early neurula

Conclusion: labeled area is destined to become skeletal muscle (somites)

tadpole (differentiated muscle)

14

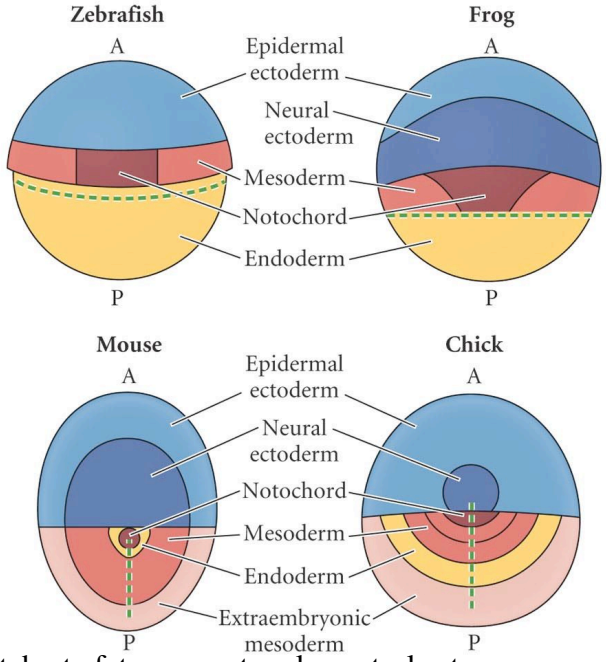


Fate mapping in zebrafish
- what cells will become

1. Inject one/few cell(s) in early embryo

2. Score labeled cell positions in older embryo

15



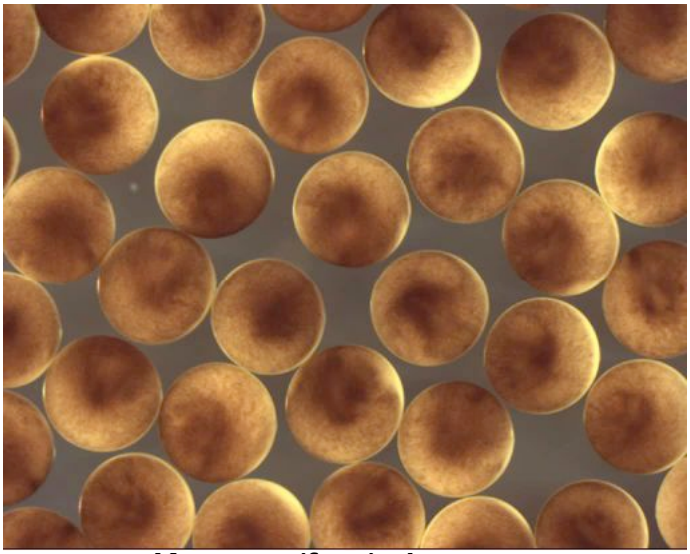
Vertebrate fate maps at early gastrula stage

4. Dorsoventral axis:

When is this determined?

