



Model Organisms and Innovative Approaches In Developmental Biology



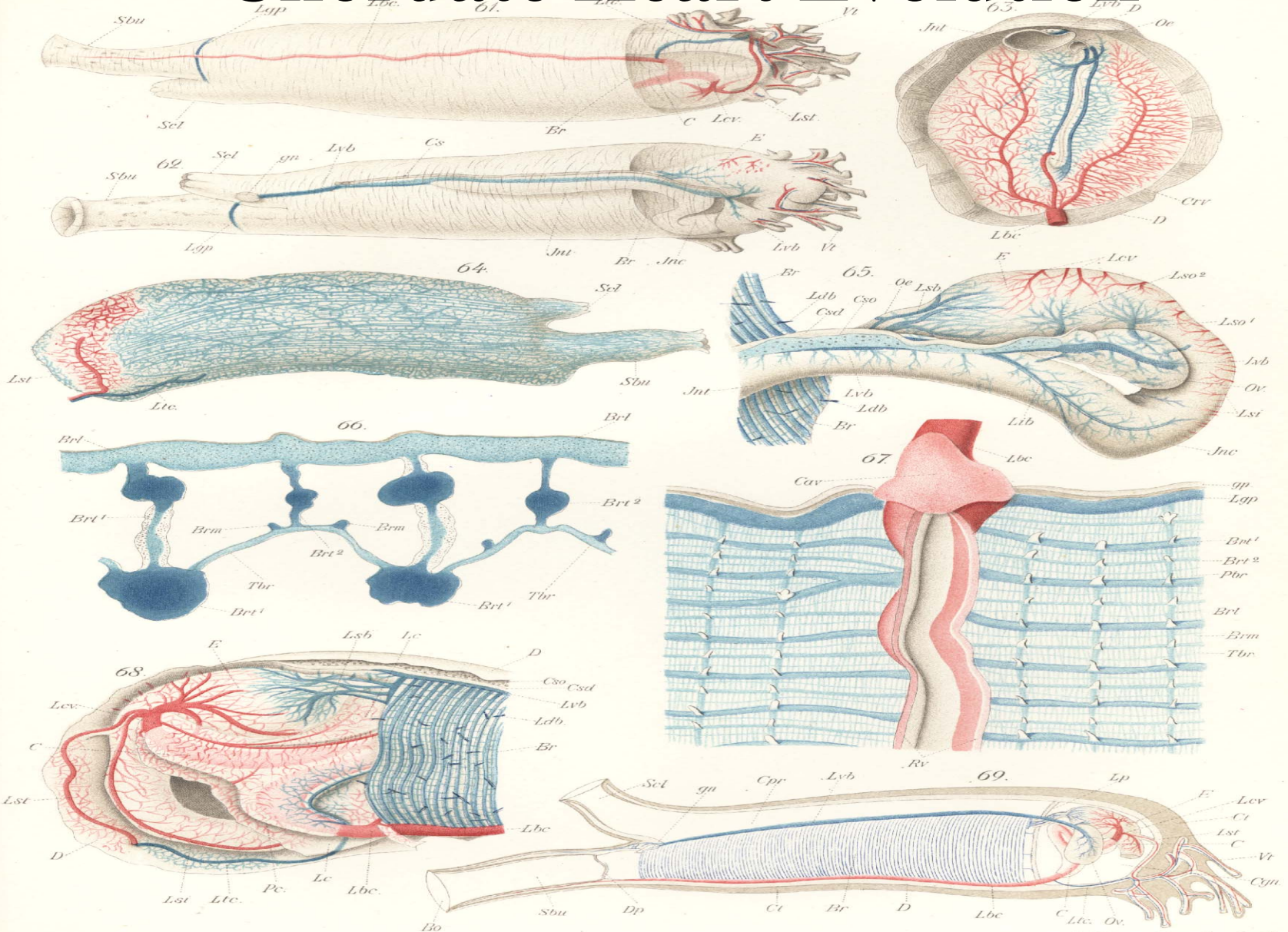
Chordate heart evolution: A basal view

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Chordate Heart Evolution

Annales du Muséum National d'Histoire Naturelle N° 1. PL. 7



A Basal View

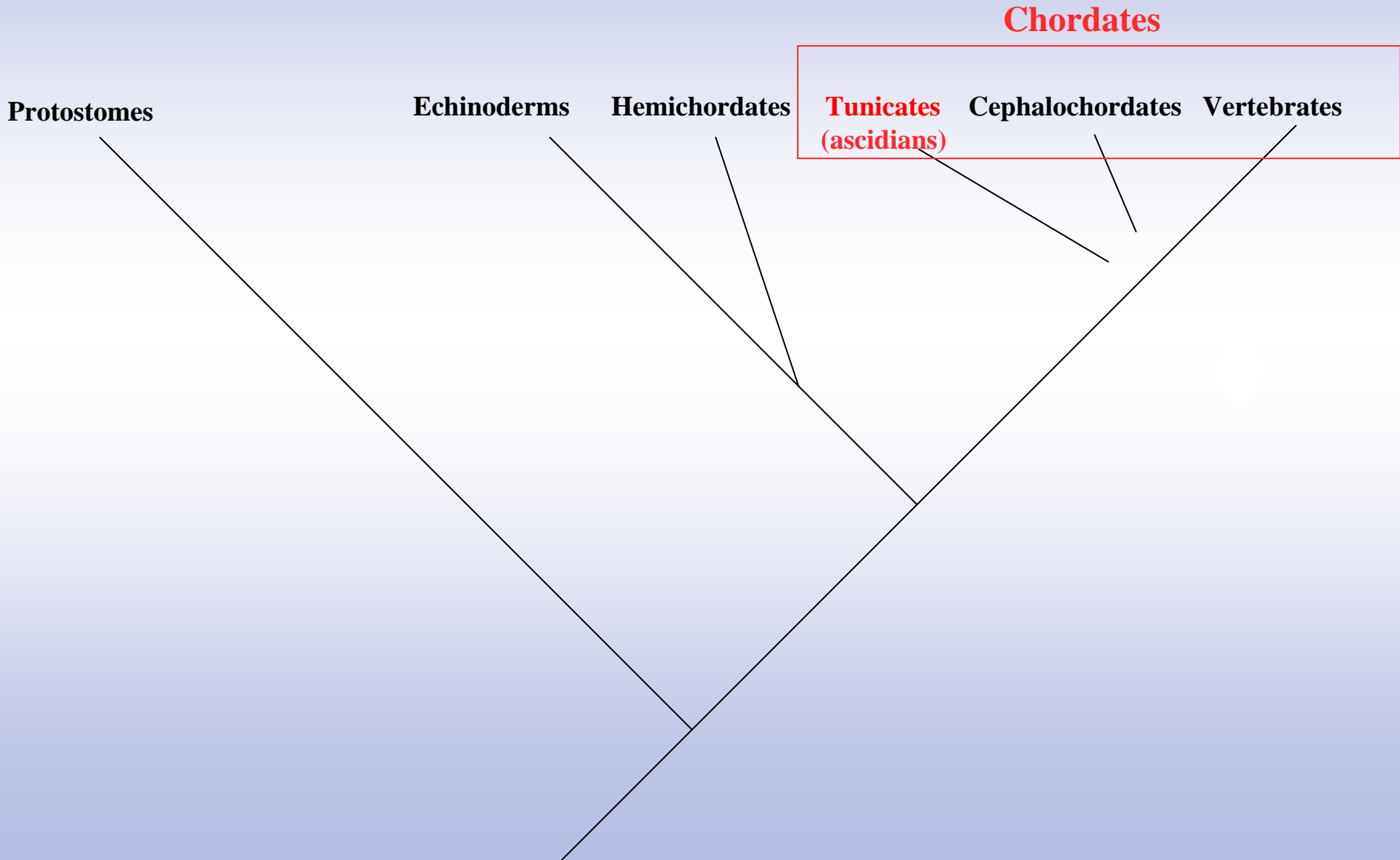
L. Roule del.

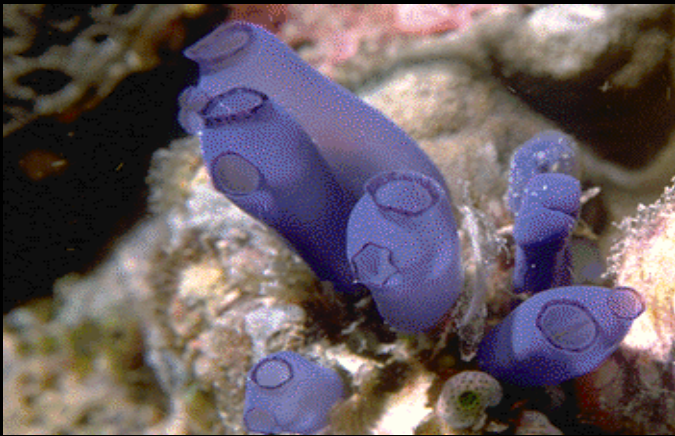
Lith. Werner & Wenter, Frankfurt a. M.

Outline

1. Introduction to the Tunicates
2. Why study Ciona?
3. The Ciona Heart
4. Mesp regulation
5. Mesp function
6. Evolutionary implications

Phylogeny

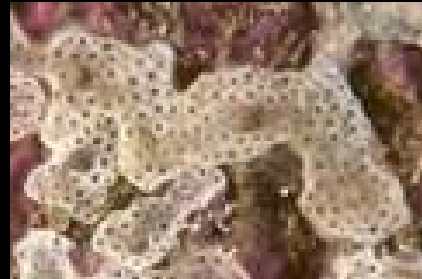


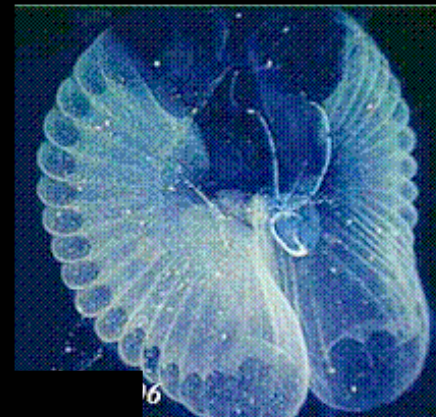
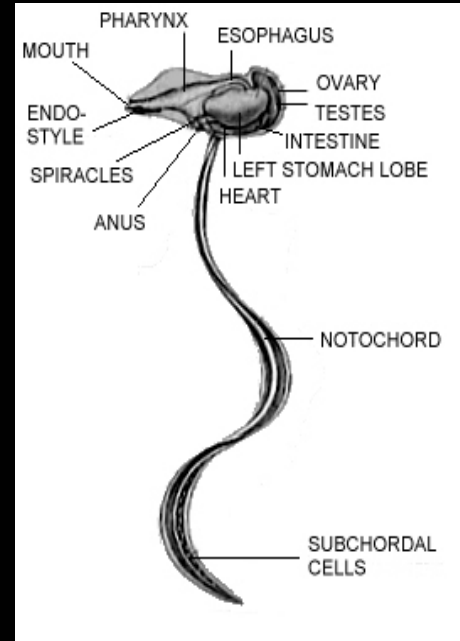


