



Analysis of cell lineage stability during *X.laevis* tadpoles tail regeneration

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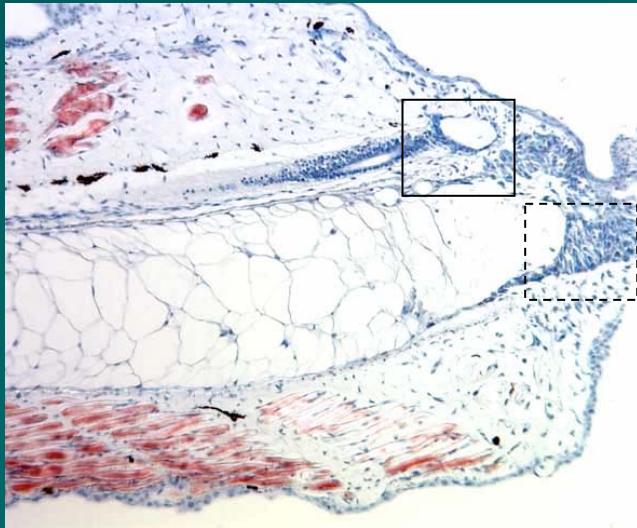


Regenerating Processes

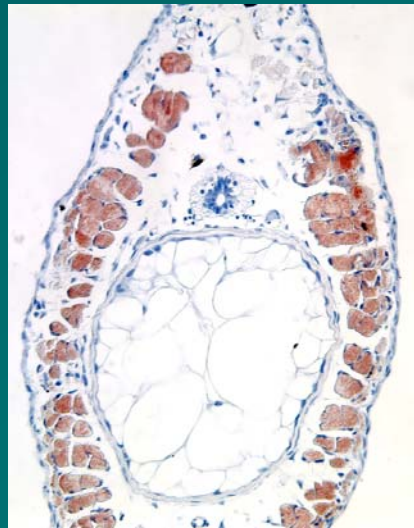
- **Preblastematic:** wound healing from epidermis
- **Blastematic:** cellular dedifferentiation and proliferation
- **Morphogenetic:** cellular redifferentiation or transdifferentiation. Is Metaplasia occurring?

Metaplasia: the conversion of one differentiated cell type into another

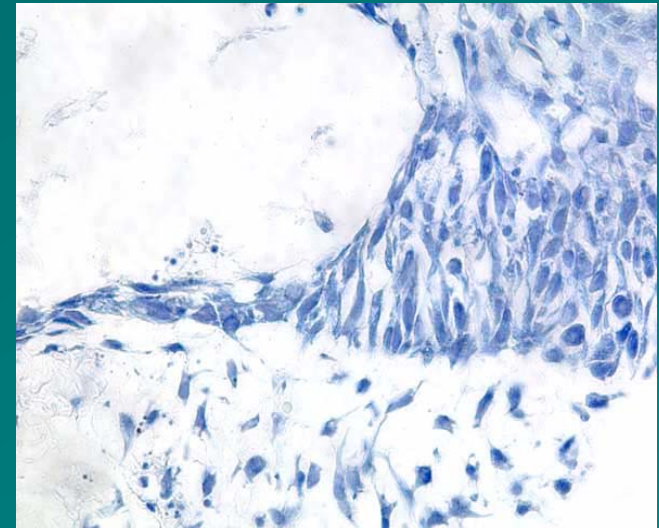
Tail Regeneration



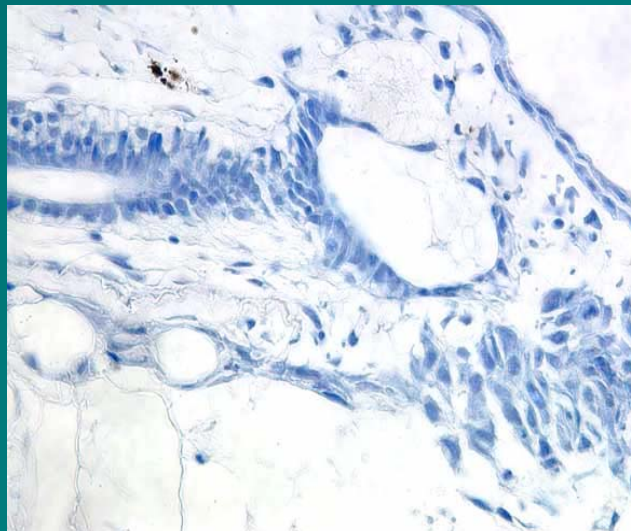
**3dd reg.
longitudinal
section**



**Wt tail
transverse section**



**3dd reg.
notochord**



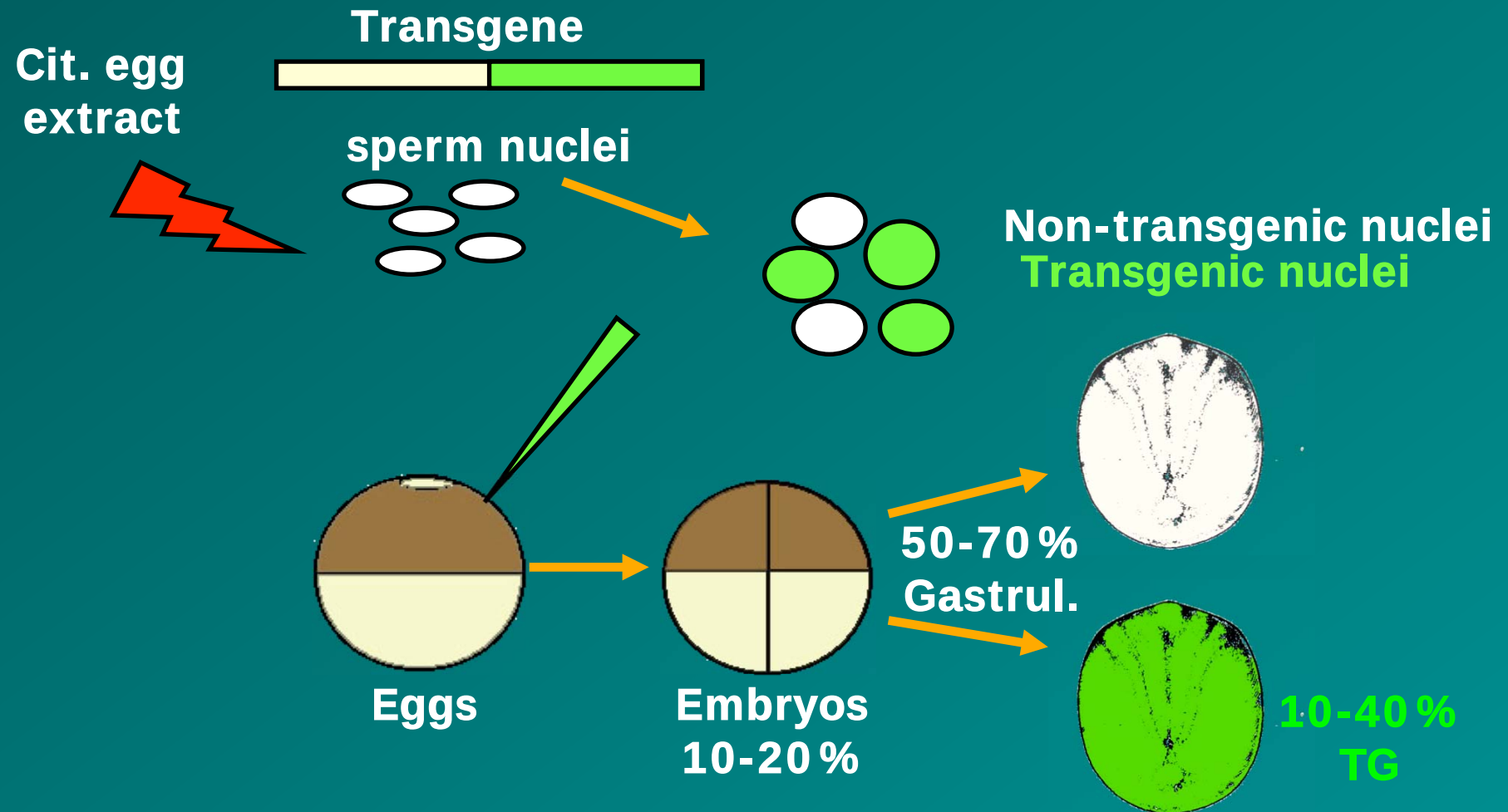
**3dd reg.
Neural Ampulla**

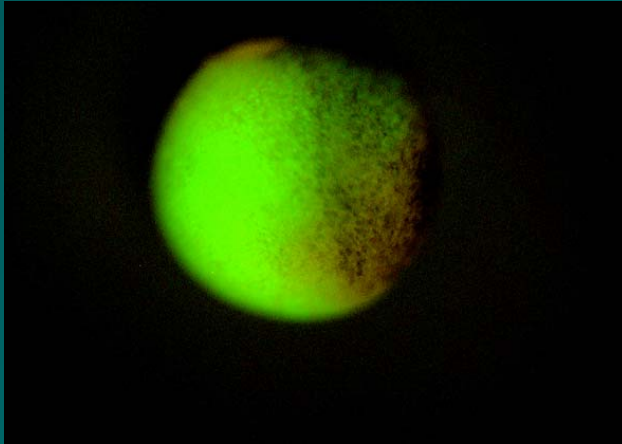
Research Aim

Does metaplasia occur during *Xenopus* tail regeneration?

- Specific tail core tissue labeling by grafting tissue segment from CMV-GFP transgenic donor into Wt hosts
- Analysis of the GFP expression stability in the regenerating labeled tissues
- Investigation of the cell populations directing the tail regeneration process