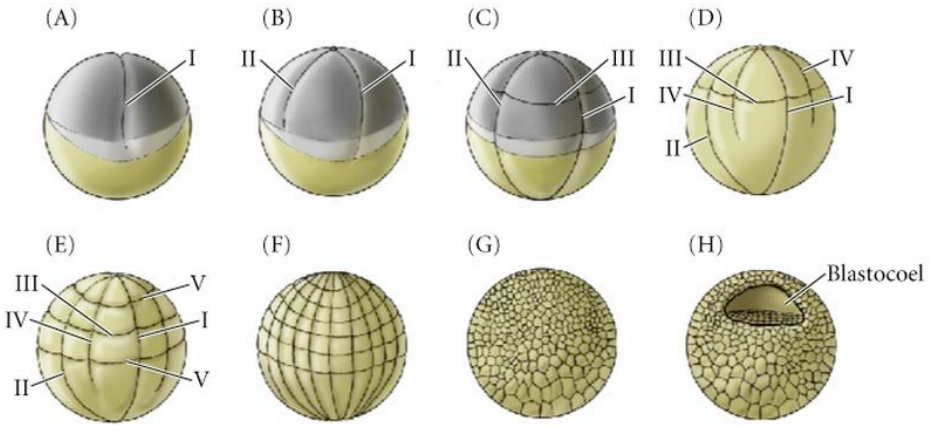
16

Pink Floyd/ another brick in the wall



***Xenopus* (frog) cleavage:**
0 - 10 hours after fertilization/s
dorsal and ventral decided by 2 cell stage!

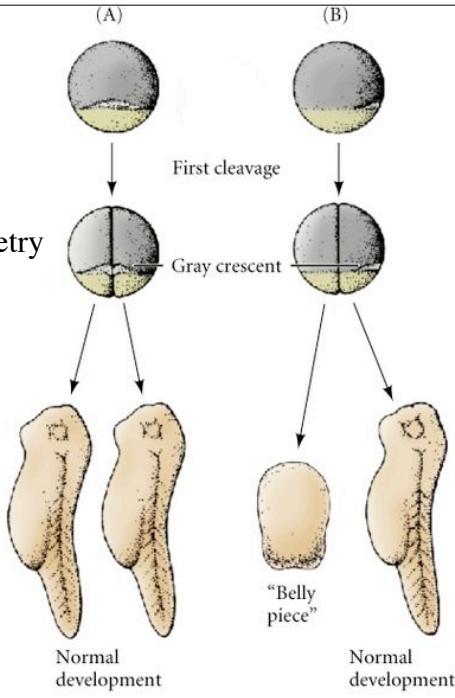
16a



Gilbert: Xenopus cleavage- when initial D/V patterning takes place

17

Gilbert: Xenopus early D/V asymmetry



18

Animal pole

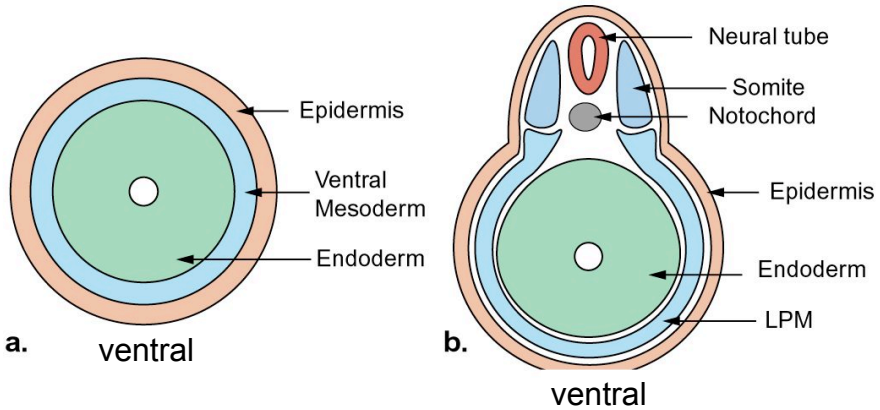
V  D

Vegetal pole

Movement of pigment away from dorsal side in frog zygote (20 min after fertilization) = grey crescent

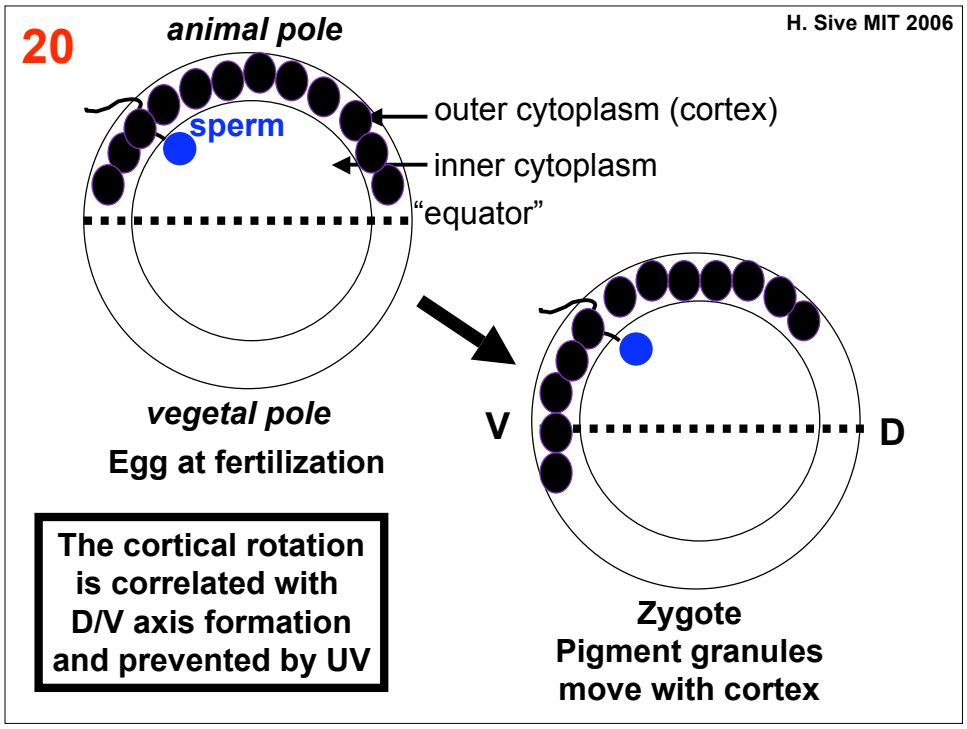
19

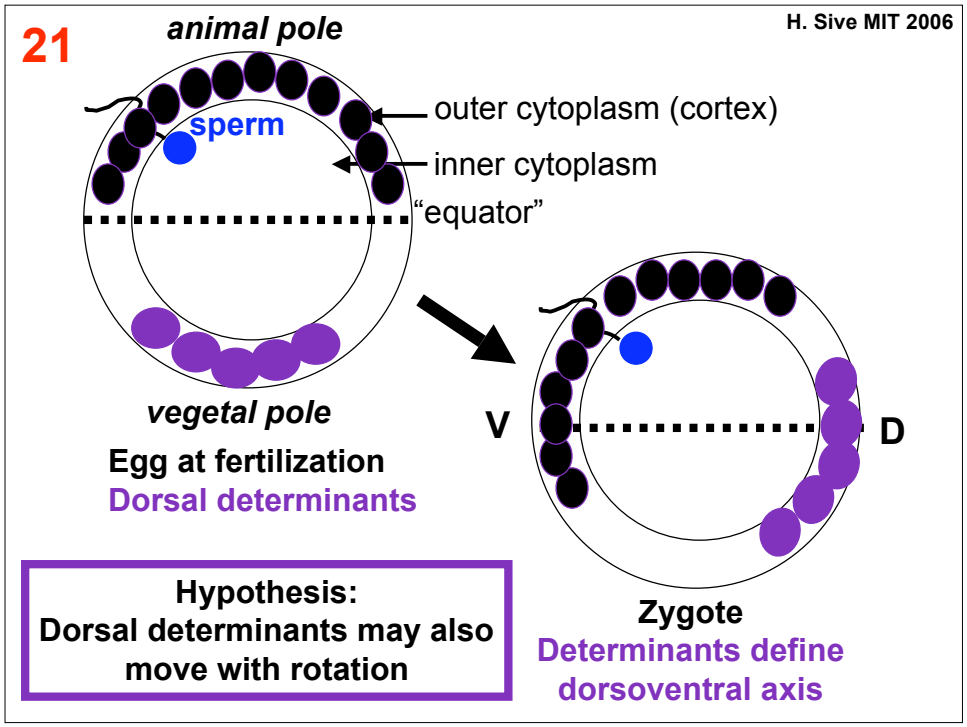
UV treatment of vegetal pole by 20 minutes after fertilization prevents formation of dorsal structures