A. Gittenberger

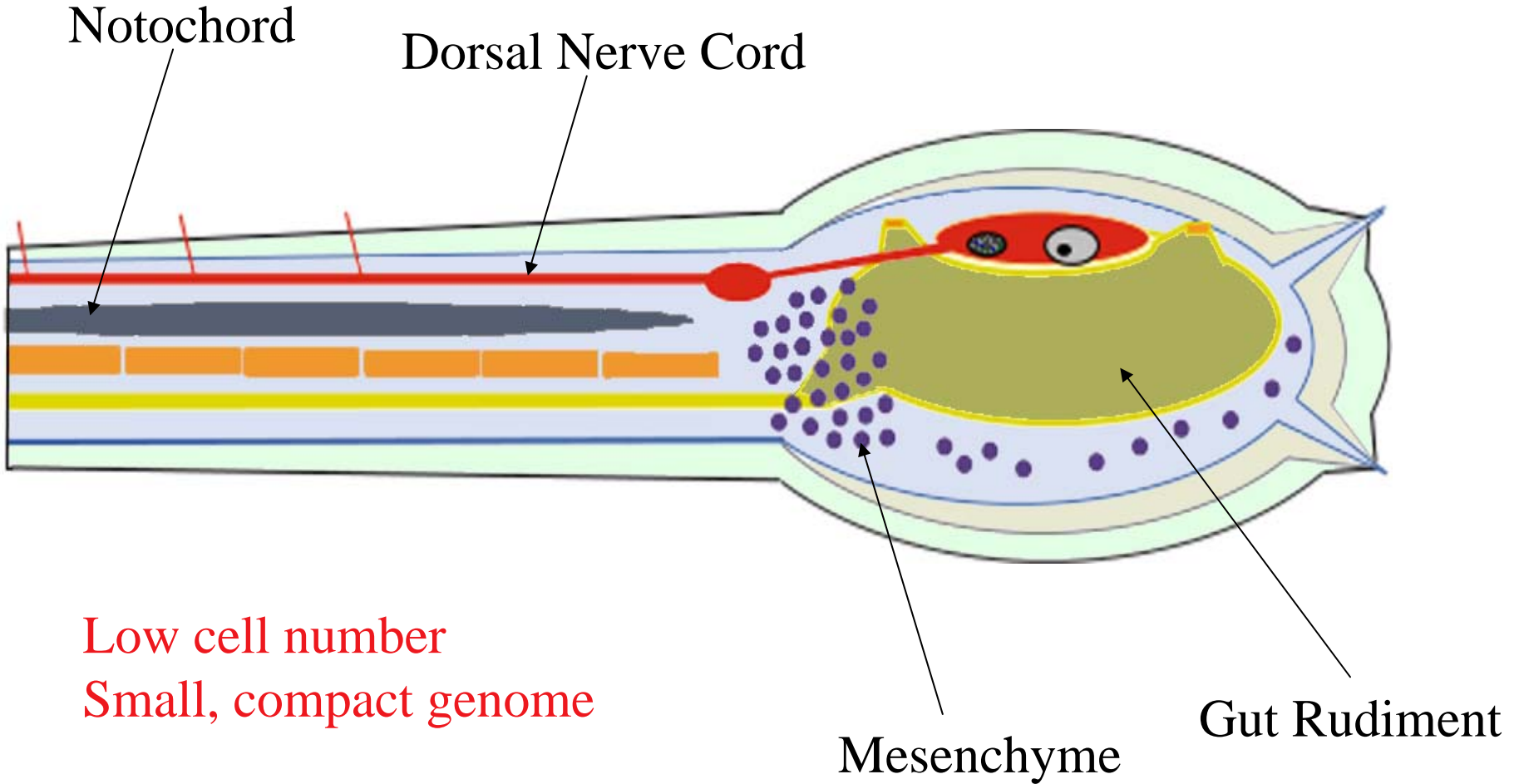


A. Gittenberger

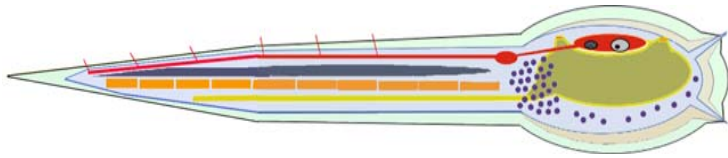




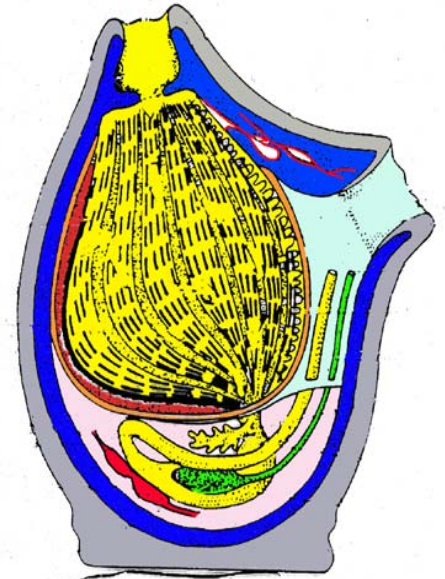
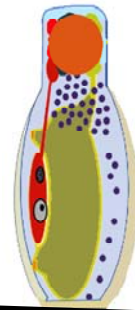
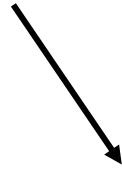
Chordate Features in Ascidian Larvae



Ascidian Metamorphosis



Settlement (20 minutes)
(Loss of Chordate Tail)

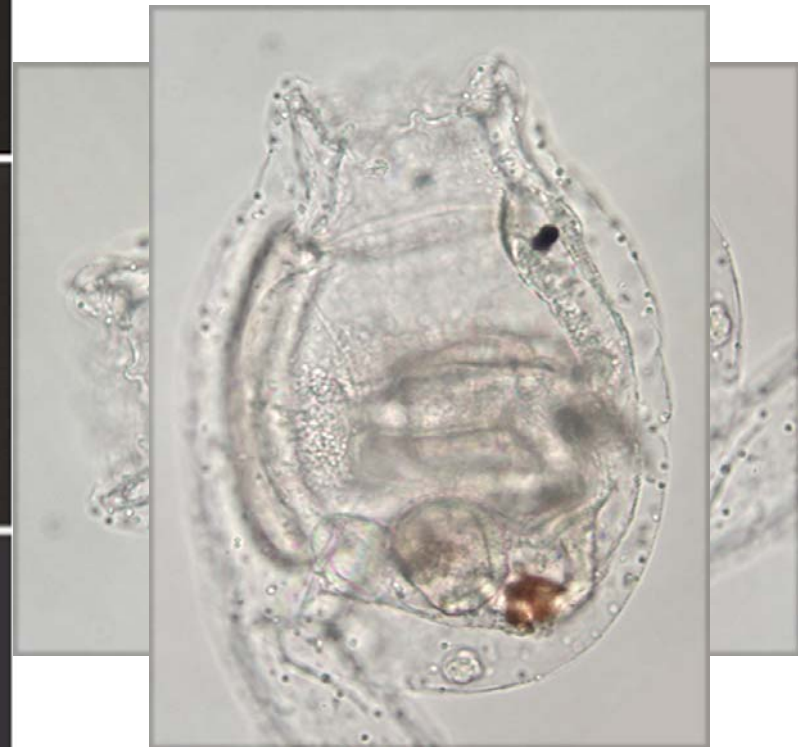


Juvenile Differentiation
(3-7 days)

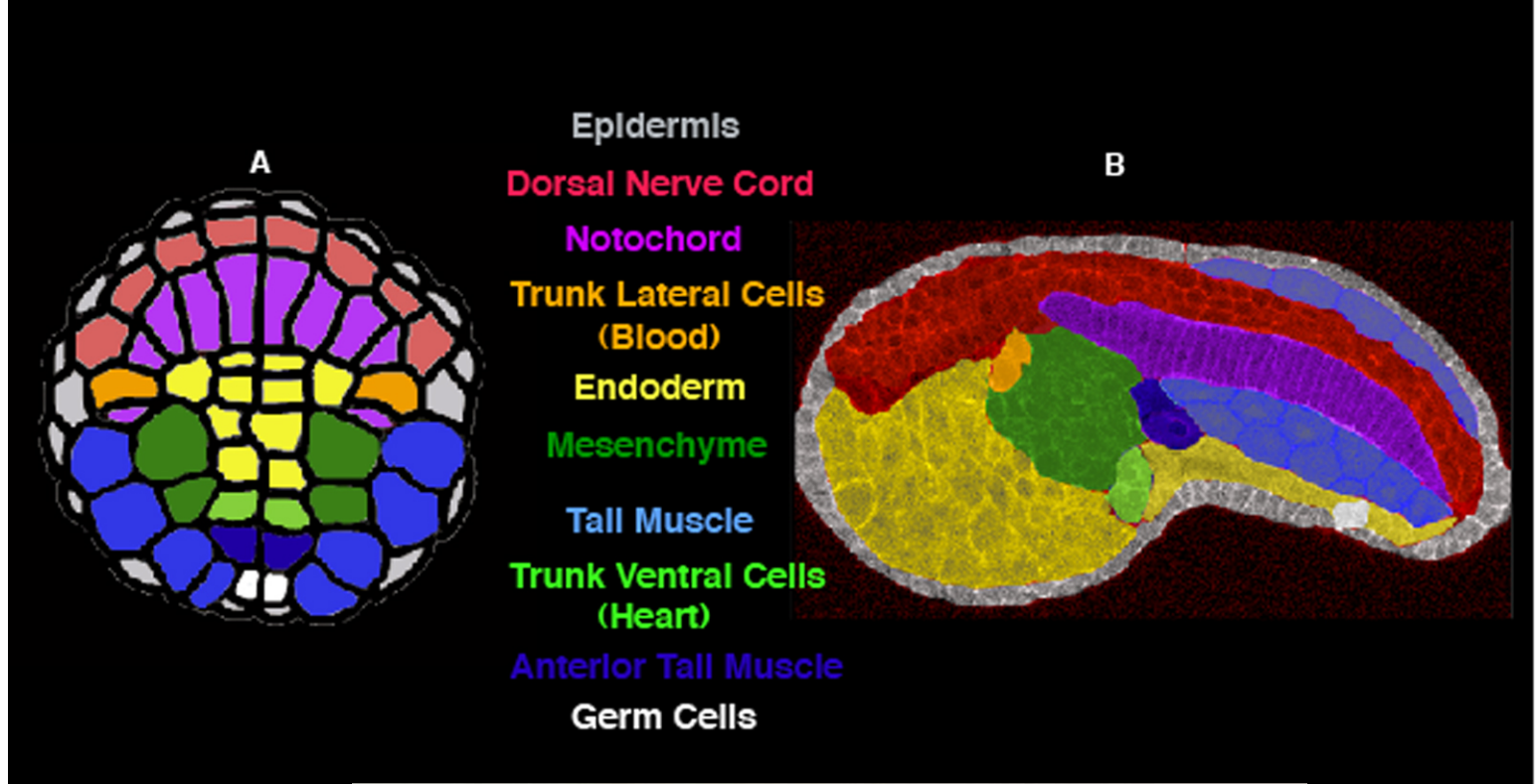
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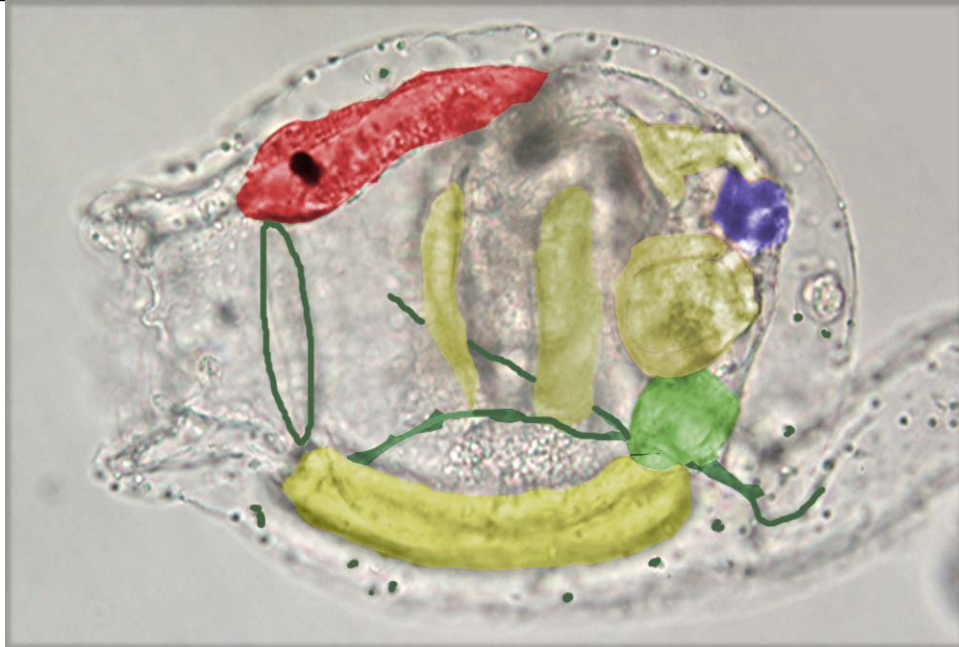
- Rapid Embryogenesis
- Low Cell number
- Transparent blastomeres
- Can isolate blastomeres
- Settle and rear in lab