X. laevis Transgenesis

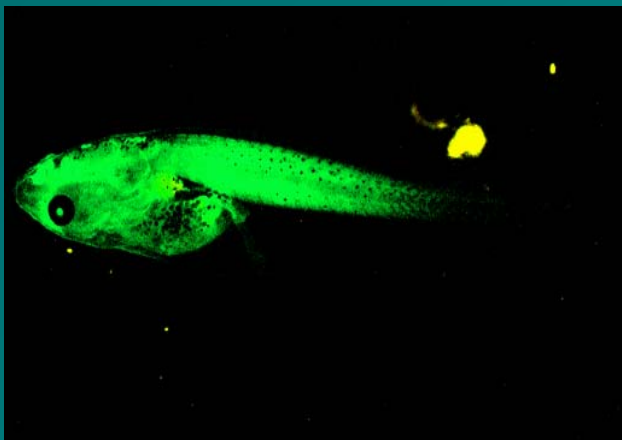




CMV-GFP TG
St. 13



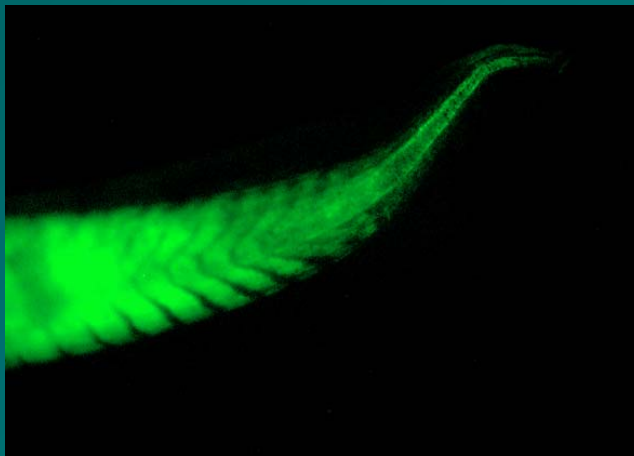
CMV-GFP TG
St. 27



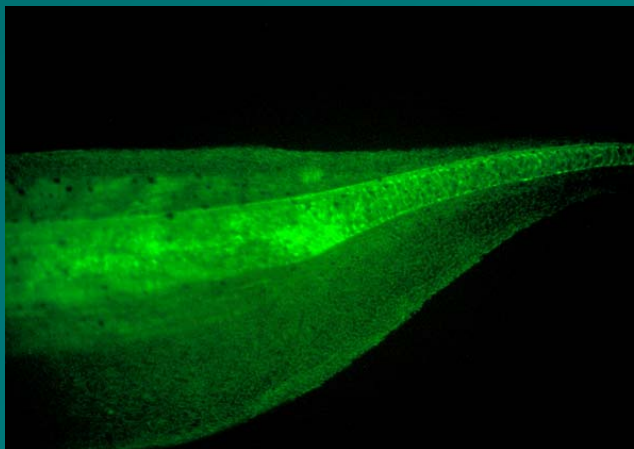
CMV-GFP TG
St. 48



CMV-GFP TG
5 d. post-amputation

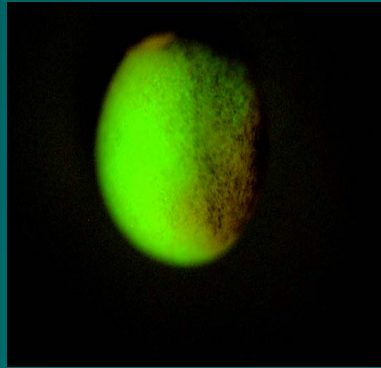


CMV-GFP TG
10 d. post-amputation



CMV-GFP TG
15 d. post-amputation

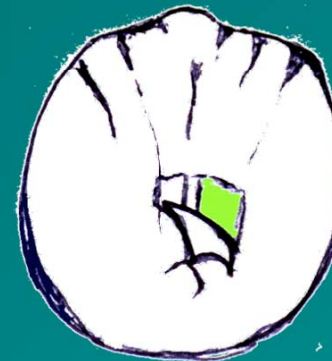
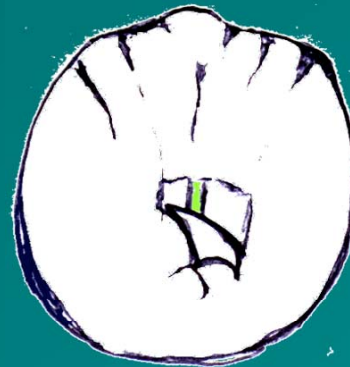
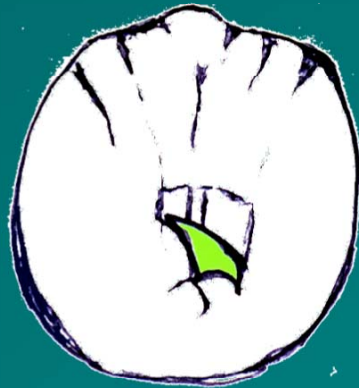
Grafts from transgenic embryos (CMV-GFP)



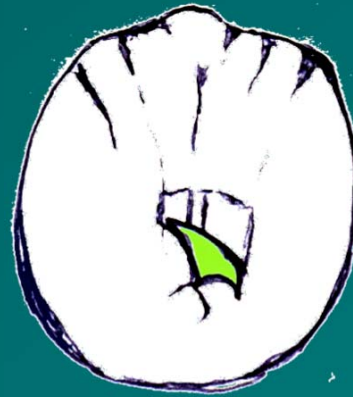
Neural-Plate

Somites

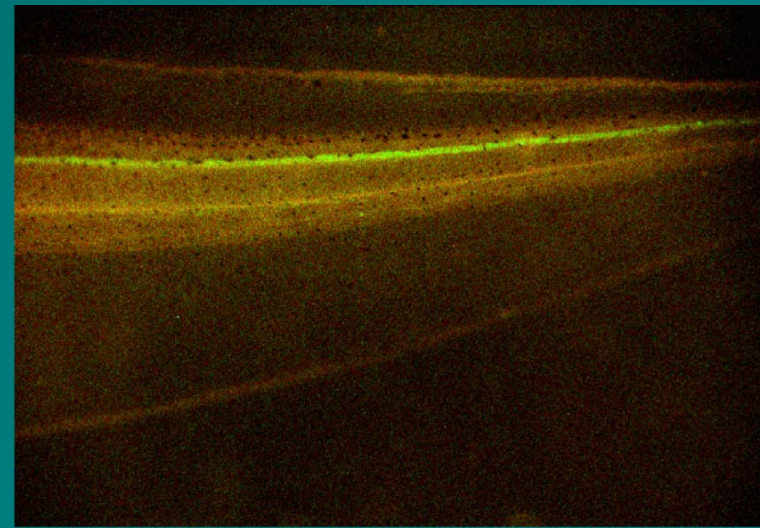
Notochord



Neural-Plate

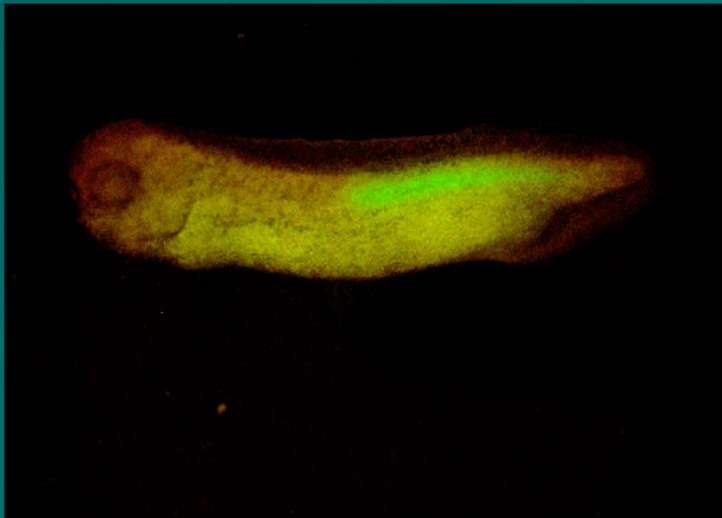
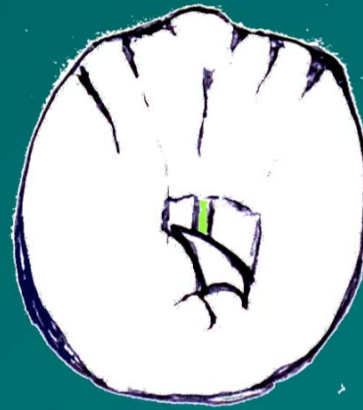


CMV-GFP NP
St. 32

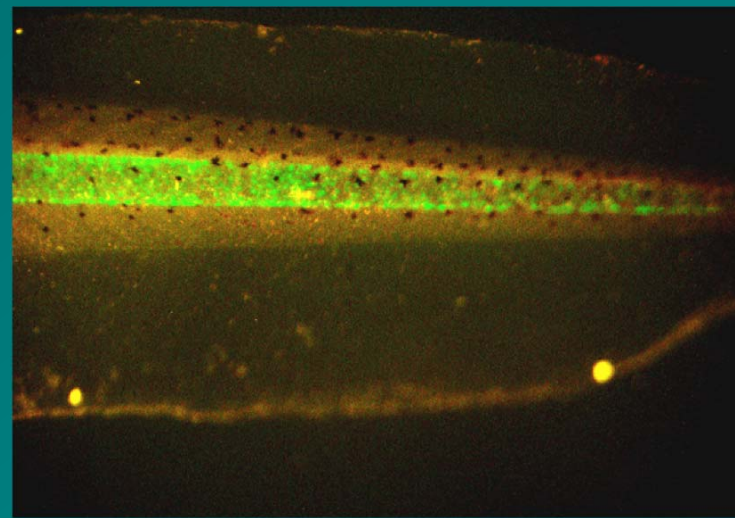


CMV-GFP NP
St. 49

Notochord

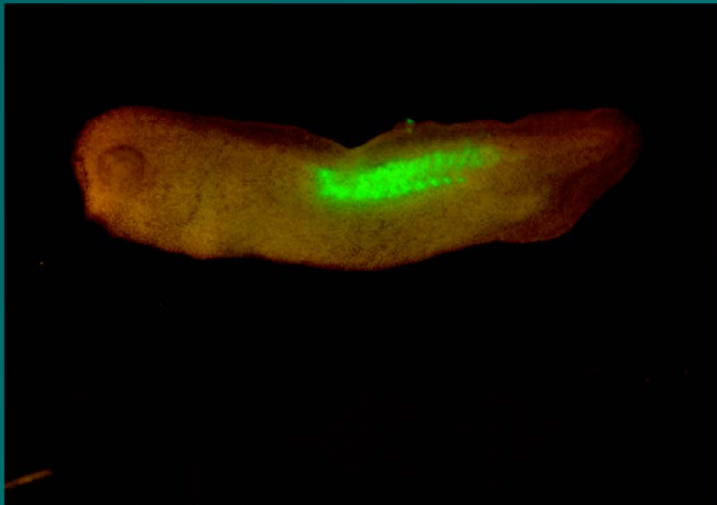
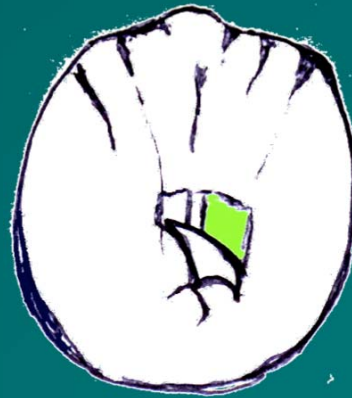