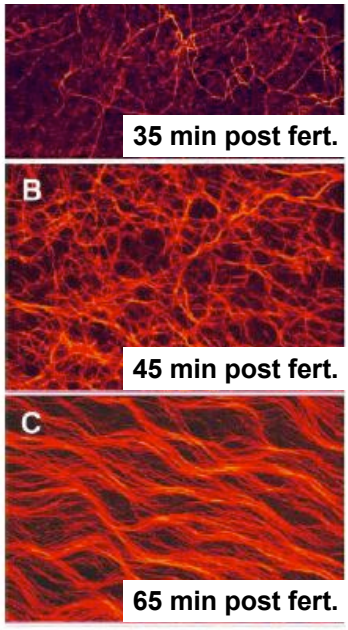
UV-treated: No rotation

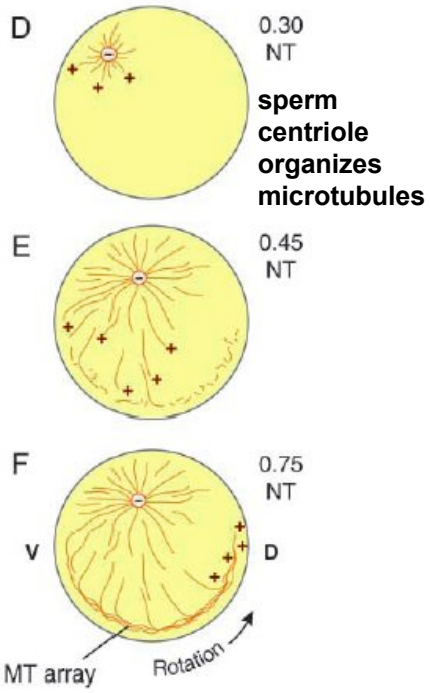
Normal: Rotation


20
animal pole
H. Sive MIT 2006
sperm
outer cytoplasm (cortex)
inner cytoplasm
"equator"
vegetal pole
Egg at fertilization
V
D
The cortical rotation is correlated with D/V axis formation and prevented by UV
Zygote
Pigment granules move with cortex


21
animal pole
H. Sive MIT 2006
sperm
outer cytoplasm (cortex)
inner cytoplasm
"equator"
vegetal pole
Egg at fertilization
Dorsal determinants
V
D
Hypothesis:
Dorsal determinants may also move with rotation
Zygote
Determinants define dorsoventral axis

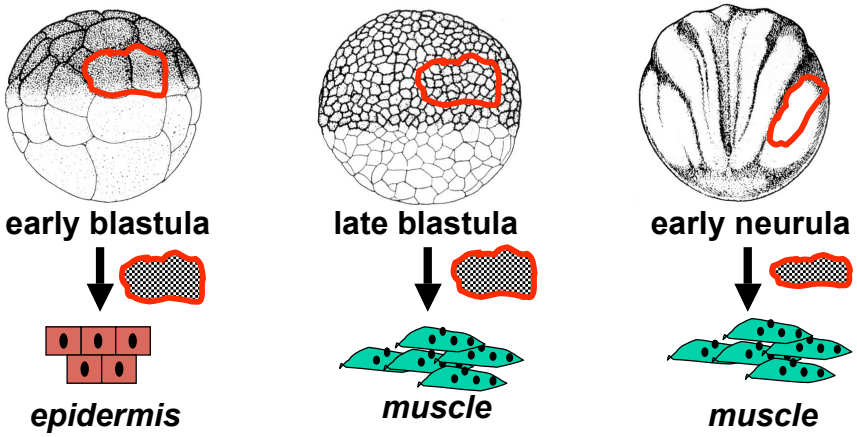
22 Formation of microtubule array that carries determinants





23 **Explant assays show when cells decide their fate**

Remove same relative region
culture in saline, later, assay fates



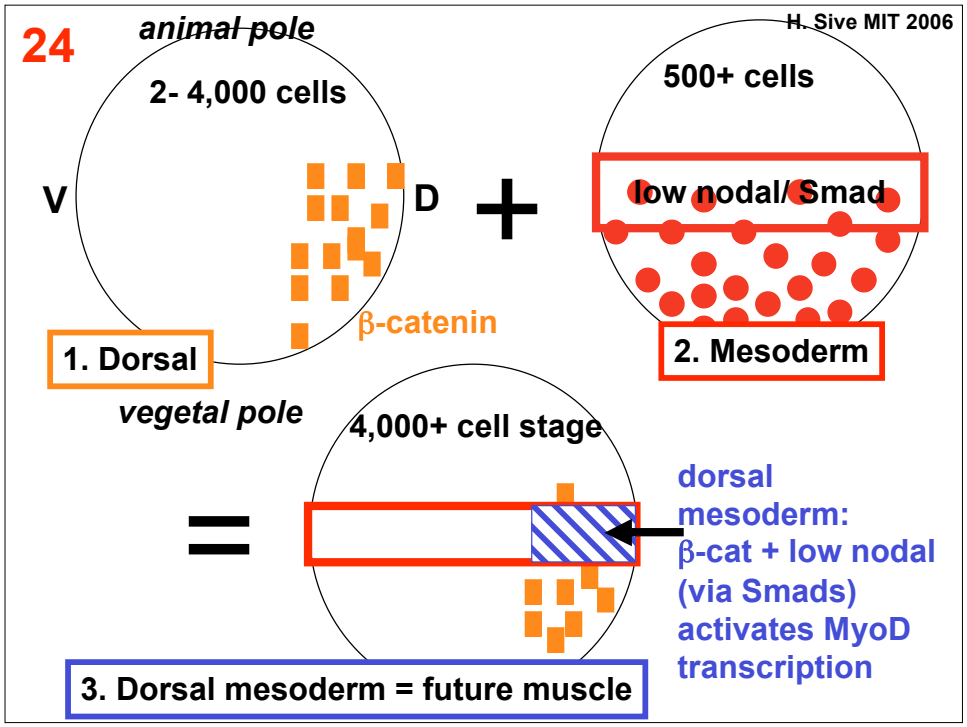
Conclusion: cell fate decision (determination)
is made by late blastula