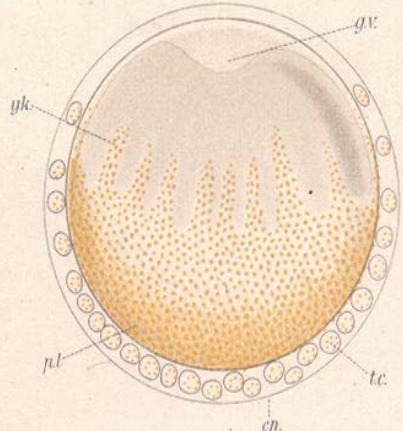
From Satoh '04



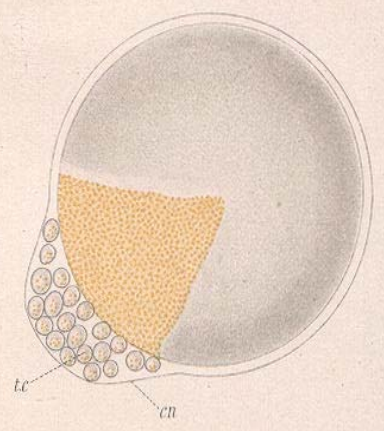
- Defined Lineages



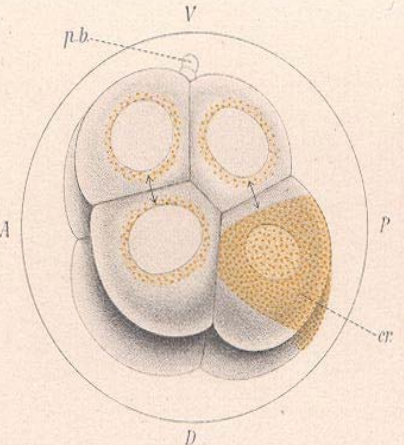
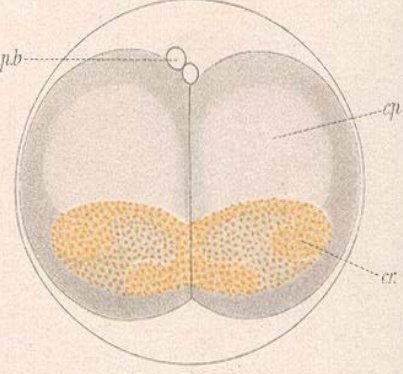
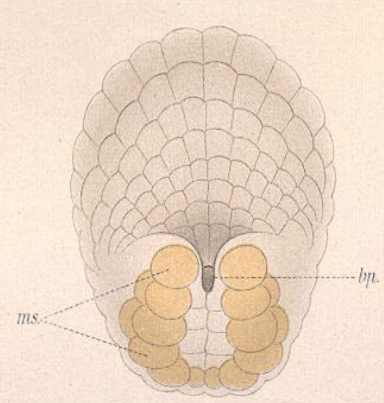
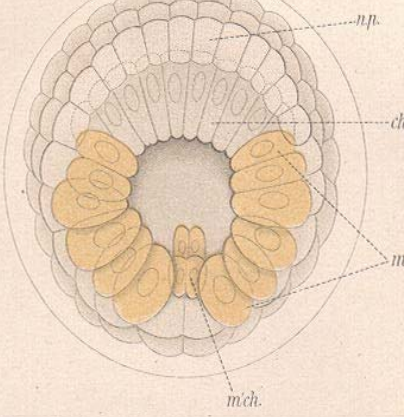
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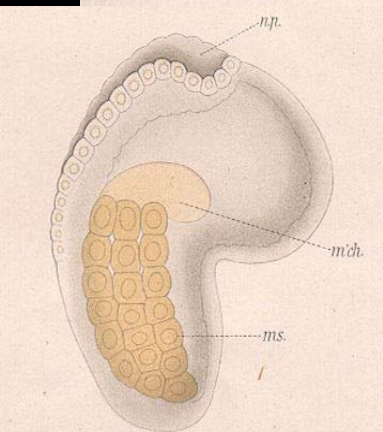
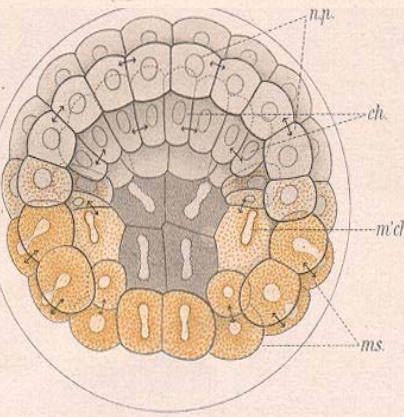
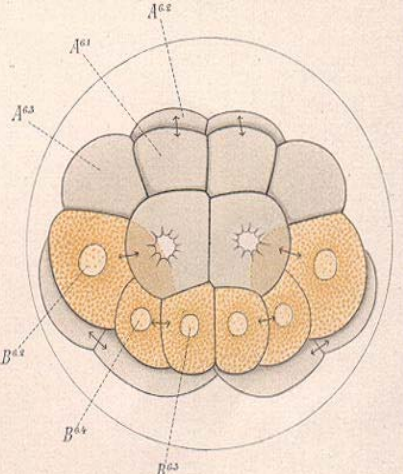
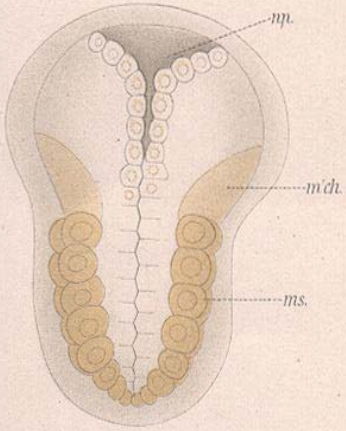
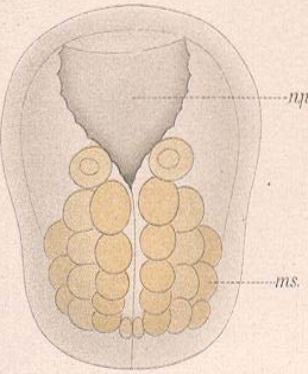
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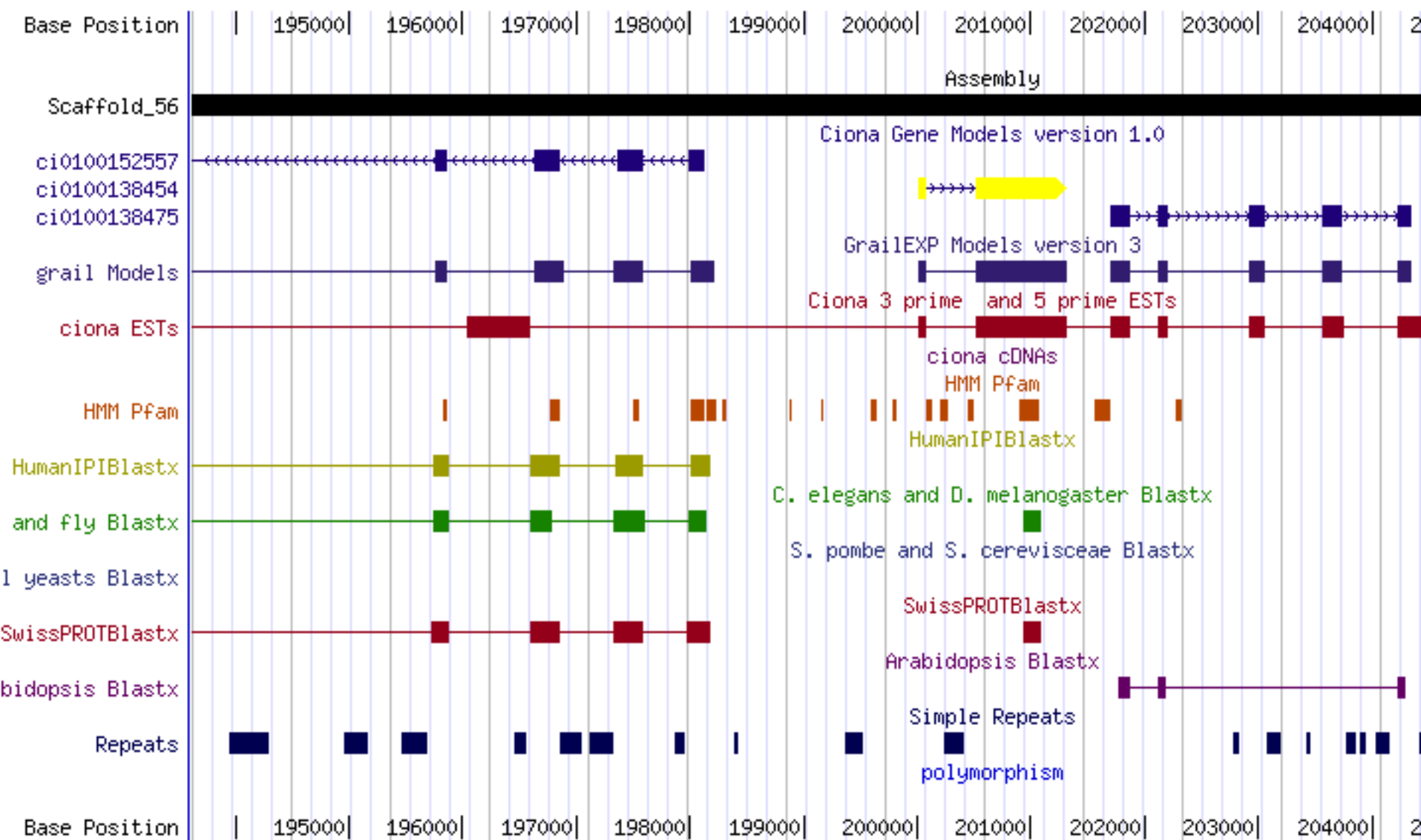
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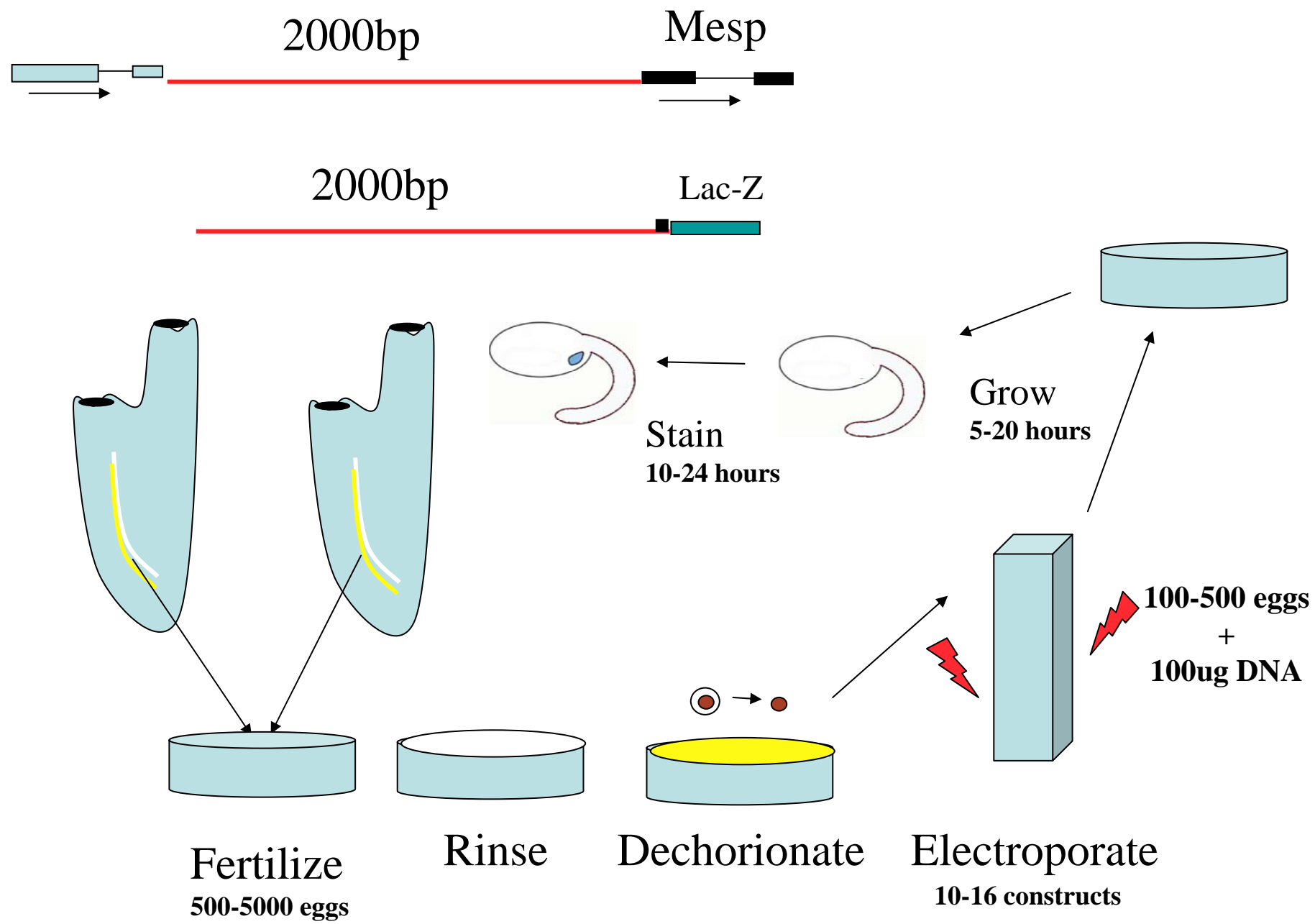
55