CMV-GFP Notochord
St. 32

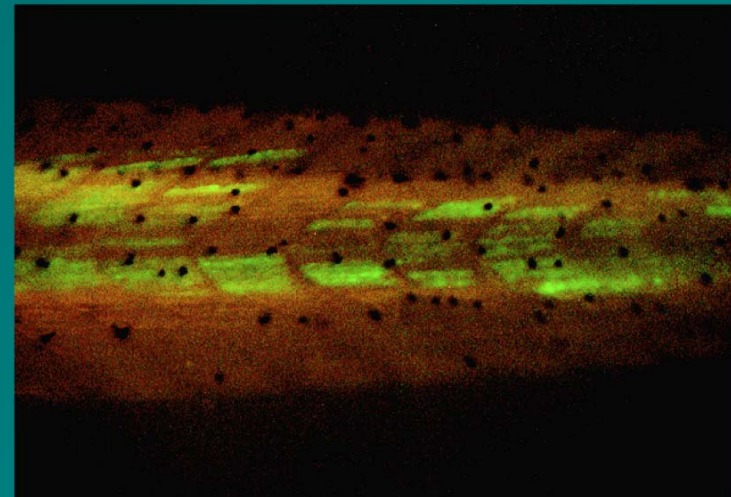


CMV-GFP Notochord
St. 49

Somites

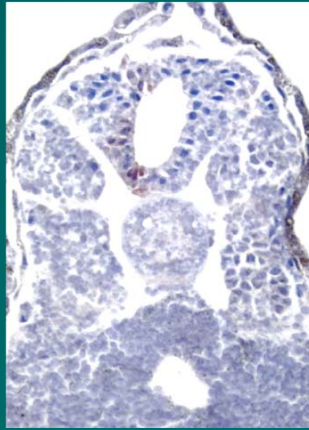


CMV-GFP Somites
St. 32

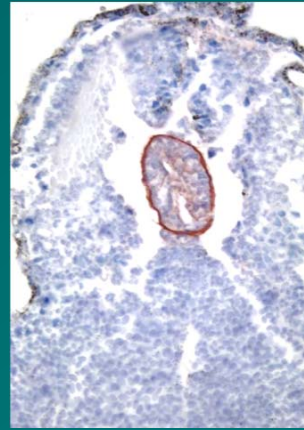


CMV-GFP Somites
St. 49

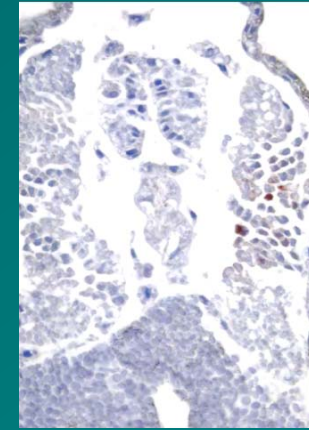
Anti-GFP Immunostaining



NP-GFP st.28



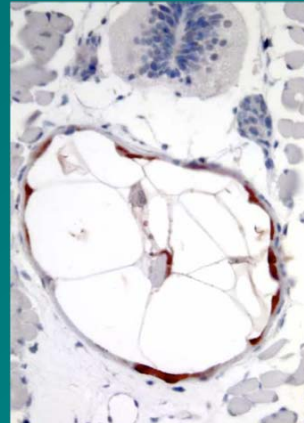
Not-GFP st.28



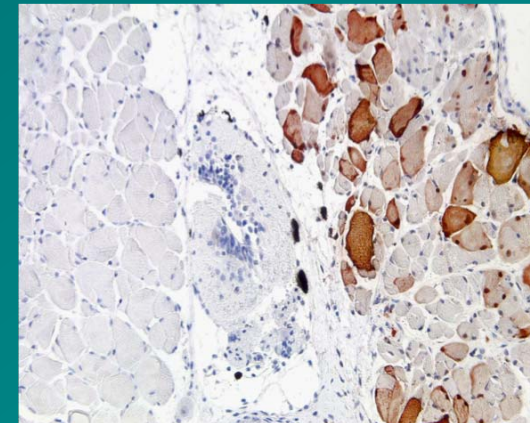
Som-GFP st.28



NP-GFP st.49

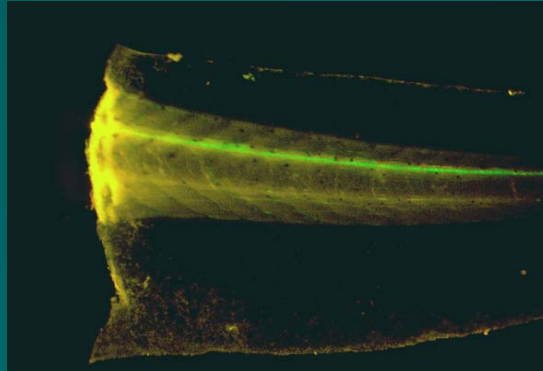


Not-GFP st.49



NP-GFP st.49

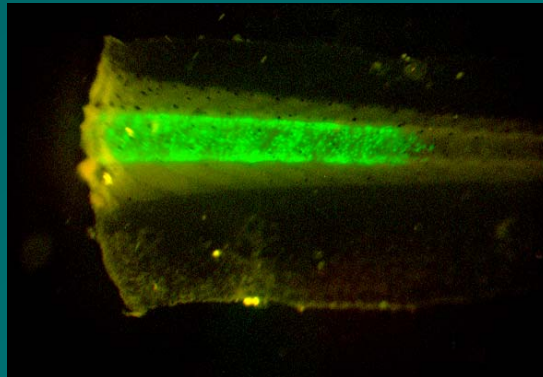
**CMV-GFP
NT amp.**



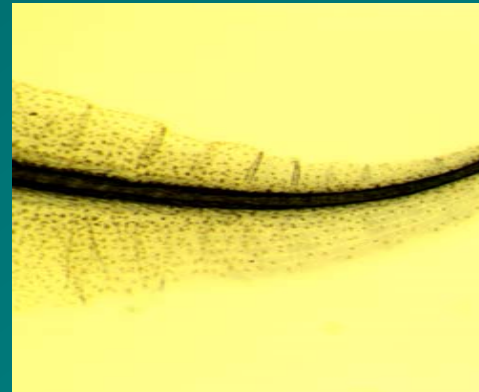
**2G9
mAb α NT**



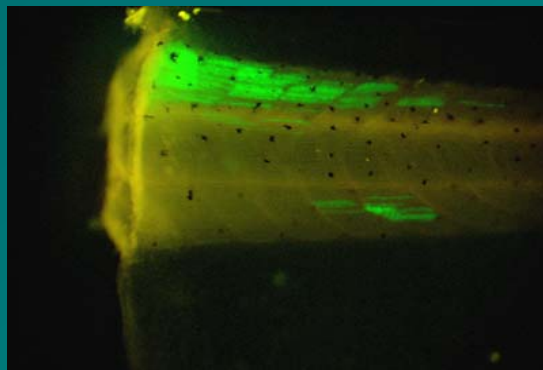
**CMV-GFP
Not amp.**



**α Col II
mAb α Not**

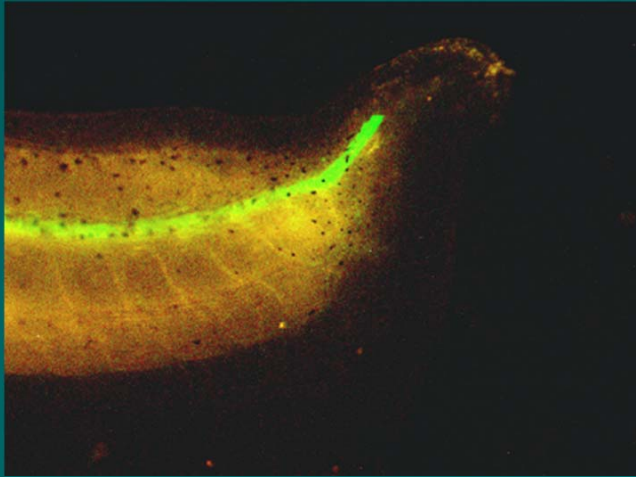


**CMV-GFP
Som amp.**

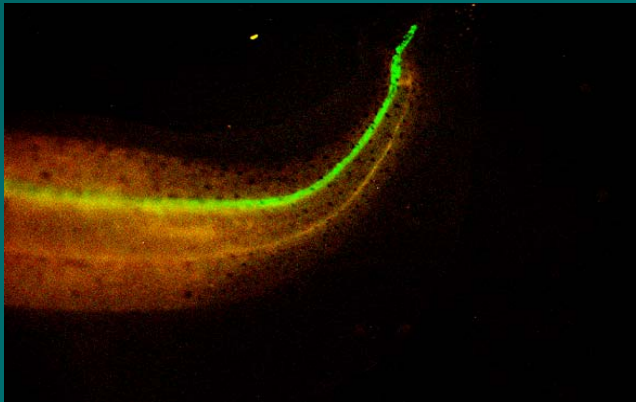


**12/101
mAb α Som**

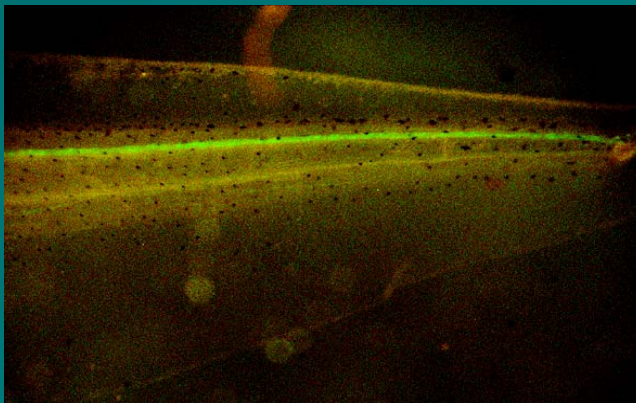




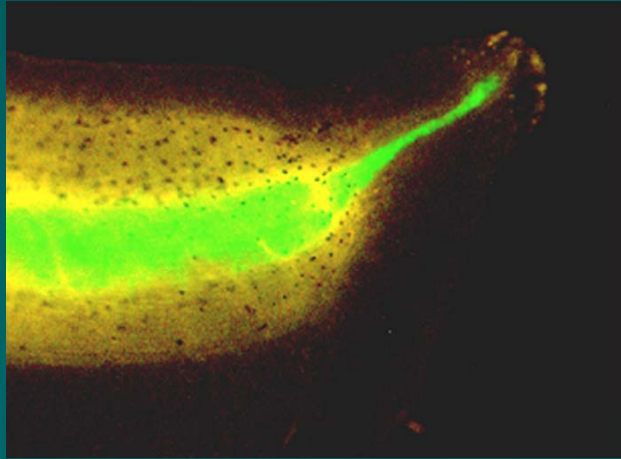
CMV-GFP NT
5 d. post-amputation



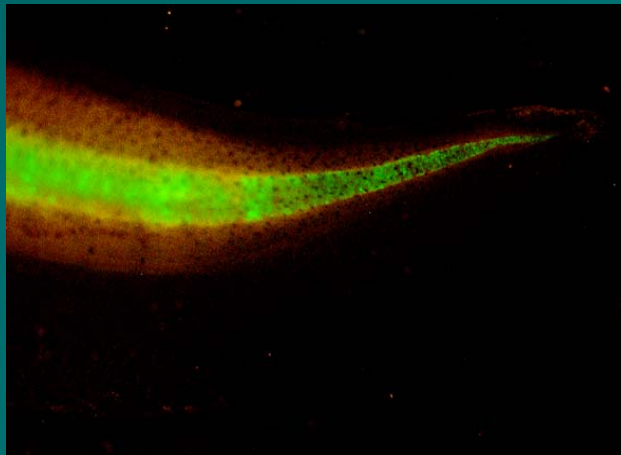
CMV-GFP NT
10 d. post-amputation



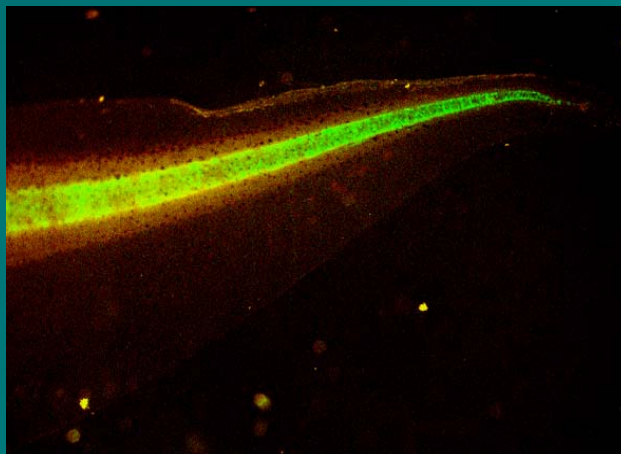
CMV-GFP NT
20 d. post-amputation



**CMV-GFP Not
5 d. post-amputation**



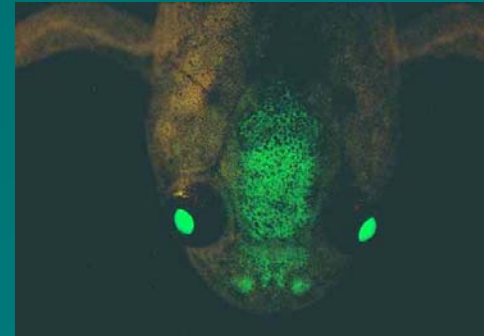
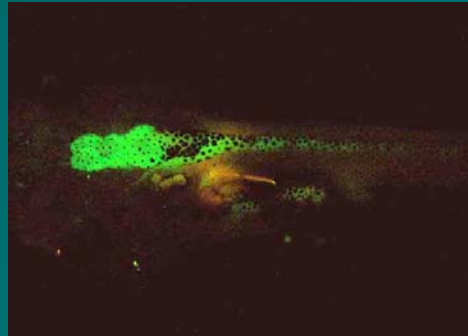
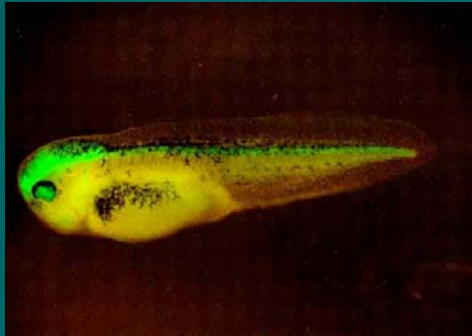
**CMV-GFP Not
10 d. post-amputation**