H. Sive MIT 2006

4. Dorsoventral axis:

Molecules that determine

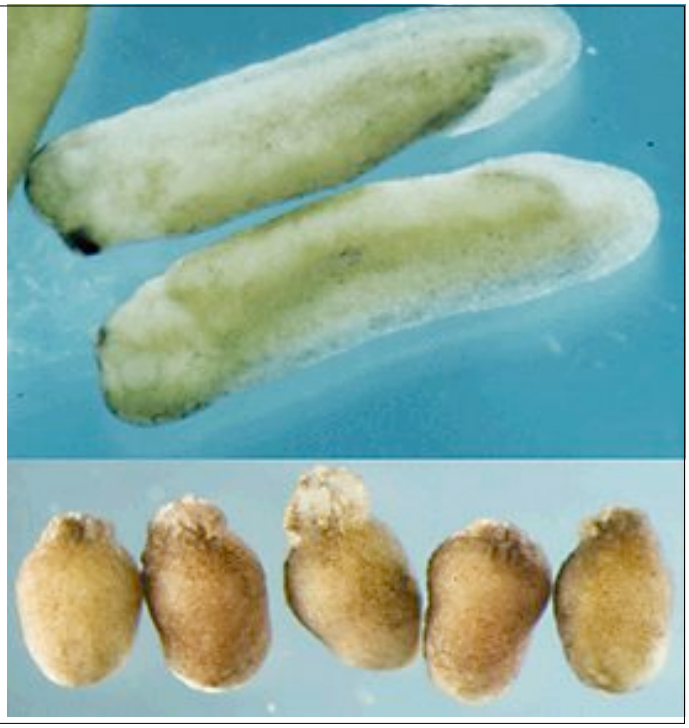
D/V position



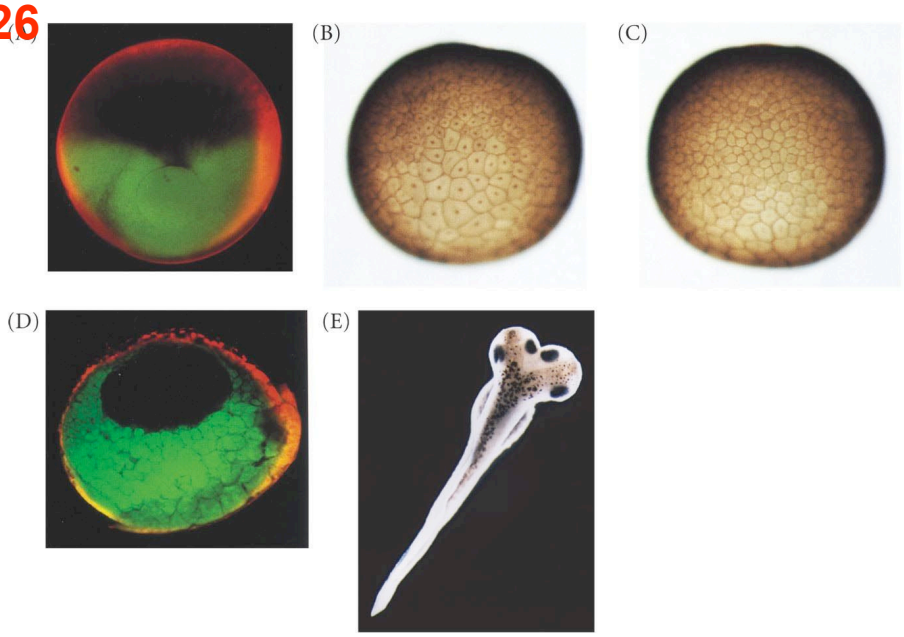
25

Wild type embryos

Depleted of β-catenin: no dorsal structures (ventralized)