Regulatory DNA identification - Compact Genome/electroporation

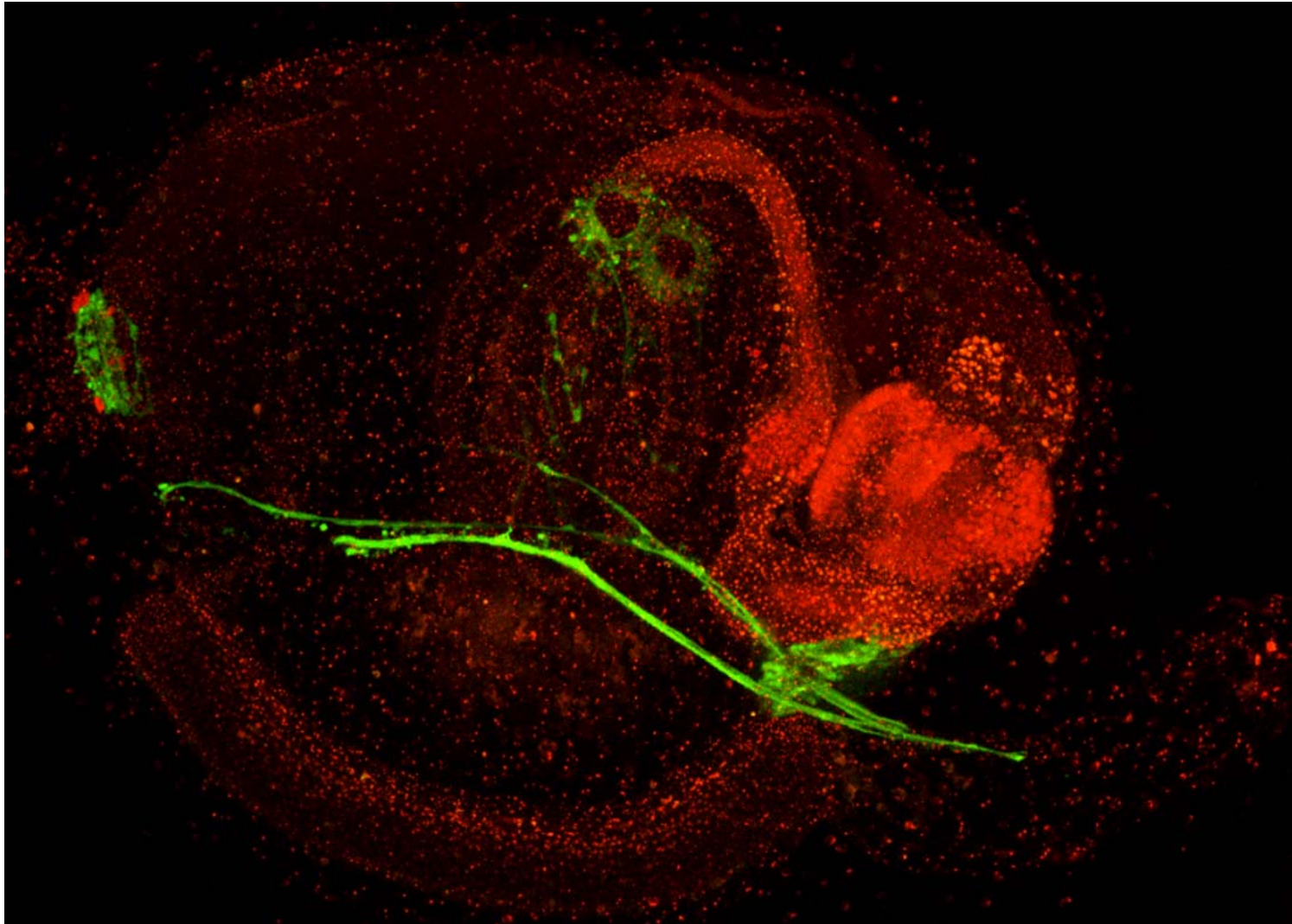


Electroporation - < 48 Hour reporter assay

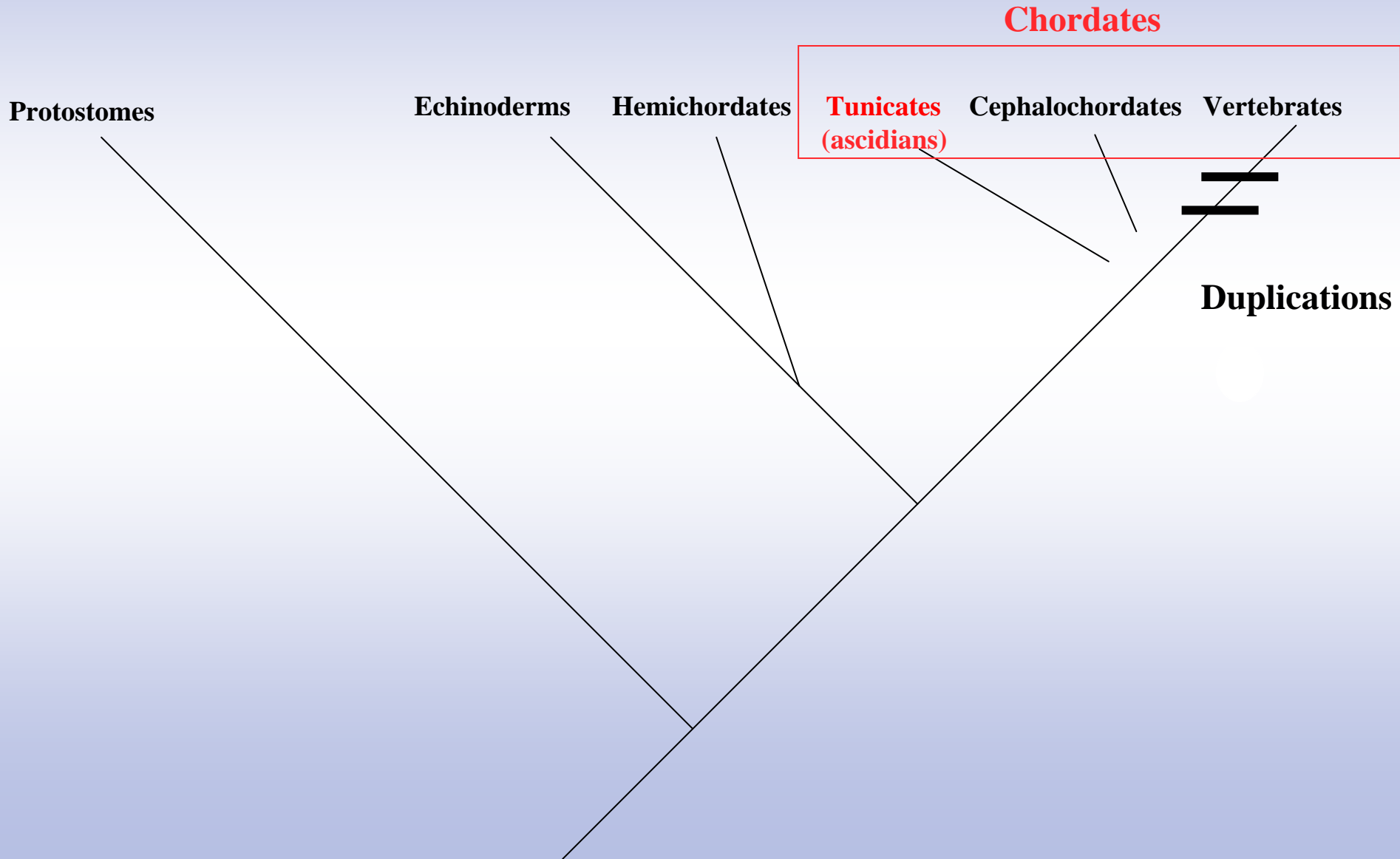


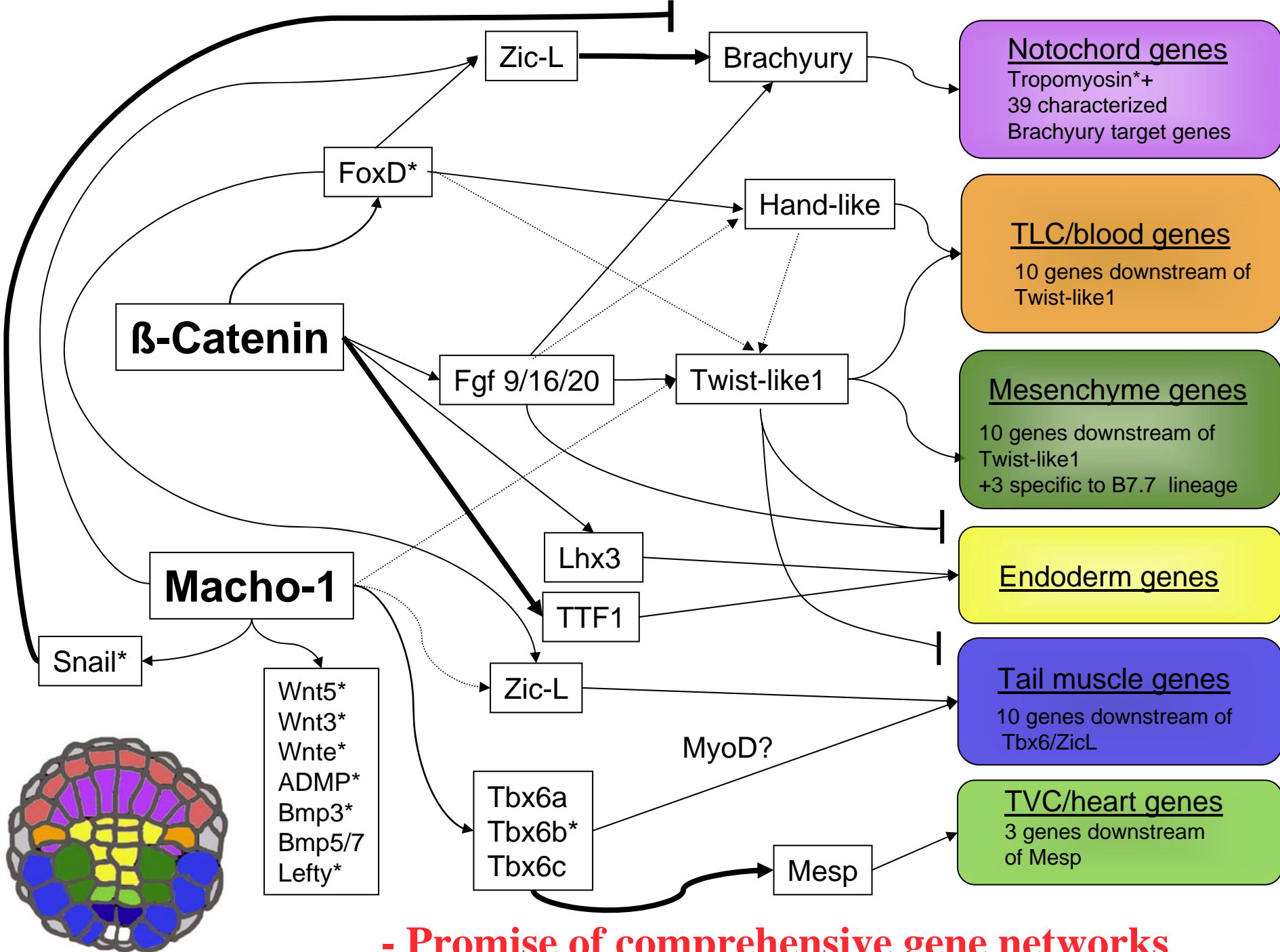
Stable Transgenics

Time to maturation : 3 months; Life span 1-2years



Single orthologs/morpholino injections





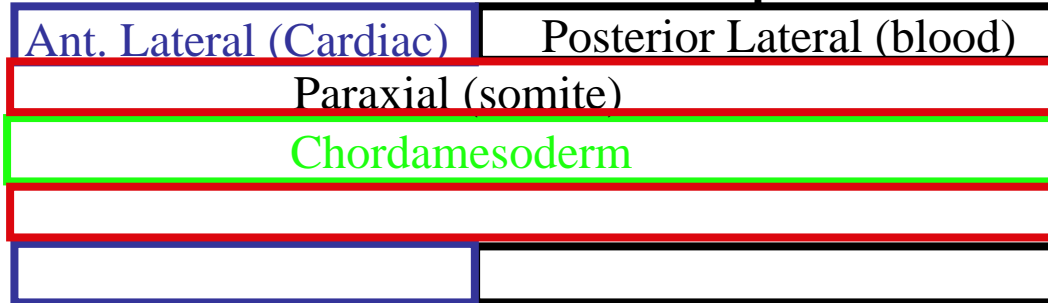
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Current Model for Vertebrate Heart Specification

Fly

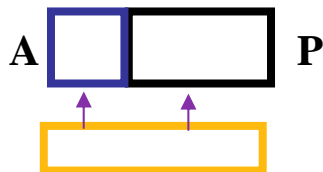
1. Prior to gastrulation mesodermal cell fates are unspecified.



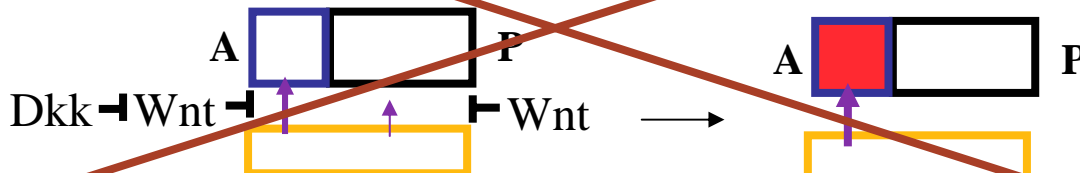
Dpp

Ectoderm maintains

2. During gastrulation **Bmp (+ ?)** secreted by ~~endoderm initiates~~ cardiac specification.