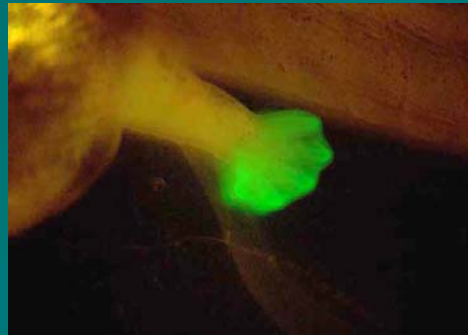
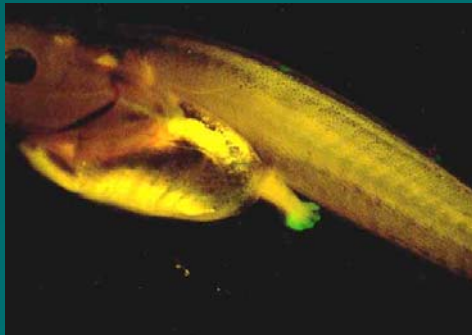
**CMV-GFP Not
20 d. post-amputation**

Tissue specific Promoter

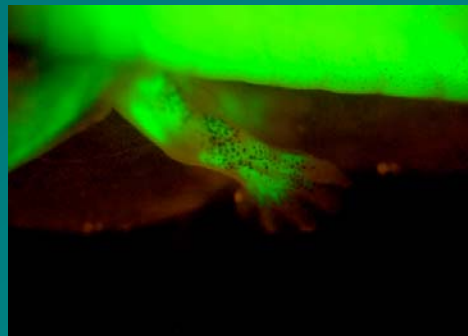
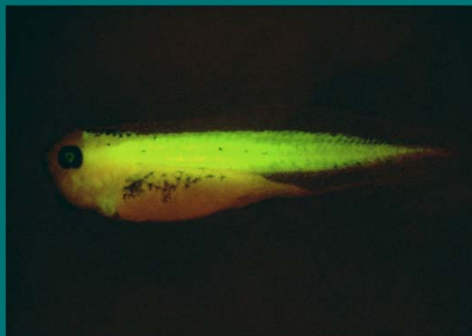
N β Tub



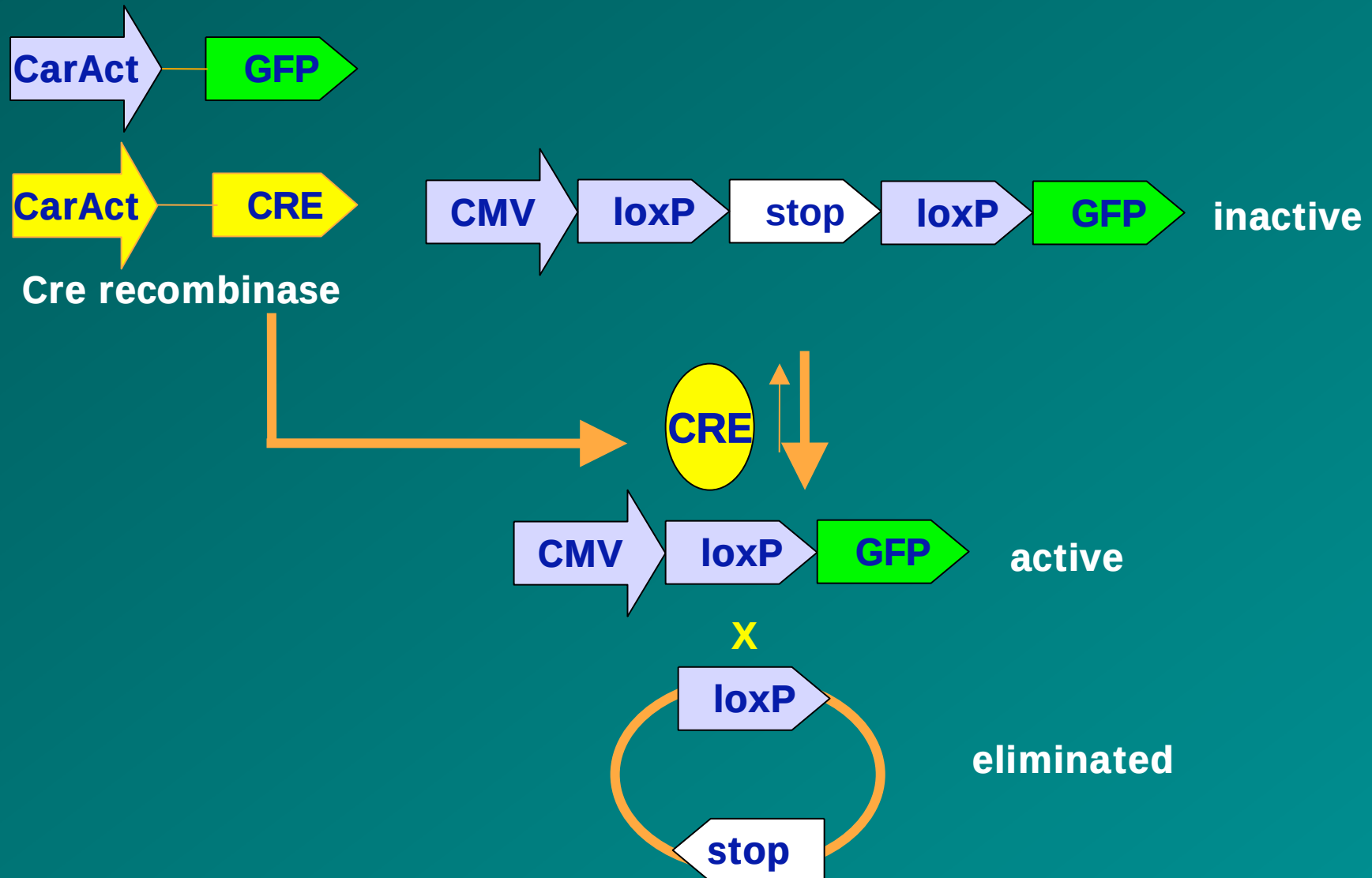
Col II



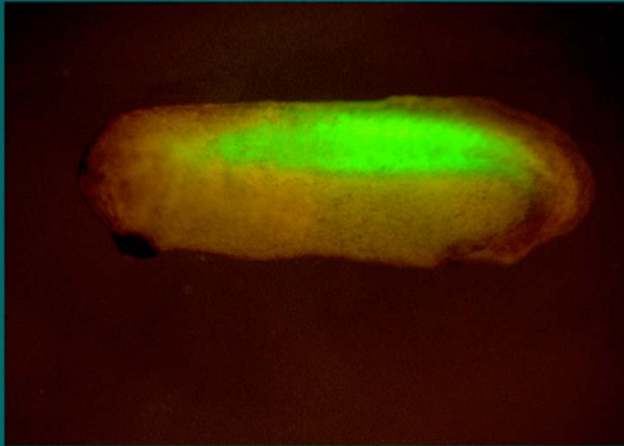
α CarAct



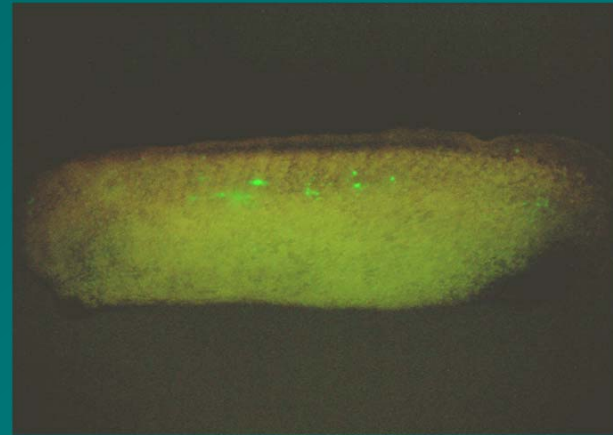
α CardAct driving GFP and CRE



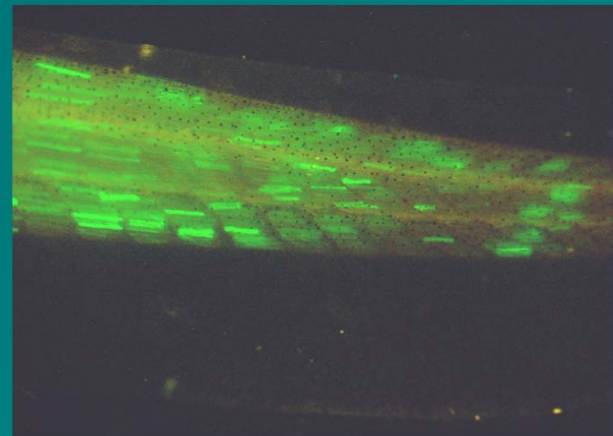
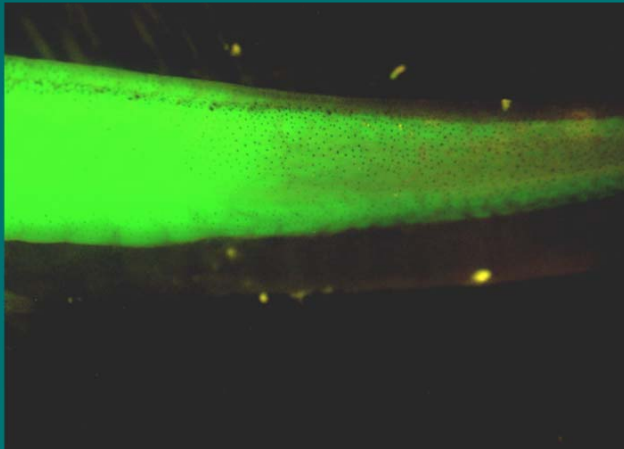
α CardAct-GFP