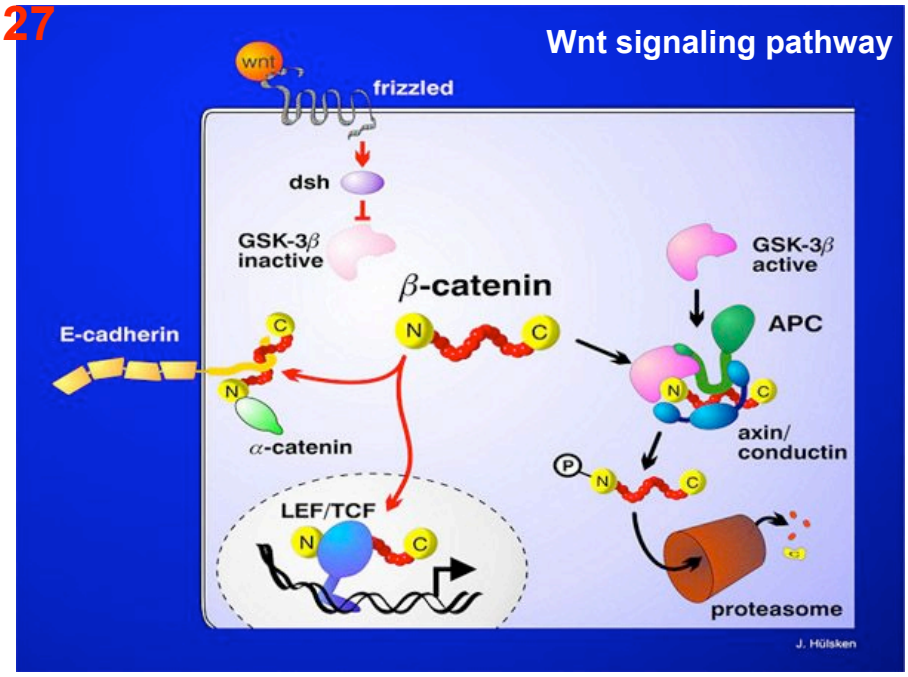


26



Dorsal localization of nuclear βcatenin protein in Xenopus

27



28

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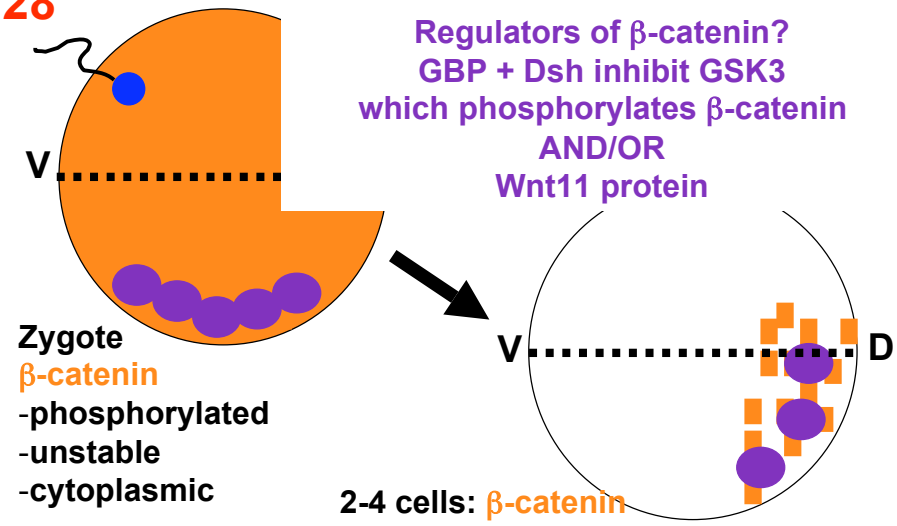
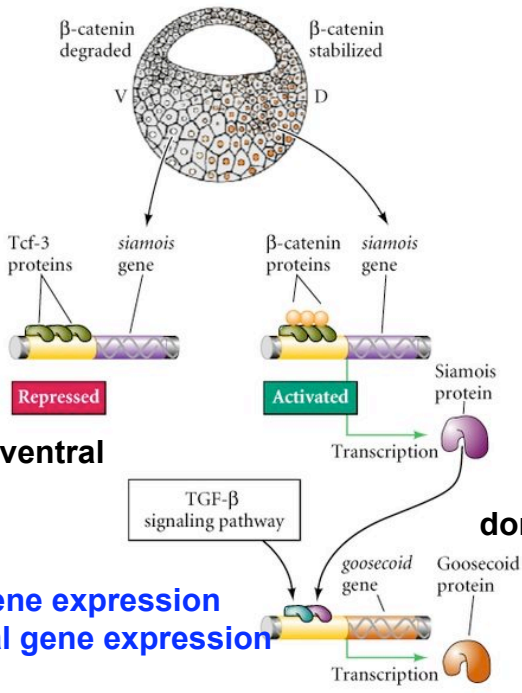
Regulators of β-catenin?
GBP + Dsh inhibit GSK3
which phosphorylates β-catenin
AND/OR
Wnt11 protein



Zygote
β-catenin
-phosphorylated
-unstable
-cytoplasmic

2-4 cells: β-catenin
-dephosphorylated dorsally
-stabilized
-nuclear
-activates dorsal-specific transcription

29



β-catenin + Tcf3 = transcription factor
>> activates dorsal gene expression
Tcf3 represses ventral gene expression

4. Dorsoventral axis: Relationship of specific cell types to position (mesoderm/ muscle)

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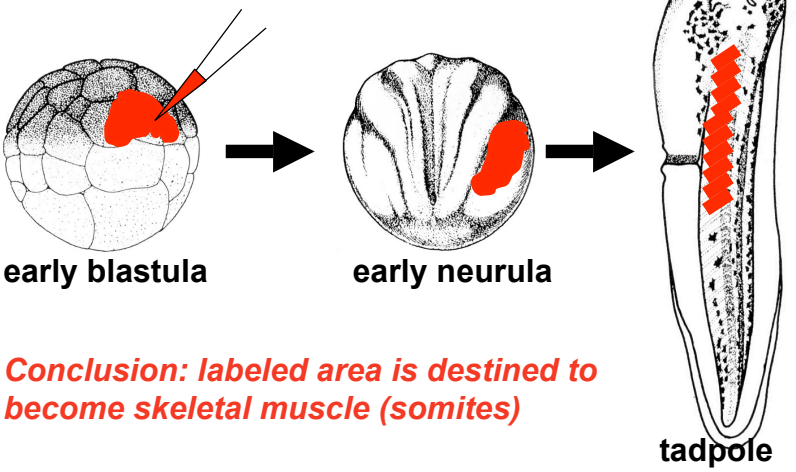
12 **Fate mapping shows what cells will become**