~~3. Induction is prevented in posterior by Wnt signals (blocked by DKK in anterior)~~



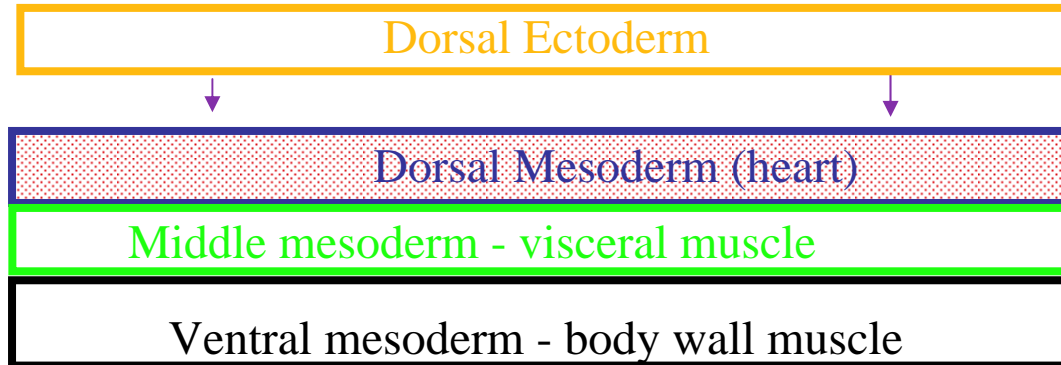
Heart genes:
Nkx2.5, Gata, etc.
Tinman, Pannier

Current Model for Fly Heart Specification

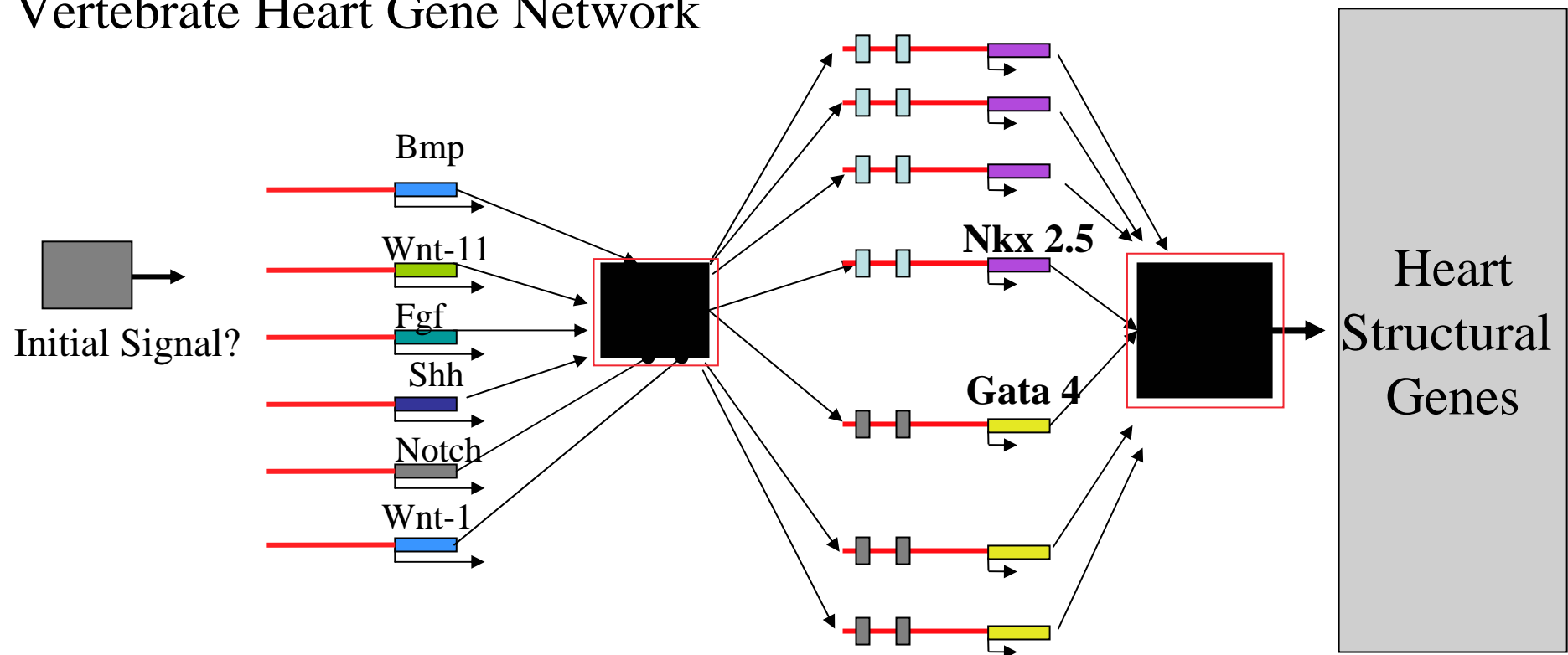
1. Prior to gastrulation the heart factor **Tinman** (Nkx2.5) is expressed in all mesoderm.



2. During gastrulation **Dpp** (+ ?) secreted by dorsal ectoderm maintains **Tinman**, activates **Pannier**.



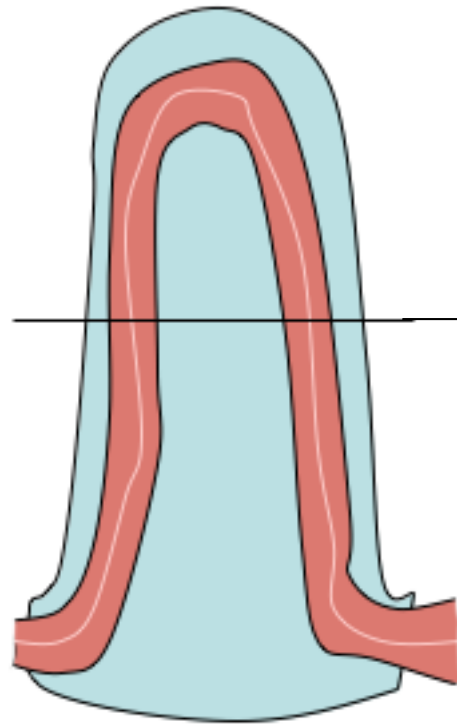
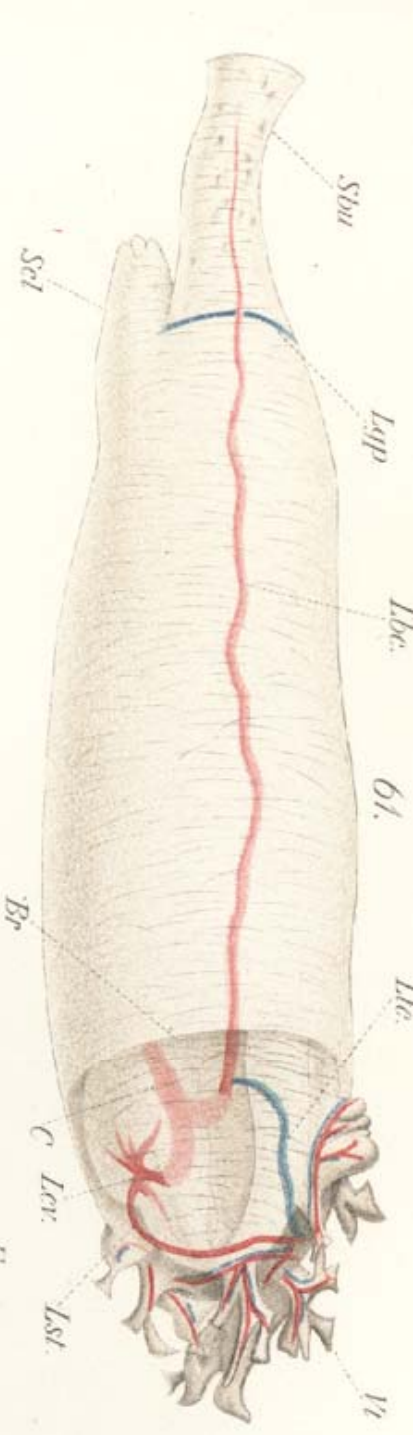
Vertebrate Heart Gene Network



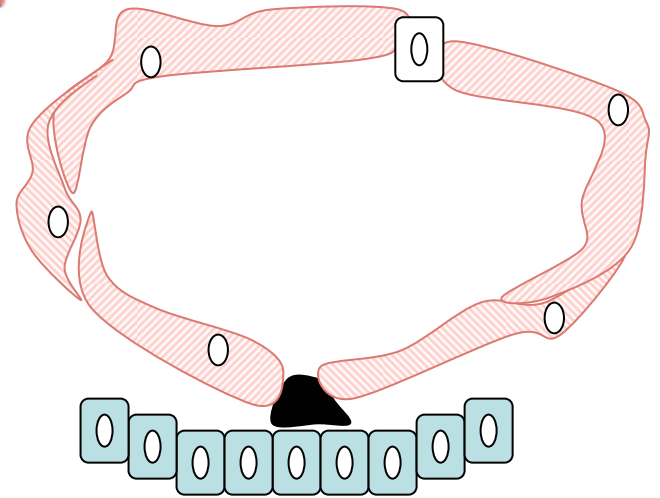
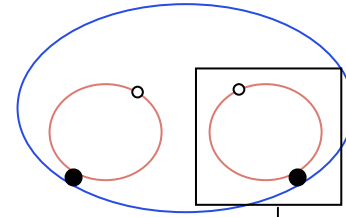
Conundrums...