α CardAct-CRE
+
CMV-loxSTOPllox-GFP

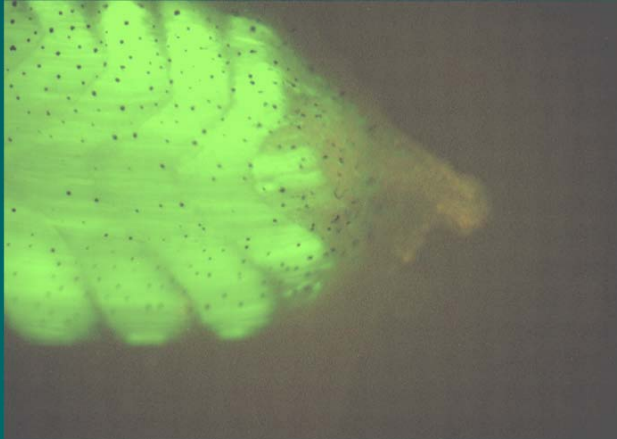


St. 28

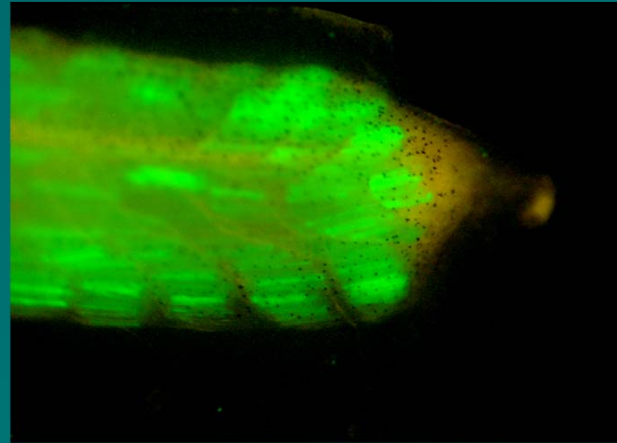


St. 49

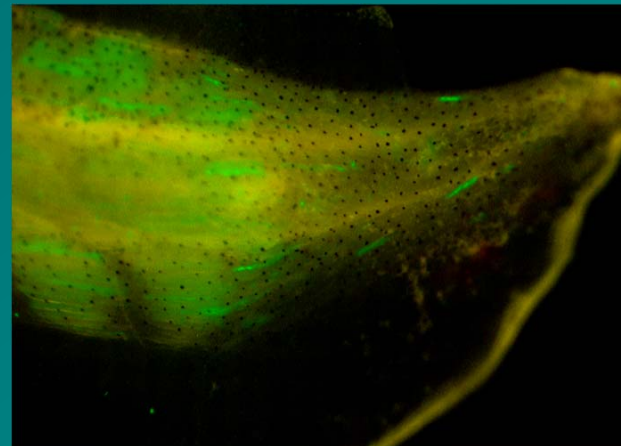
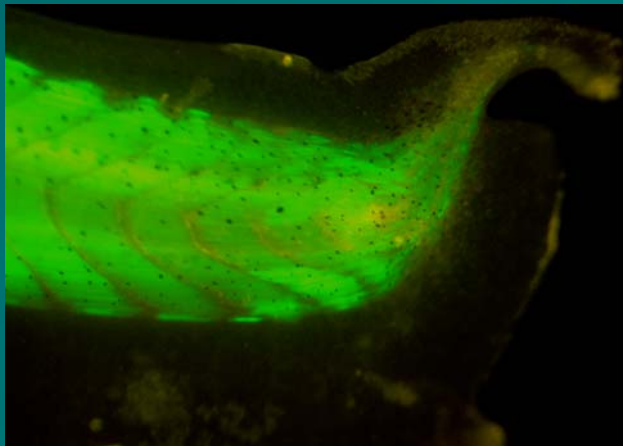
α CardAct-GFP



α CardAct-CRE
+
CMV-loxSTOPlox-GFP

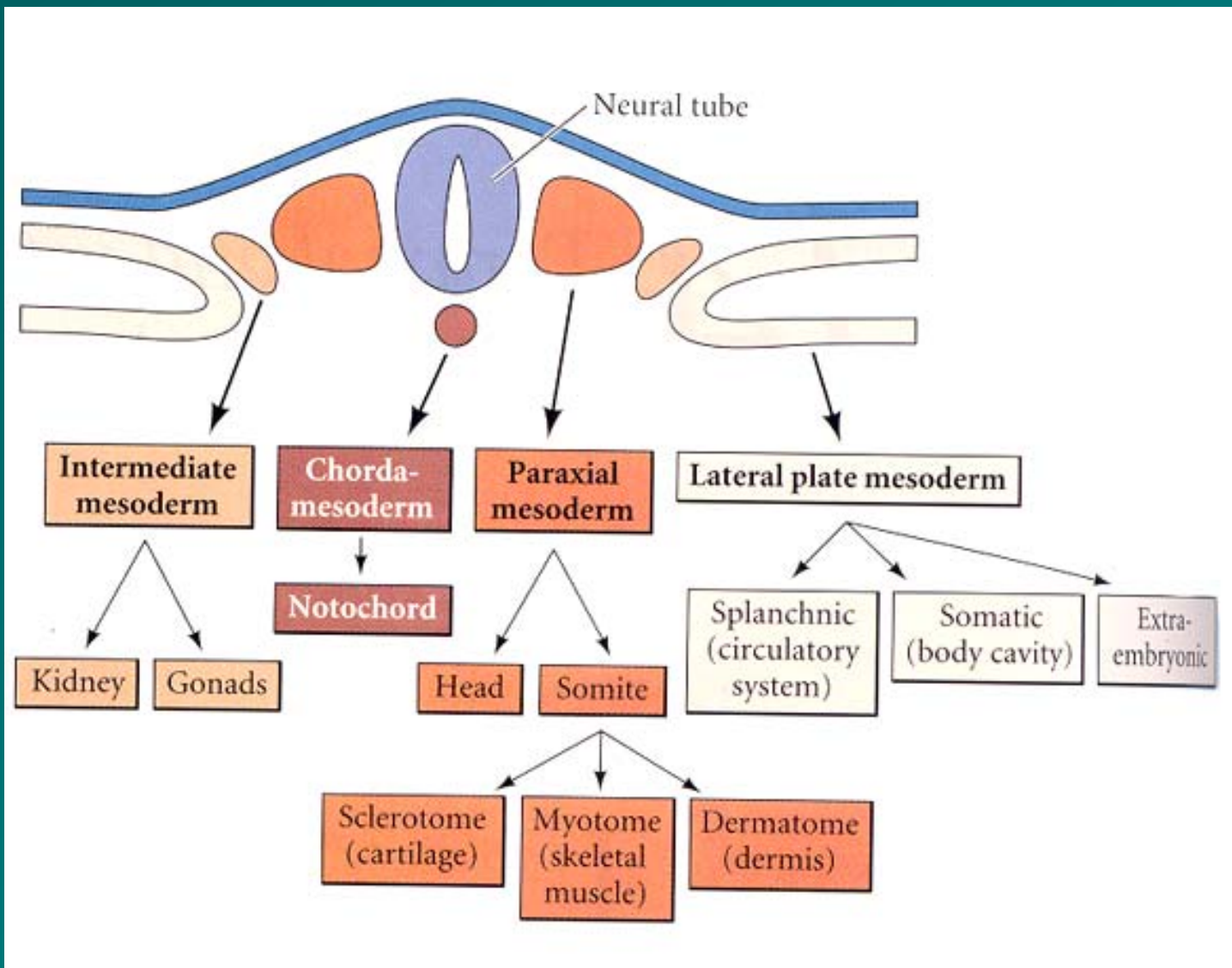


3 d.
p.amp.

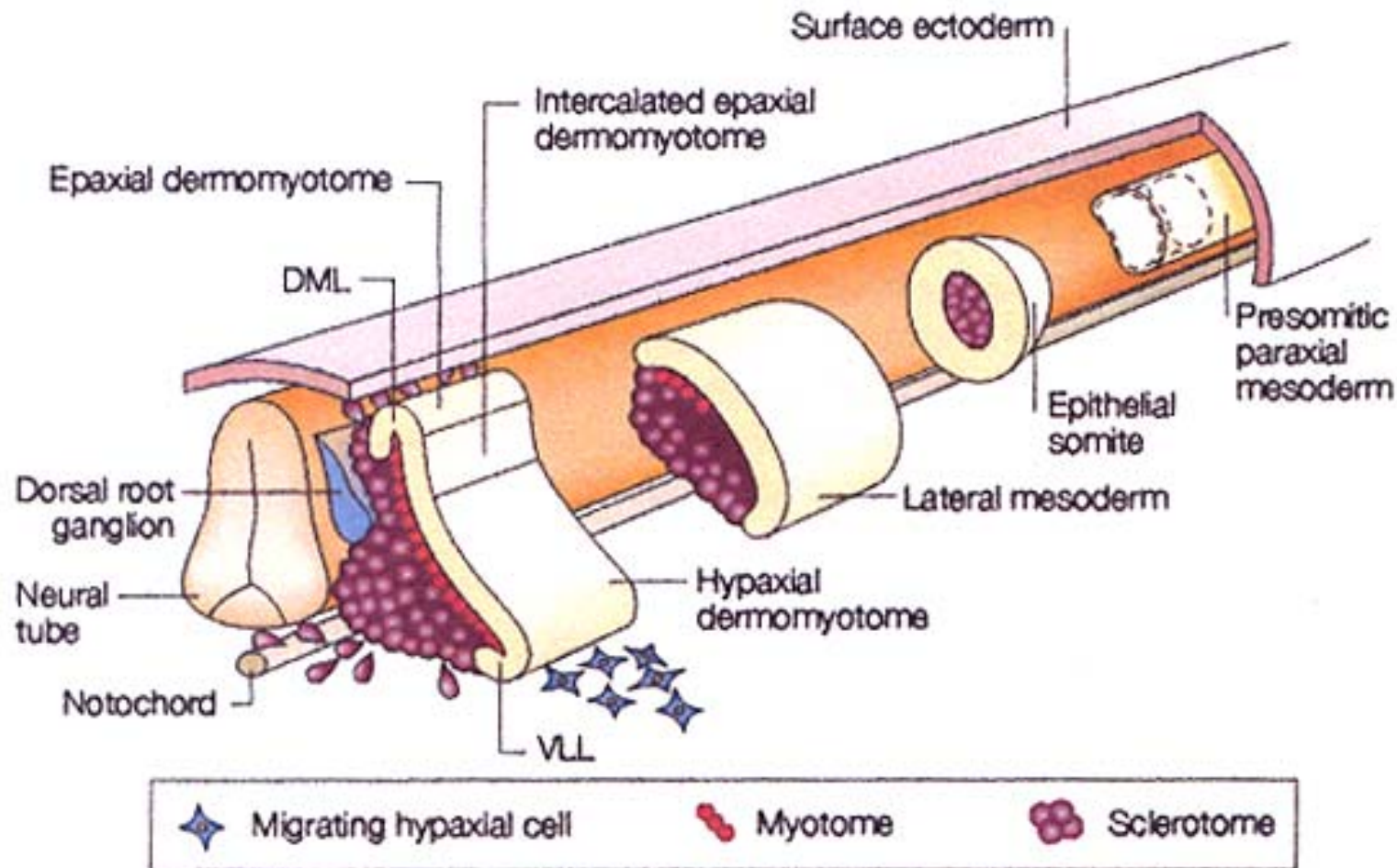


5 d.
p.amp.