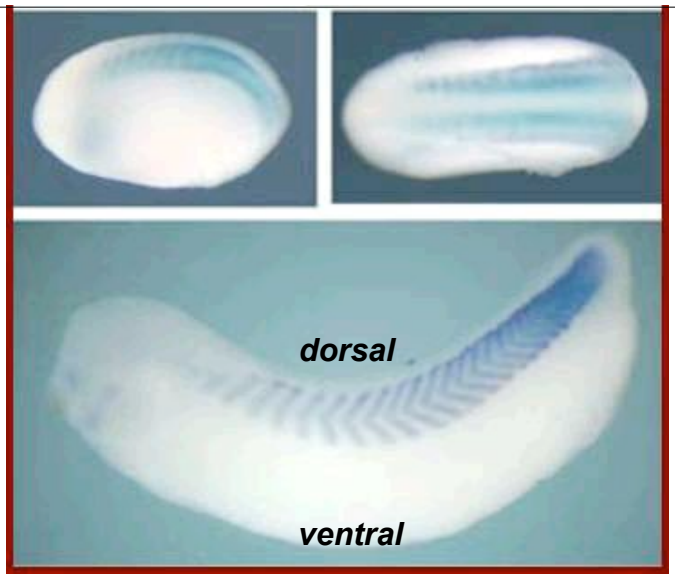
Experiment: inject non-diffusible dye, ask what labeled area becomes



early blastula **early neurula** **tadpole (differentiated muscle)**

Conclusion: labeled area is destined to become skeletal muscle (somites)

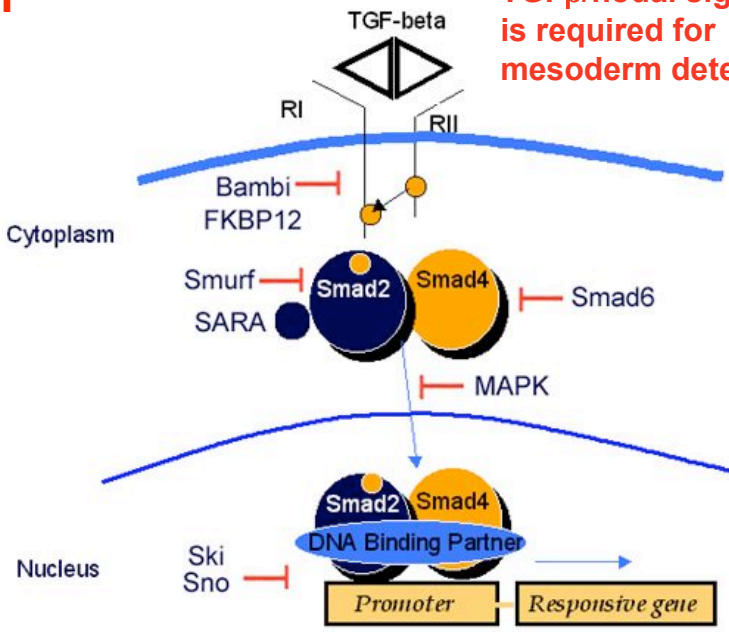
30



Future skeletal muscle (somites) is located dorsally in the embryo and arise from mesoderm

31

TGFβ/nodal signaling is required for mesoderm determination



32

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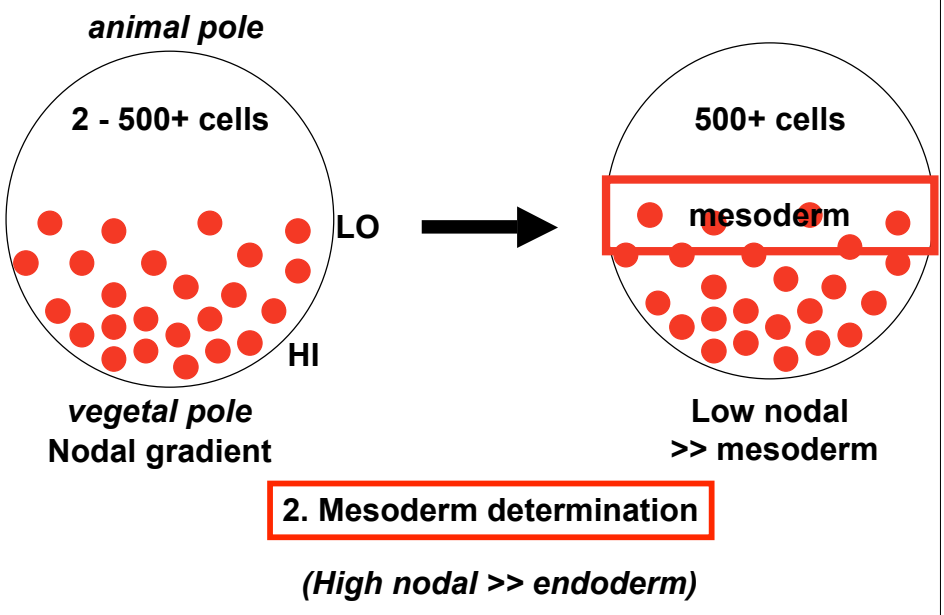
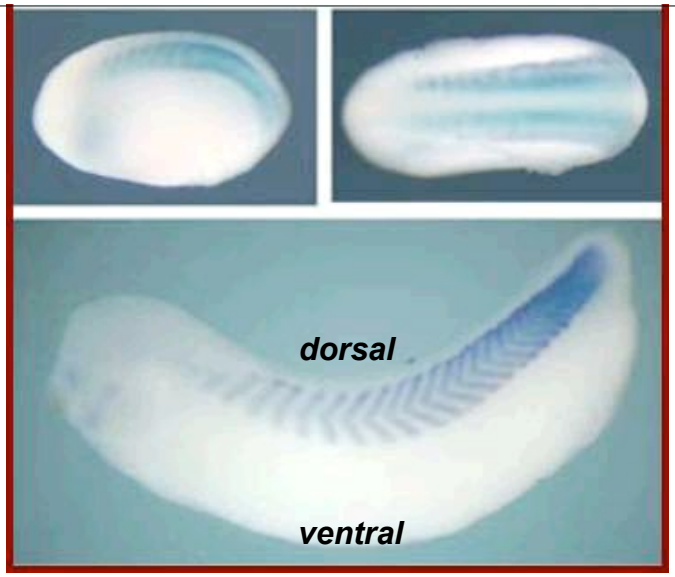