1. Initial signals?
2. Key transcription factors are redundant.
3. What are the interconnections? (Black Boxes)

Ciona heart morphology



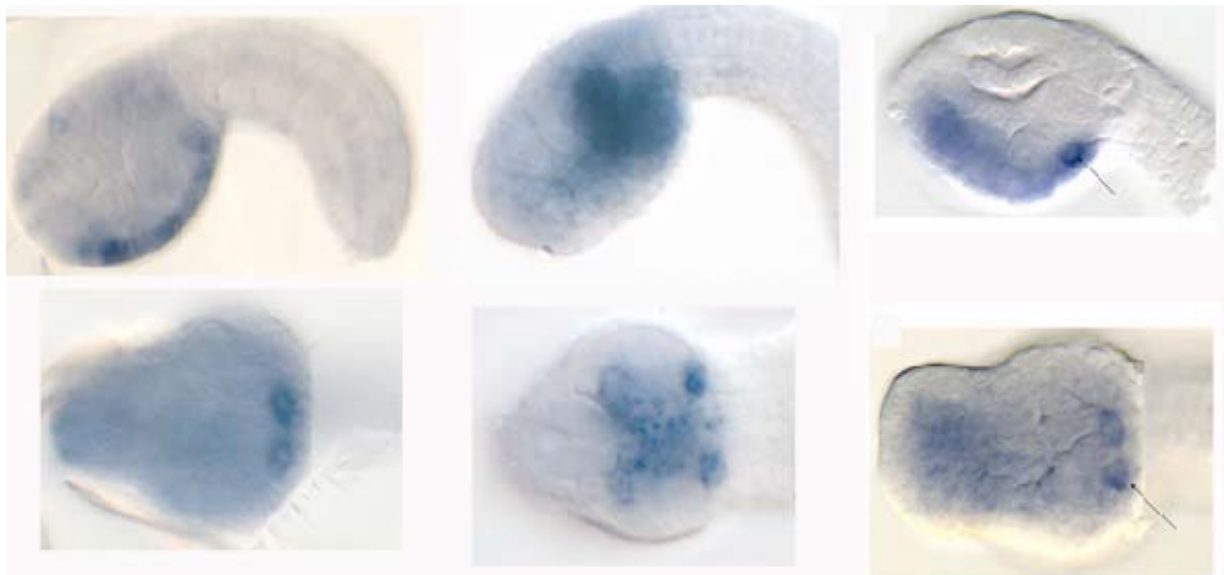
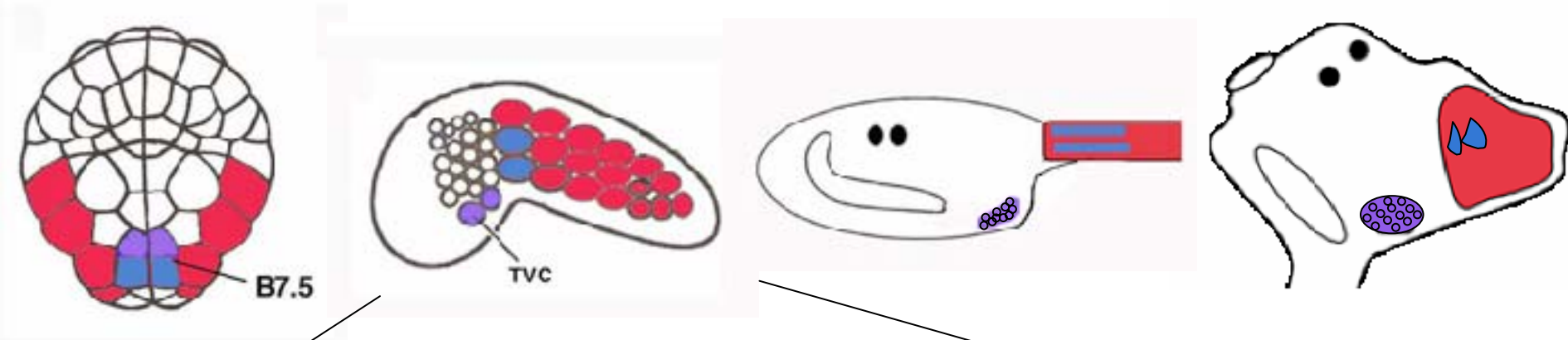
Pericardium / Myocardium



Heart movie

QuickTime™ and a
DV/DVCPRO - NTSC decompressor
are needed to see this picture.

Ciona heart lineage - conservation of “core” heart network

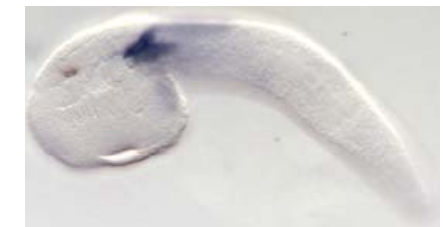


Ci-Bmp2/4

Ci-GataA

Ci-Nkx

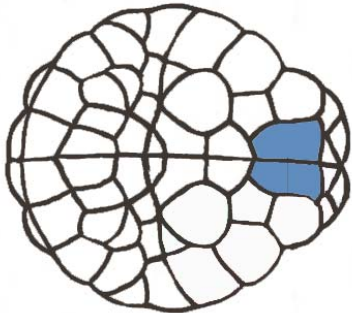
Tvc-Hand-like



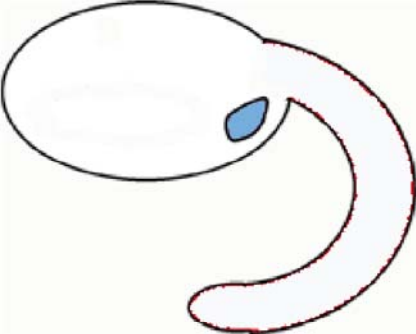
Tail-Raldh

Constructing a Ciona heart network

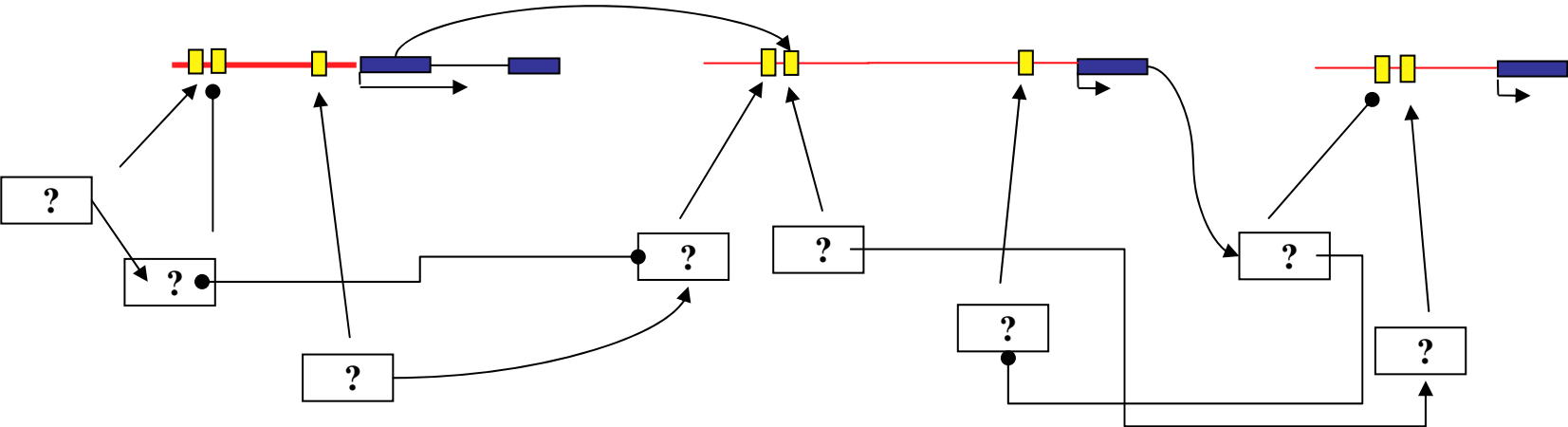
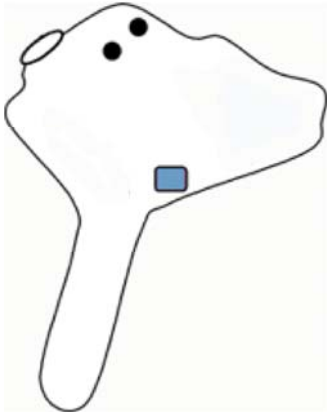
Early



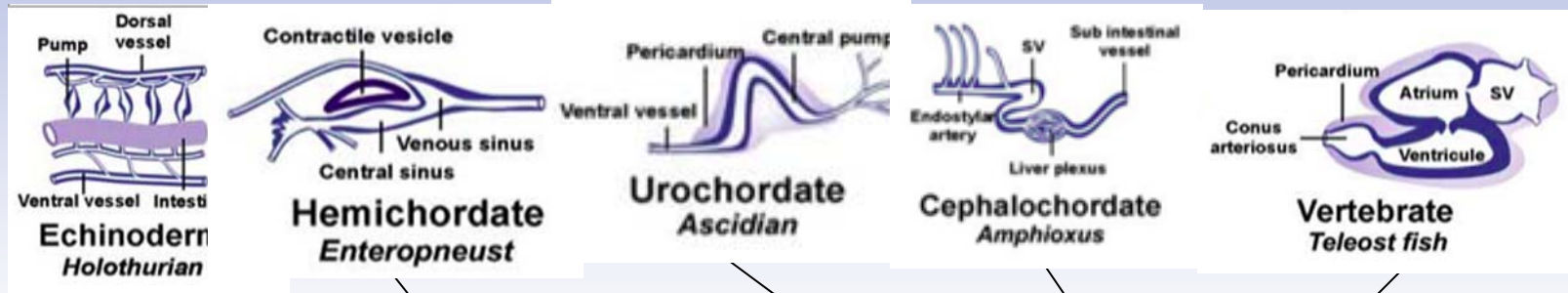
Middle



Late

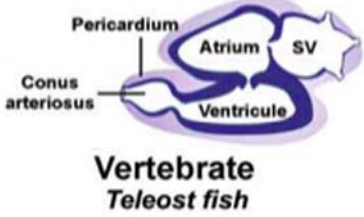
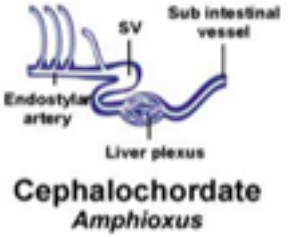
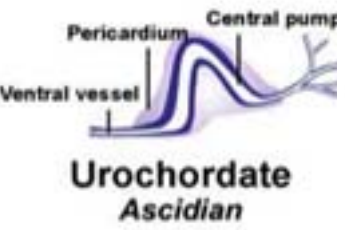
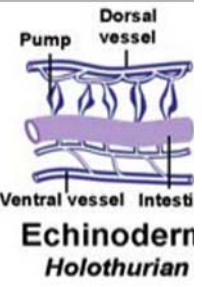


Heart Evolution



Dorsal vessel

Protostomes



Chordates

Multiple chambers (atria/ventricles)