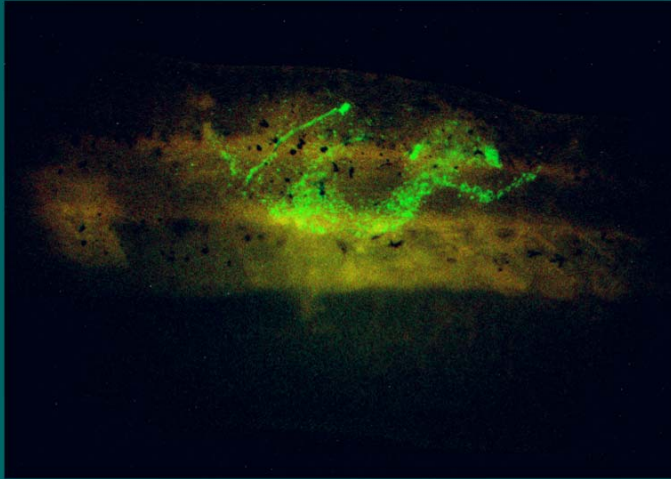
Mesodermal tissues



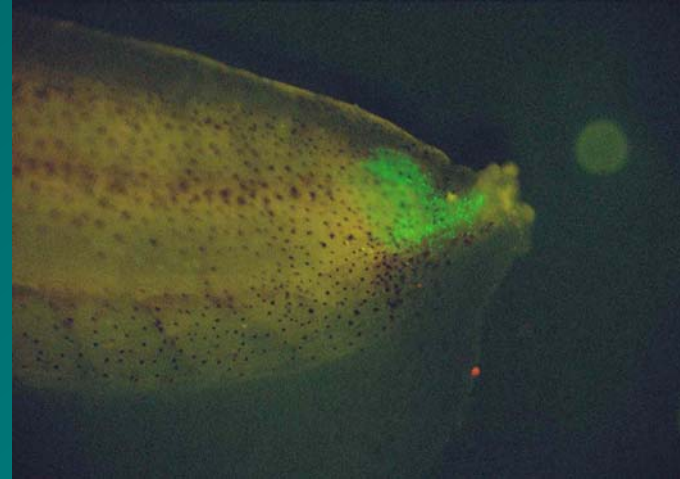
Somites Formation



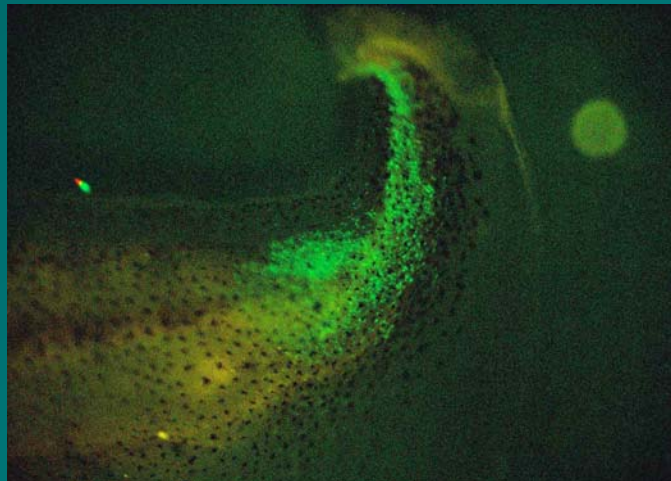
Skin Graft (CMV-GFP)



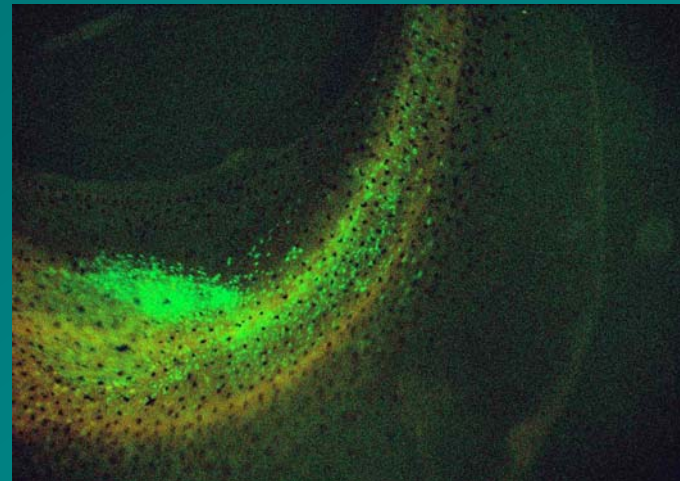
24 hr post-transplants



3 d. p.amp.

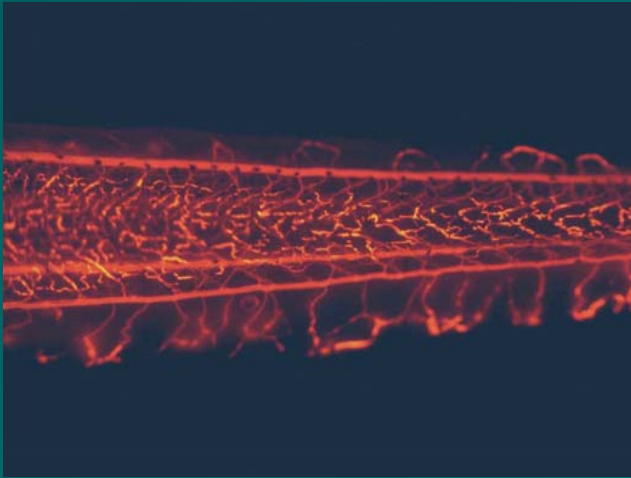


5 d. p.amp.

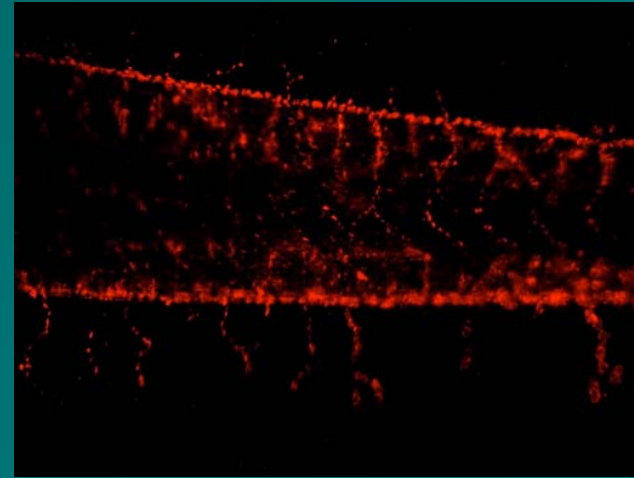


7 d. p.amp.

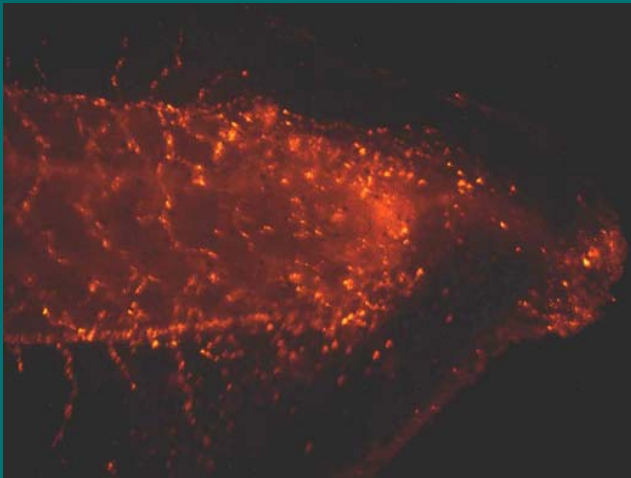
LDL-dil heart injection



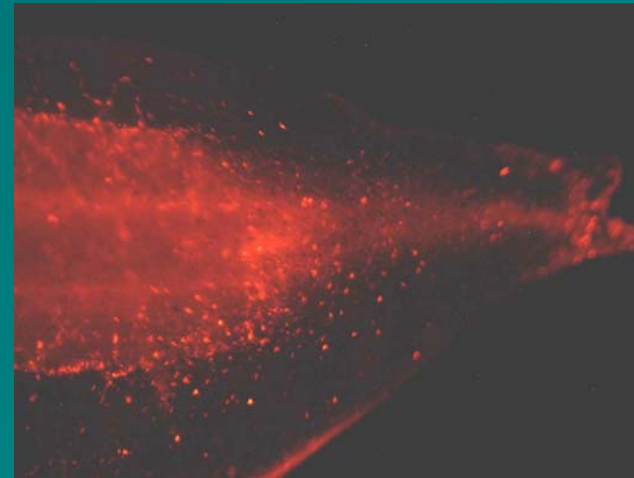
injection



24 hr post inj.



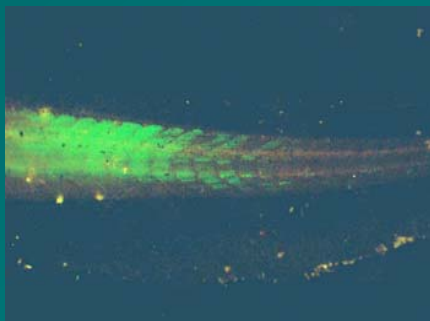
3 d. post amp.



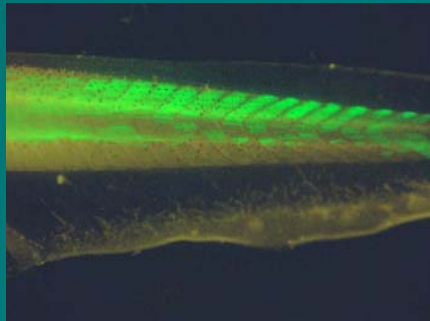
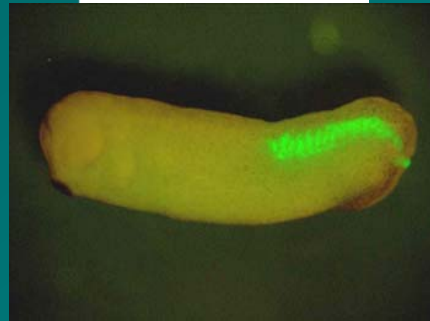
5 d. post amp.