2. Mesoderm determination

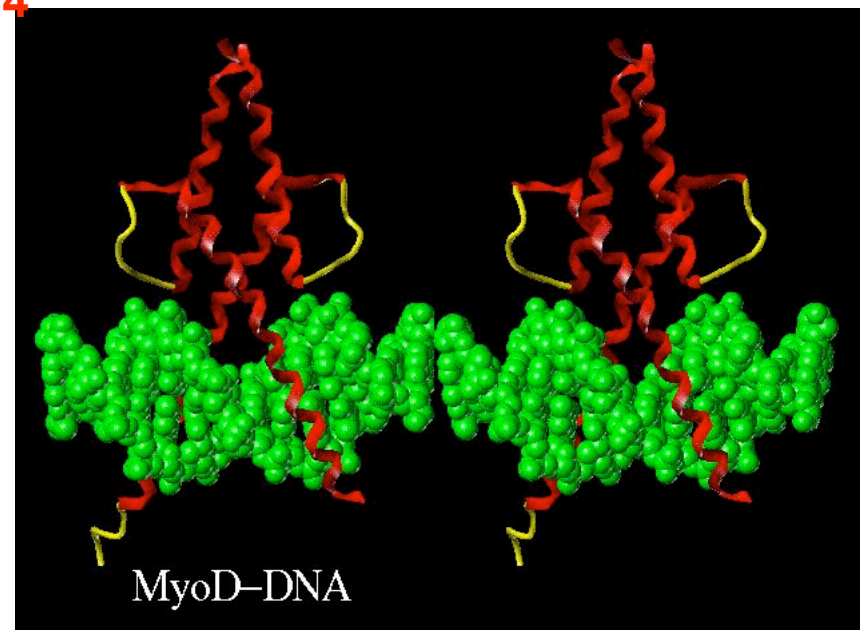
(High nodal >> endoderm)

33



MyoD is expressed only in future skeletal muscle (somites) located <u>dorsally</u> in the embryo
(Frog embryo/ in situ hybridization detects RNA)

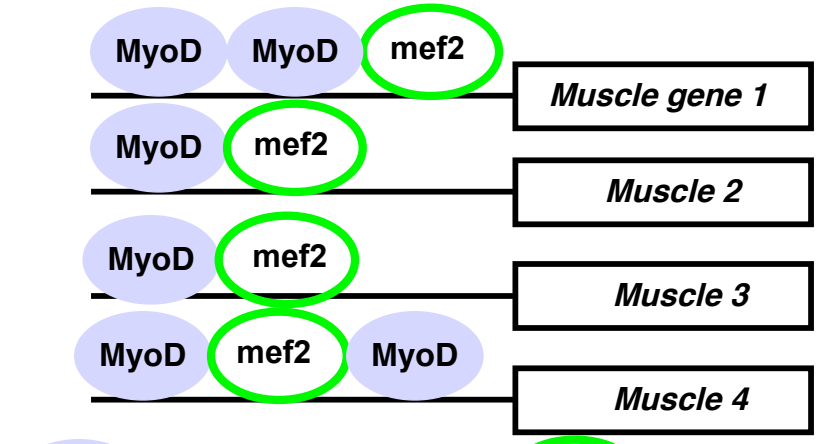
34



MyoD = basic helix/loop helix transcription factor

35

Combinatorial regulatory code for muscle includes MyoD and mef2 which activate transcription of muscle differentiation genes