Ventral/Anterior Heart

1. Chordate specific heart genes?
Why recruited?
2. Chamber evolution.
Ancestral 1 or 2?
Conserved Genes?

Conserved "initial" heart specification (Bmp/Nkx/Gata)

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Mesp (bHLH) and Heart Cell Migration:

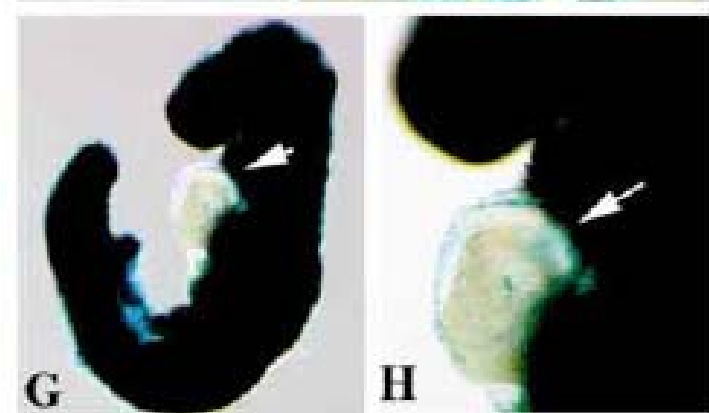
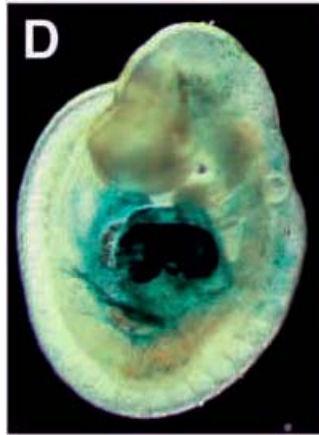
Mouse: (Kitajima, Saga, 1999, 2000)

- Mesp1 expression

Function: blue cells = Mesp1/2 KO

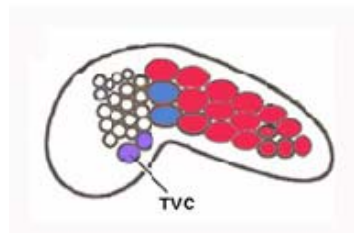
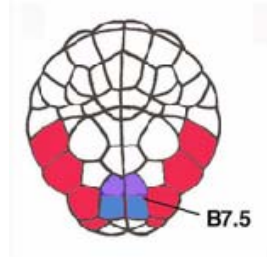
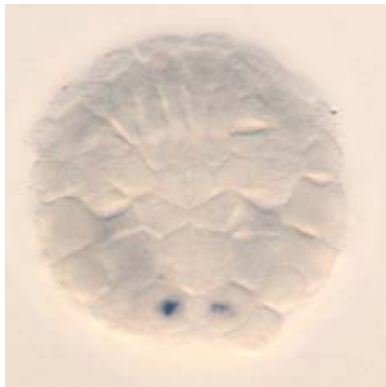


7.0 dpc



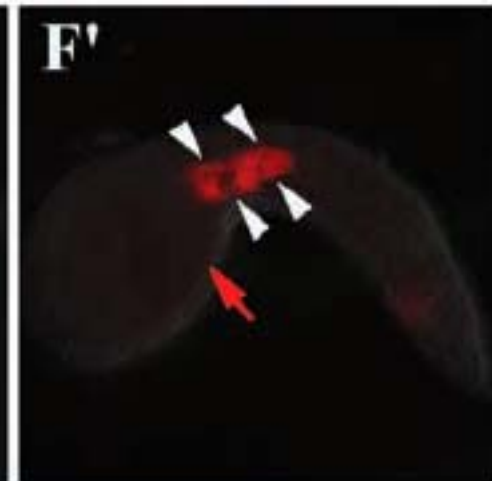
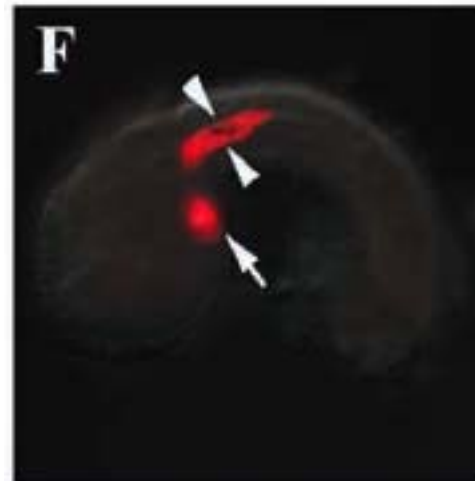
Ascidian

Mesp Expression:



Function: Normal

Mesp-KO (Satou et al., 04)



Two Questions about Mesp

- What activates Mesp?
- Is Mesp involved in Migration or Specification?

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Manipulation of Mesp function

1. Isolate DNA Binding Domain



2. Attach 3' domain

Repression

 -WRPW

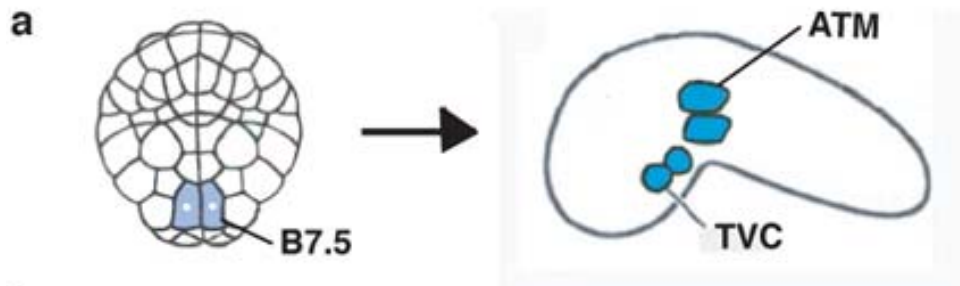
Activation

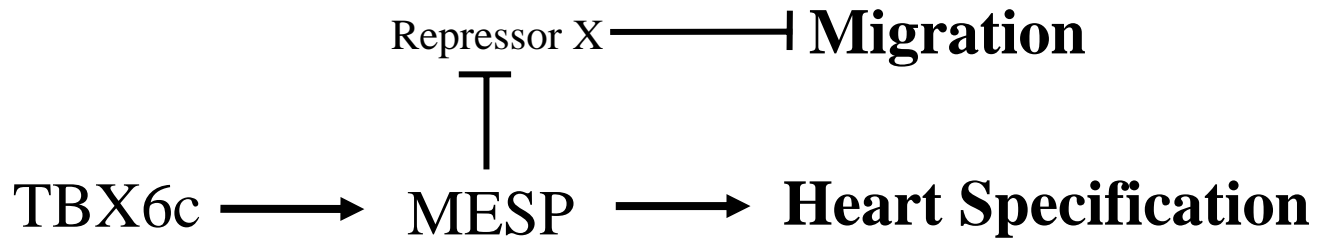
 -Vp16

3. Express in heart lineage

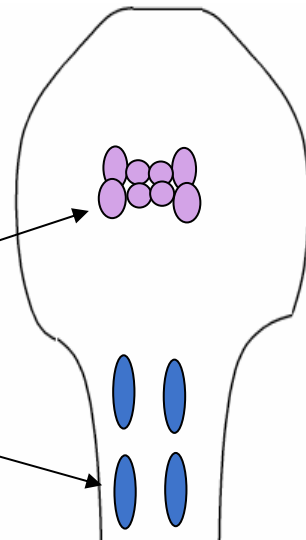
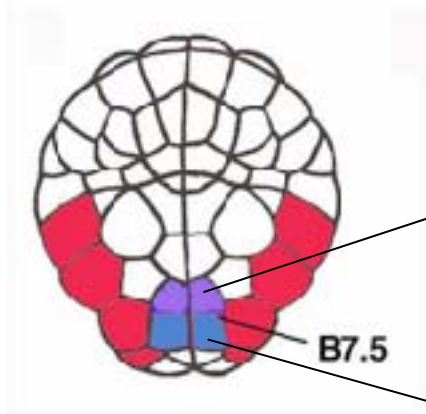
Mesp Enhancer 

Mesp Enhancer 



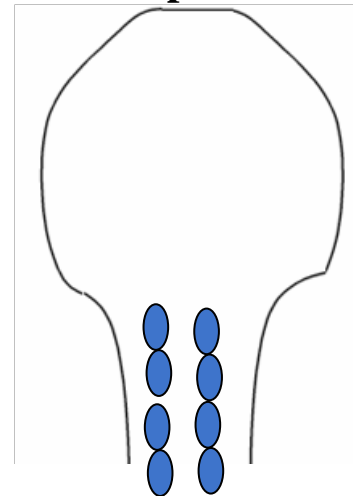


TVCs (Hndx)
migrate and
divide

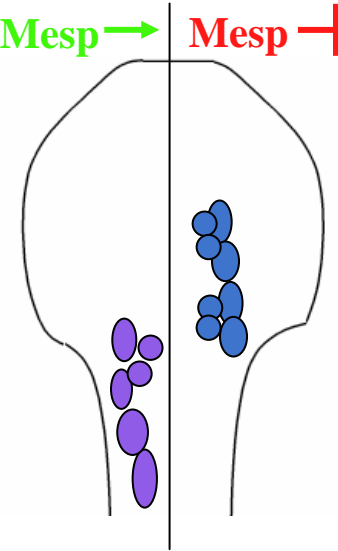


**Anterior Tail
Muscle
(Raldh2)**

Mesp KO



Mesp → **Mesp** ┆



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 - a. Early vertebrate heart specification
 - b. Recruitment of Mesp in chordate heart dev.
 - c. Mesp and the Germ Plasm
 - d. Chamber evolution

Vertebrate

Ciona

Cardiac/paraxial (somite)

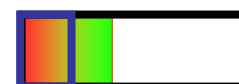
Cardiac/Tail muscle



Tbx6



Mesp on

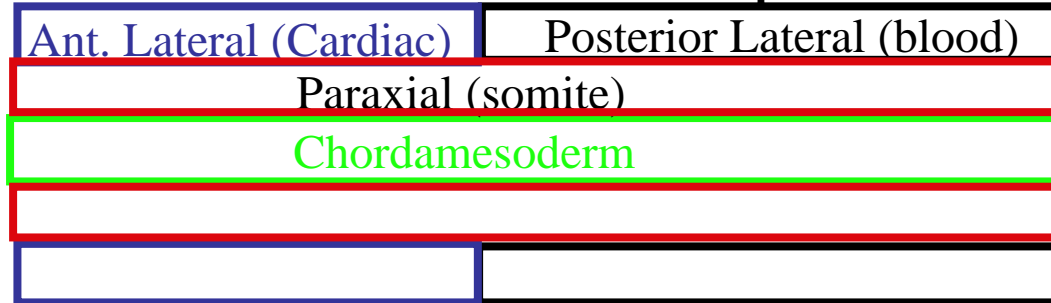


In Vertebrates:

Mesp initially induces a broad potential cardiac field

Current Model for Vertebrate Heart Specification

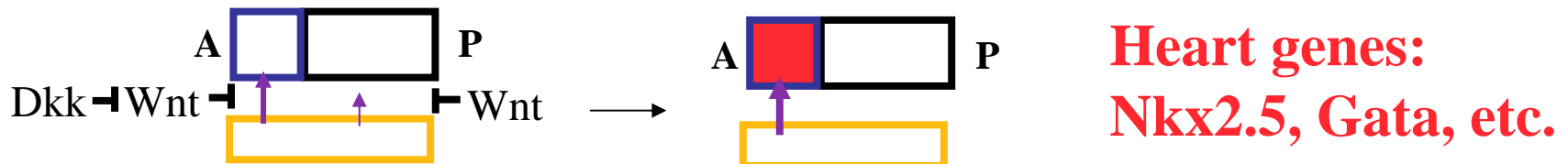
1. Prior to gastrulation mesodermal cell fates are unspecified.



2. During gastrulation **Bmp** (+ ?) secreted by endoderm initiates cardiac specification.



3. Induction is prevented in posterior by Wnt signals (blocked by DKK in anterior)



New Model for Vertebrate Heart Specification

New Vertebrate Model: Endoderm signal for maintenance, not inducer.