Presomitic Mesoderm (PSM) Graft

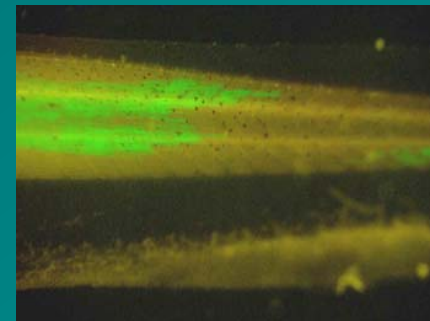
Dors Med PSM
st. 13



Dors Med PSM
st. 17



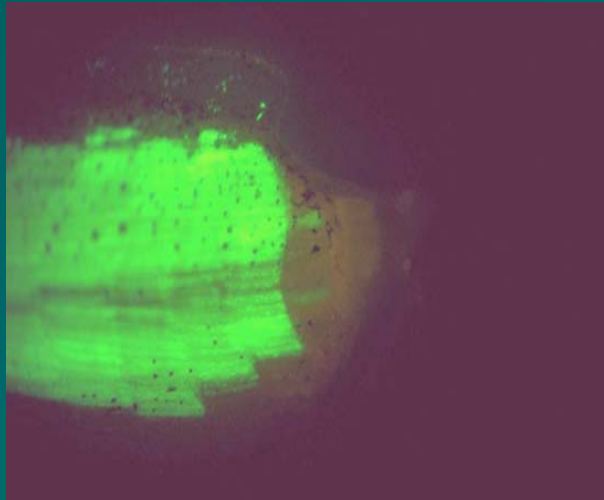
Dors Lat PSM
st. 13



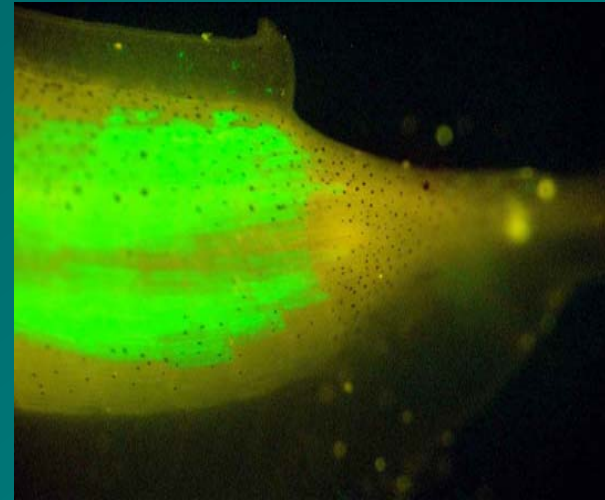
St. 28

St. 49

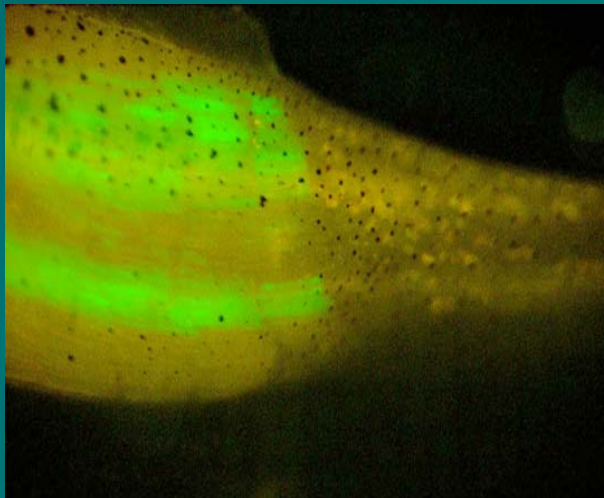
Dors Med PSM st. 13



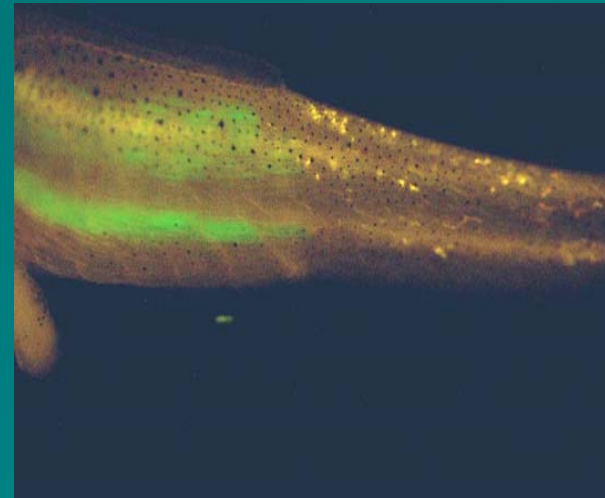
3 d. post-amputation



5 d. post-amputation

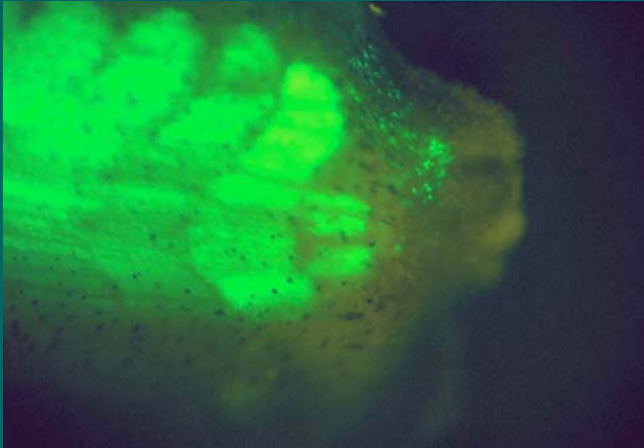


10 d. post-amputation

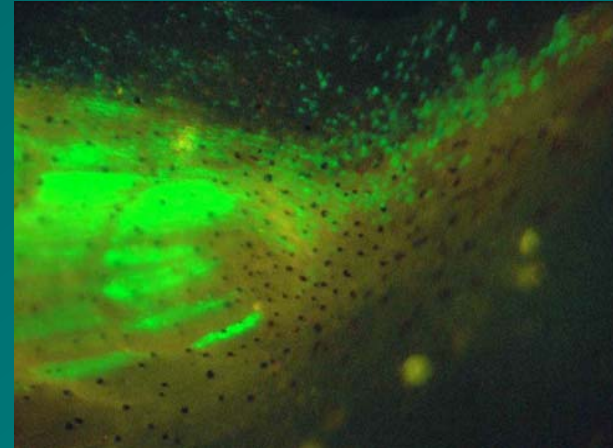


20 d. post-amputation

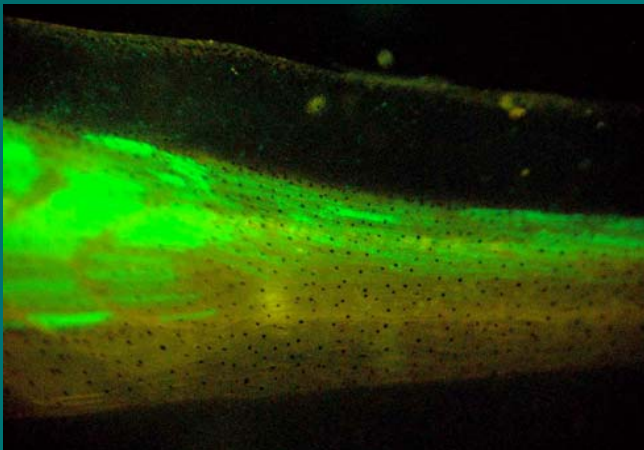
Dors Med PSM st. 17



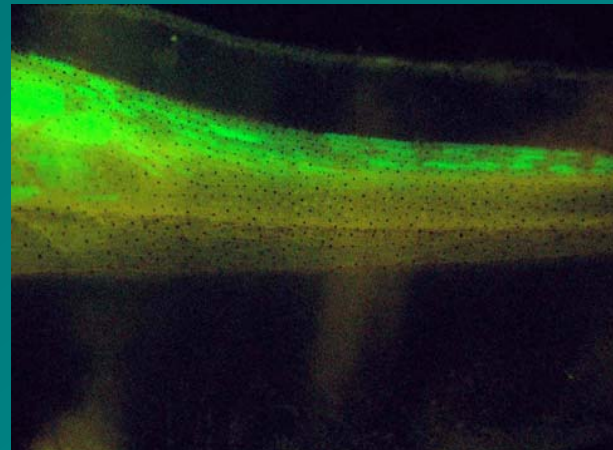
3 d. post-amputation



5 d. post-amputation

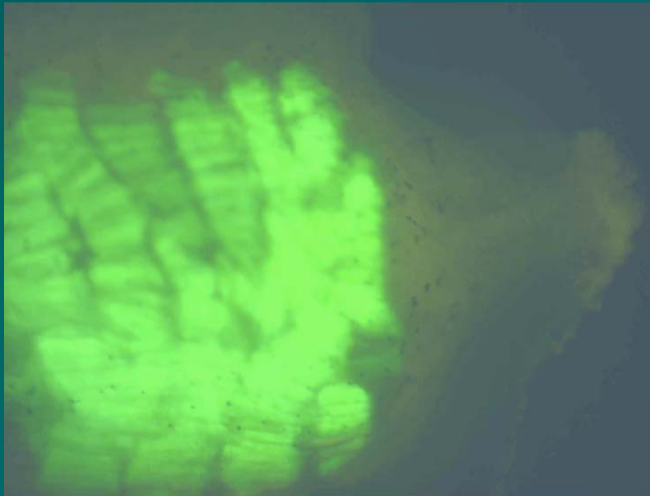


10 d. post-amputation