MyoD = *tissue specific factor* mef2 = *restricted factor*

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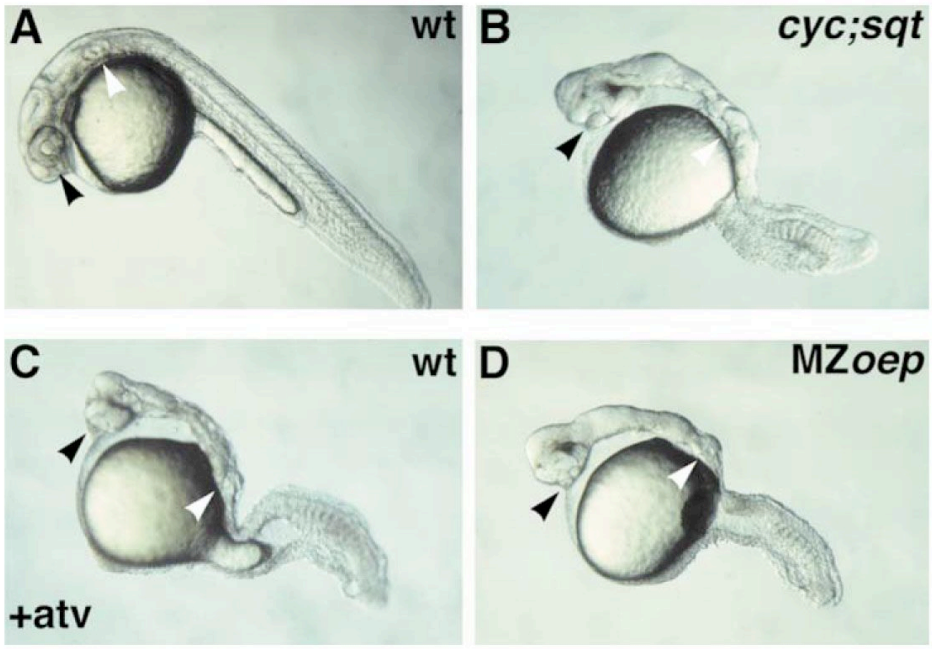
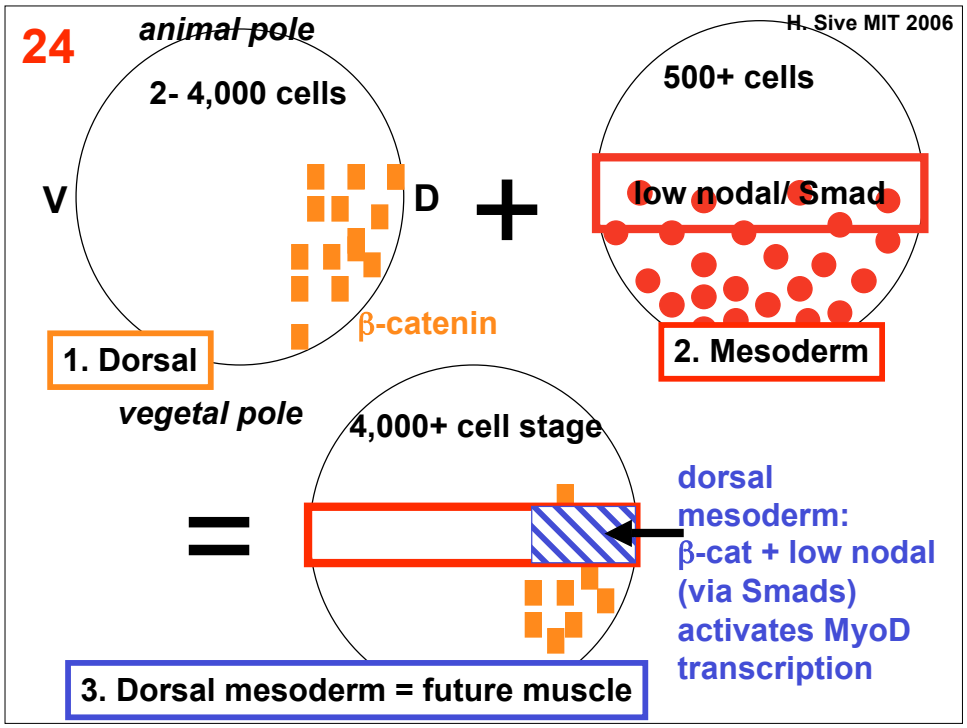
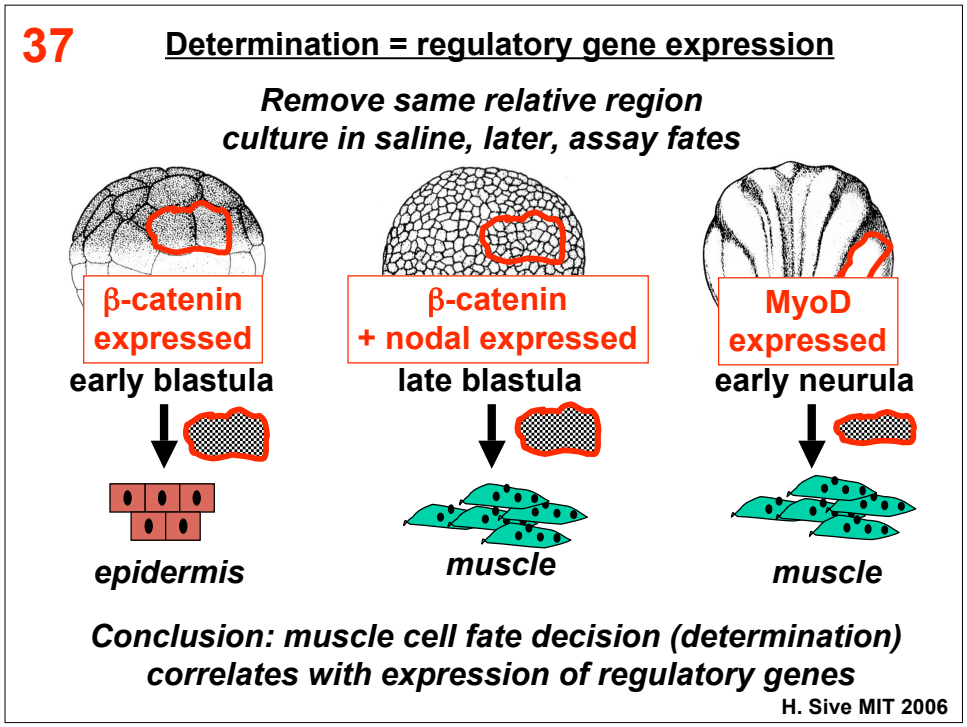


Whitman, Fig. 3 ***Zebrafish nodal mutants lack mesoderm*** 36


24
H. Sive MIT 2006
animal pole
2- 4,000 cells
V
D
β-catenin
1. Dorsal
vegetal pole
500+ cells
low nodal/ Smad
2. Mesoderm
4,000+ cell stage
dorsal mesoderm: β-cat + low nodal (via Smads) activates MyoD transcription
3. Dorsal mesoderm = future muscle


37
Determination = regulatory gene expression
Remove same relative region
culture in saline, later, assay fates
β-catenin expressed
early blastula
epidermis
β-catenin + nodal expressed
late blastula
muscle
MyoD expressed
early neurula
muscle
Conclusion: muscle cell fate decision (determination) correlates with expression of regulatory genes
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