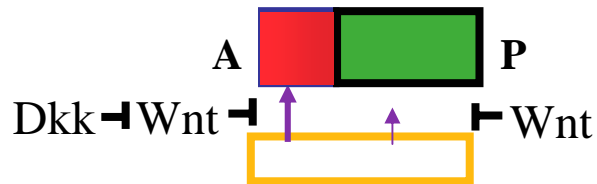
1. Tbx -> Mesp creates initial broad potential heart field.



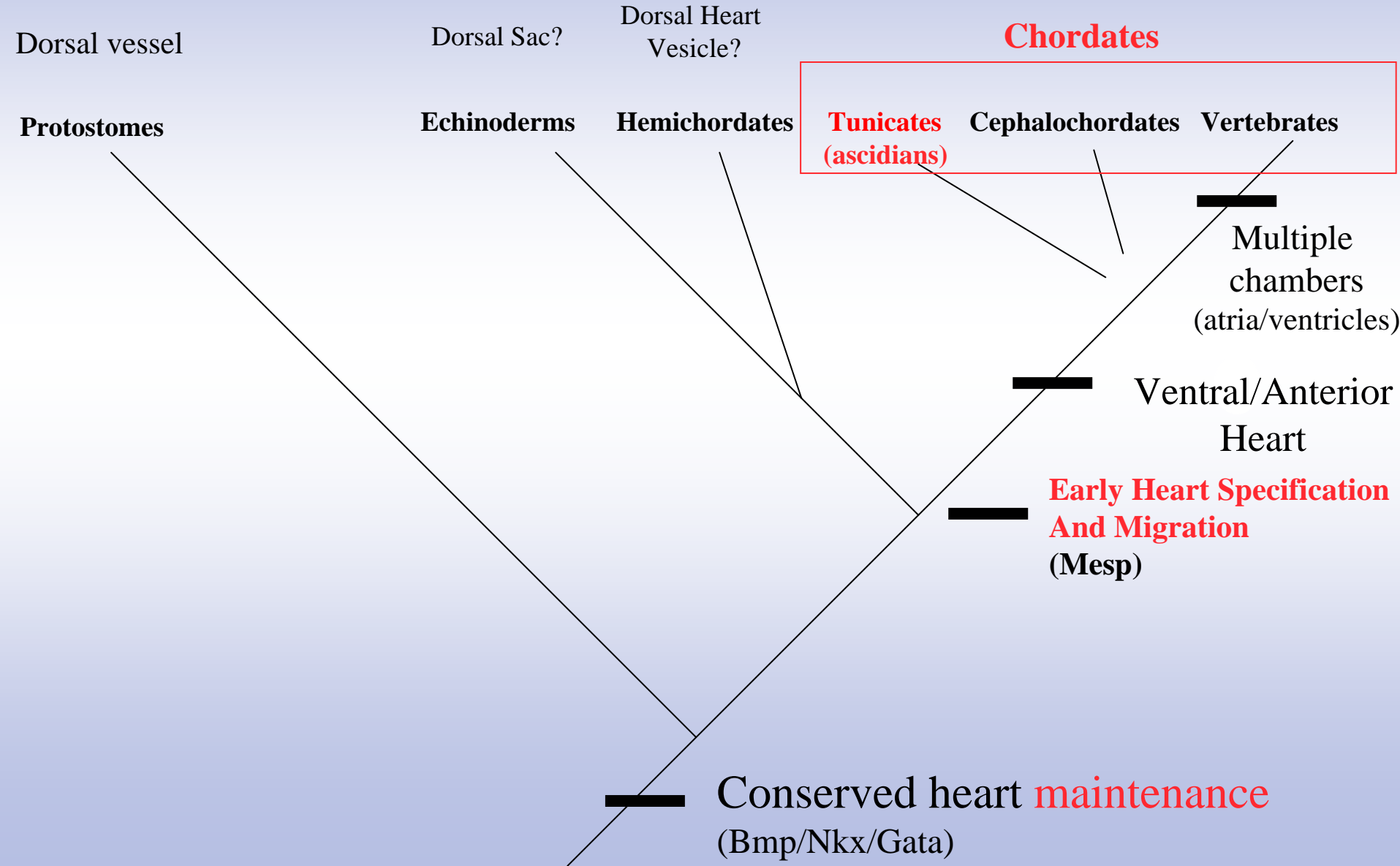
Ant. Paraxial (Cardiac) + Post. Paraxial (blood/somite)



2. Endoderm signal (**Bmp, et al.**) maintain heart fate, Wnt permits re-specification.



Heart Evolution



Recruitment of Mesp during chordate heart evolution?

Known: Only Mesp ortholog in flies (Sage) is expressed in salivary glands.

Assumption: Role for Mesp in heart/segmentation clock is specific to chordates.

{Test by expression assays in hemichordates, echinoderms, annelids and arthropods.}

Hypotheses:

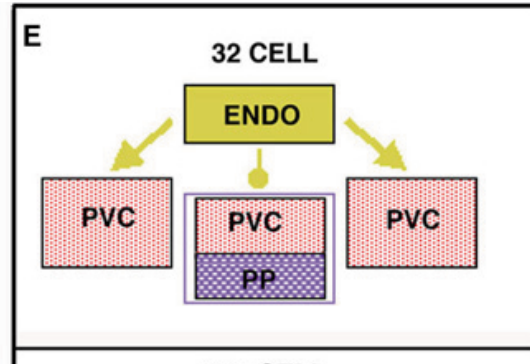
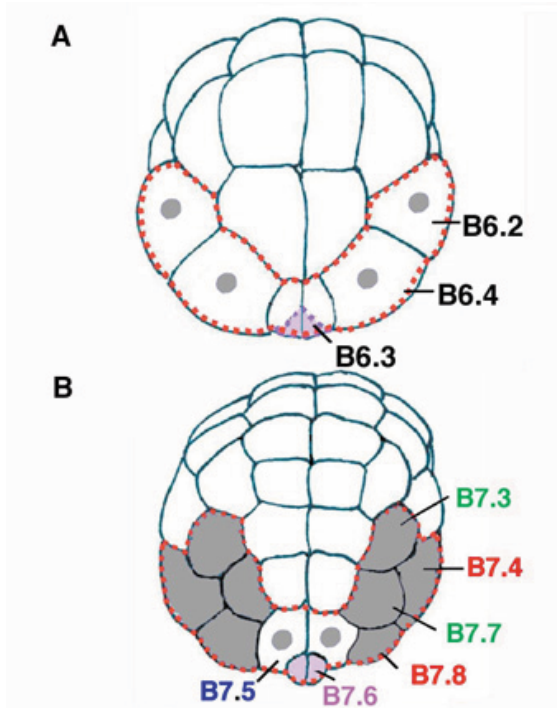
A. Evolution towards a muscular post-anal tail for chordate locomotion drove...

- Recruitment of Mesp to delay mesoderm differentiation, permitting dense clusters of muscle precursors to form = somites.

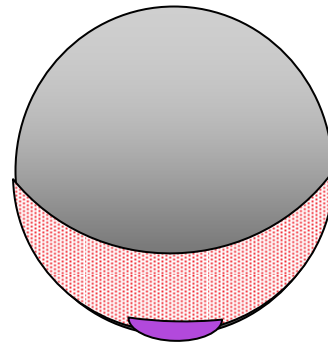
B. Lack of posterior heart lineage drove need for delayed heart differentiation.

C. Lack of Mesp in segmentation clock/heart is specific to Drosophila.

Mesp and the Germ Plasm



Germ line Silencing
(Tomioka et al; 2002)(Nishida)



Vegetal
Side

Mouse Mesp (Mesoderm Posterior) first isolated in subtractive screen for expression in primordial germ cells (Saga et al. 96)

1. Evolutionary implications

a. Early vertebrate heart specification

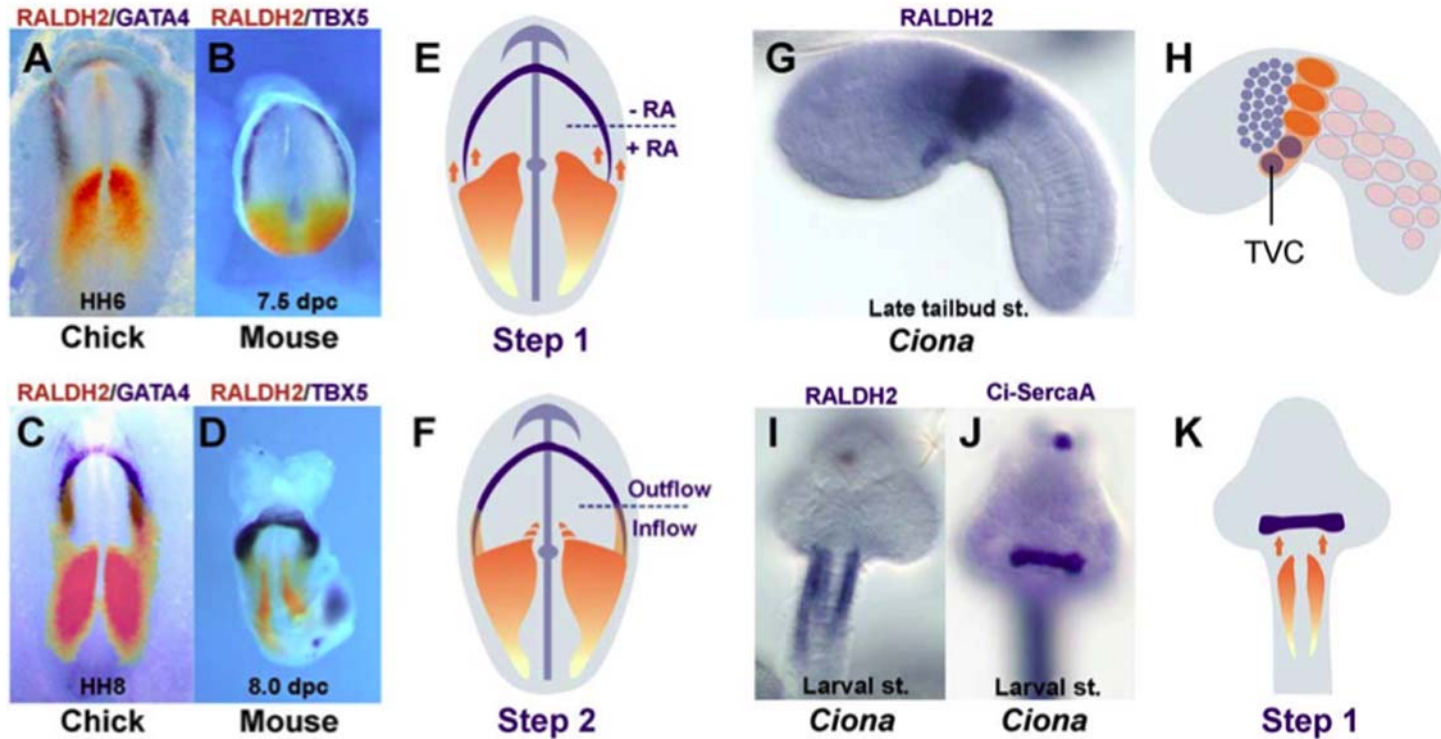
b. Recruitment of Mesp in chordate heart dev.

c. Mesp and the Germ Plasm

d. Chamber evolution

1. In vertebrates and *Ciona*, Raldh expression domain is initially caudal to heart field.

M.S. Simões-Costa et al. / Developmental Biology 277 (2005) 1–15



2. In vertebrates Raldh expression domain later spreads rostrally to help specify the atrial (inflow) chamber.

1. Raldh has conserved role in setting posterior/caudal limit of Heart
2. Raldh domain in Ciona (anterior tail muscle) is the precursor to the inflow (atrial) field in vertebrates.
3. Raldh domain in Ciona is remnant of atrial field.
4. Caudal-Rostral wave of Raldh in Ciona....
 - a. patterned the endoderm and was recruited for heart
 - b. patterned ancestral chambers and was lost in Ciona
 - c. patterns chamber precursor domains in Ciona heart

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