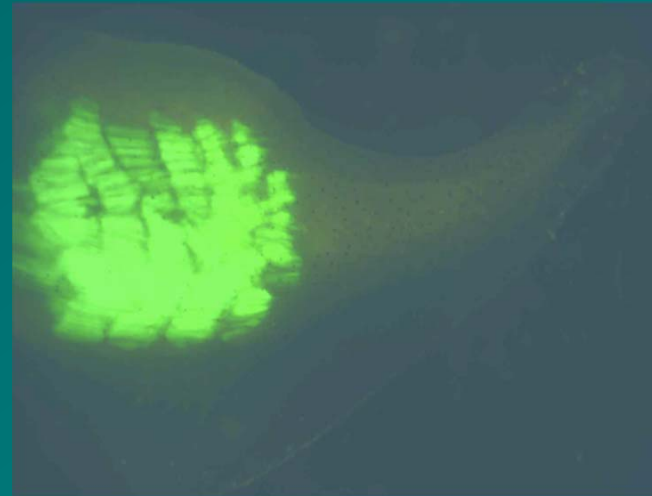


20 d. post-amputation

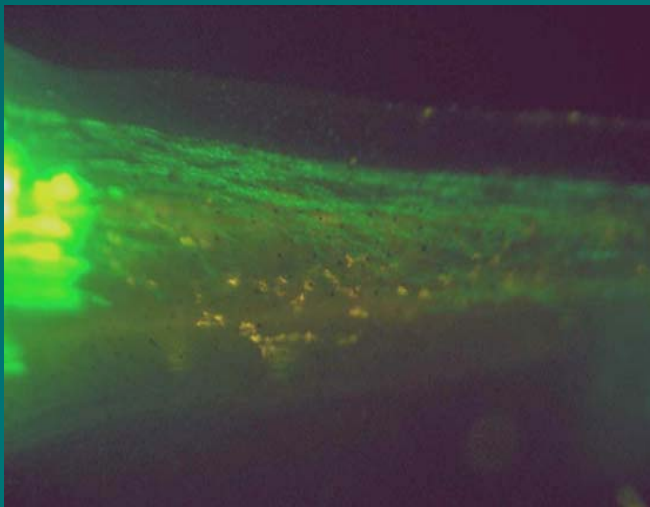
Dors Lat PSM st. 13



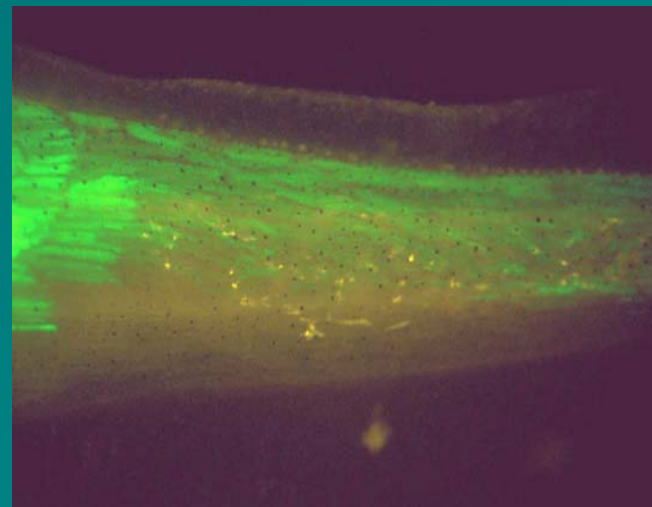
3 d. post-amputation



5 d. post-amputation

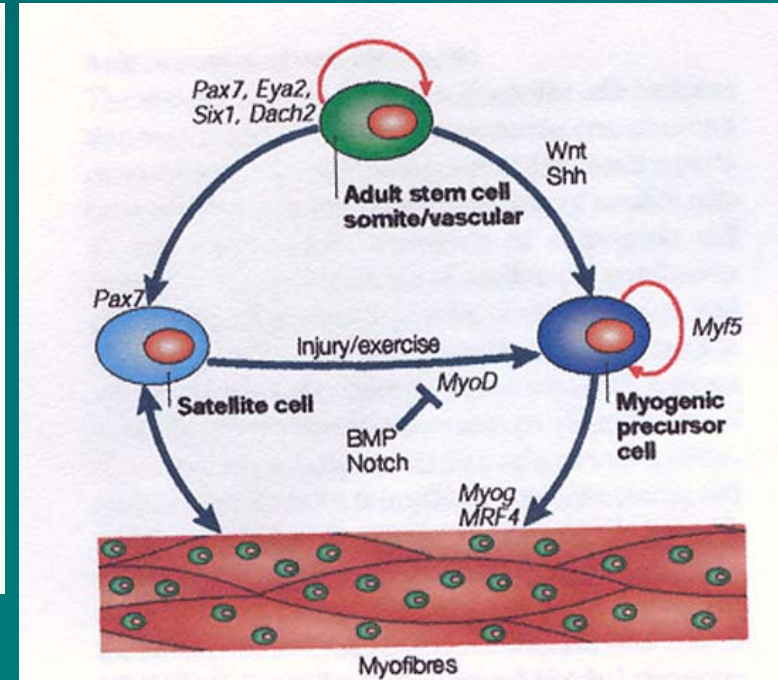
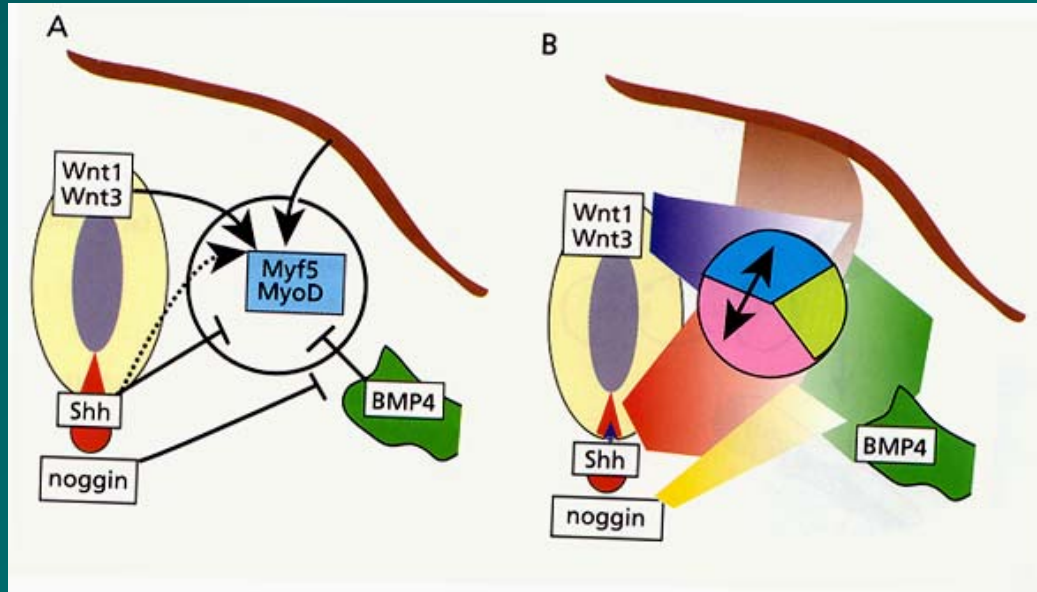


10 d. post-amputation



20 d. post-amputation

Muscle Development

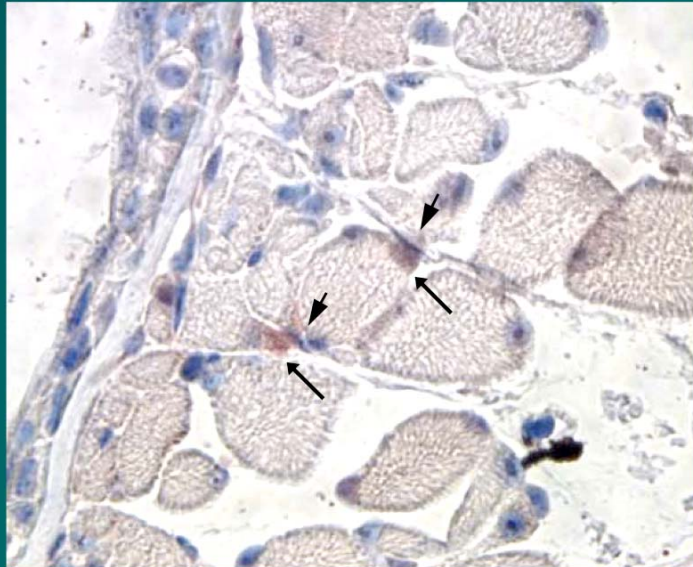


Myf5 + Pax3

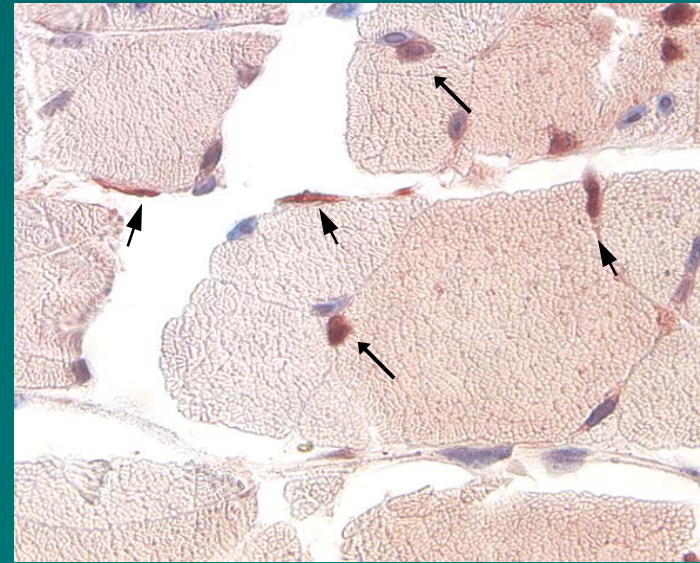
MyoD → myogenin / MRF4 → Muscles

Pax7 → Muscle Satellite Cells

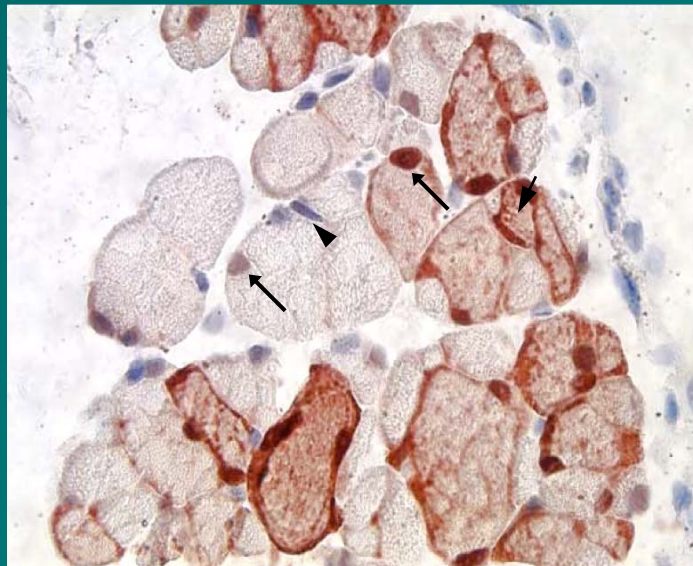
Muscle transversal sections



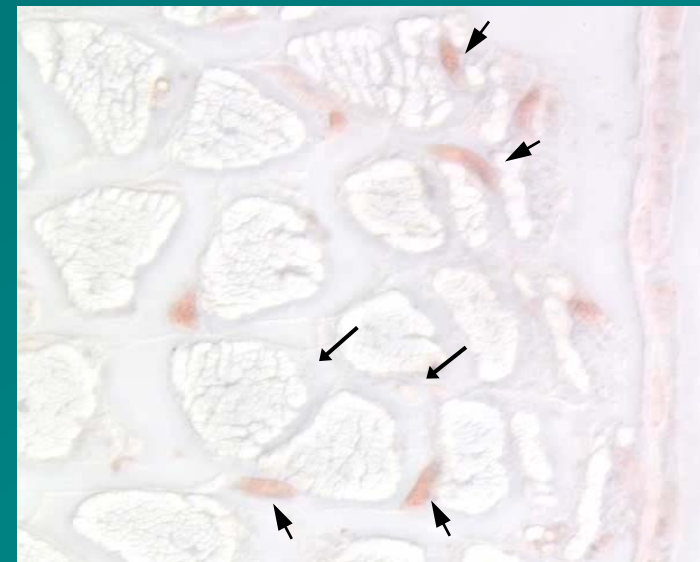
GFP Imm. Med PSM st. 13



GFP Imm. Med PSM st. 17

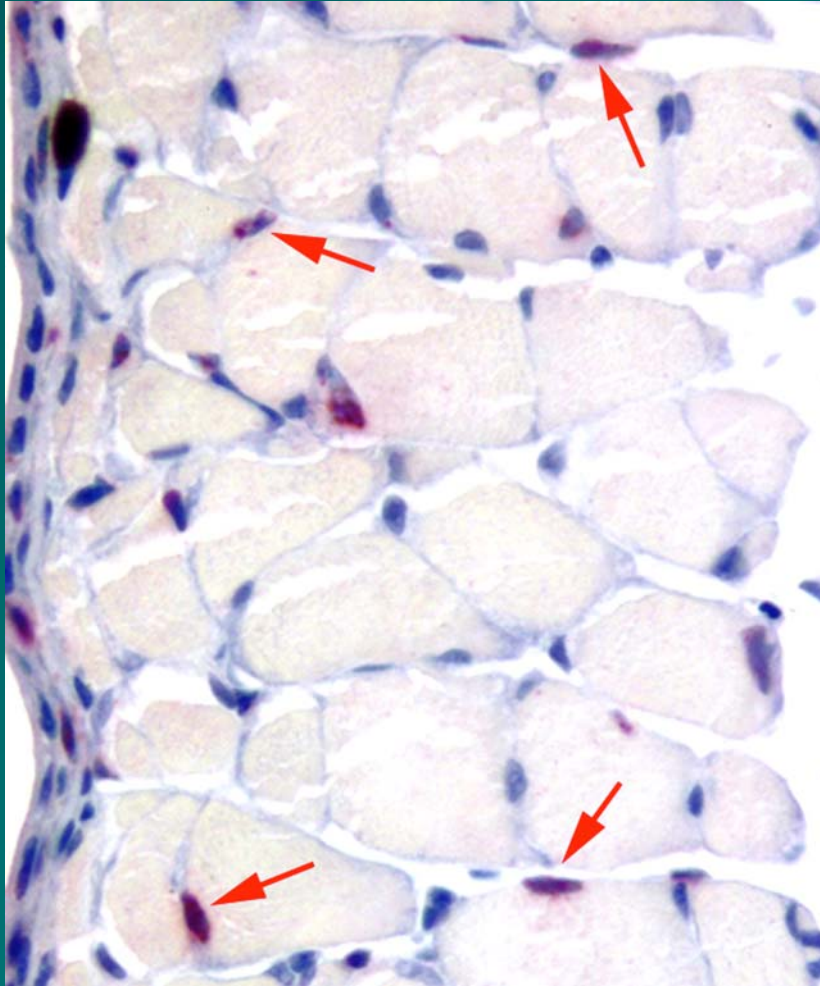


GFP Imm. Lat PSM st. 13

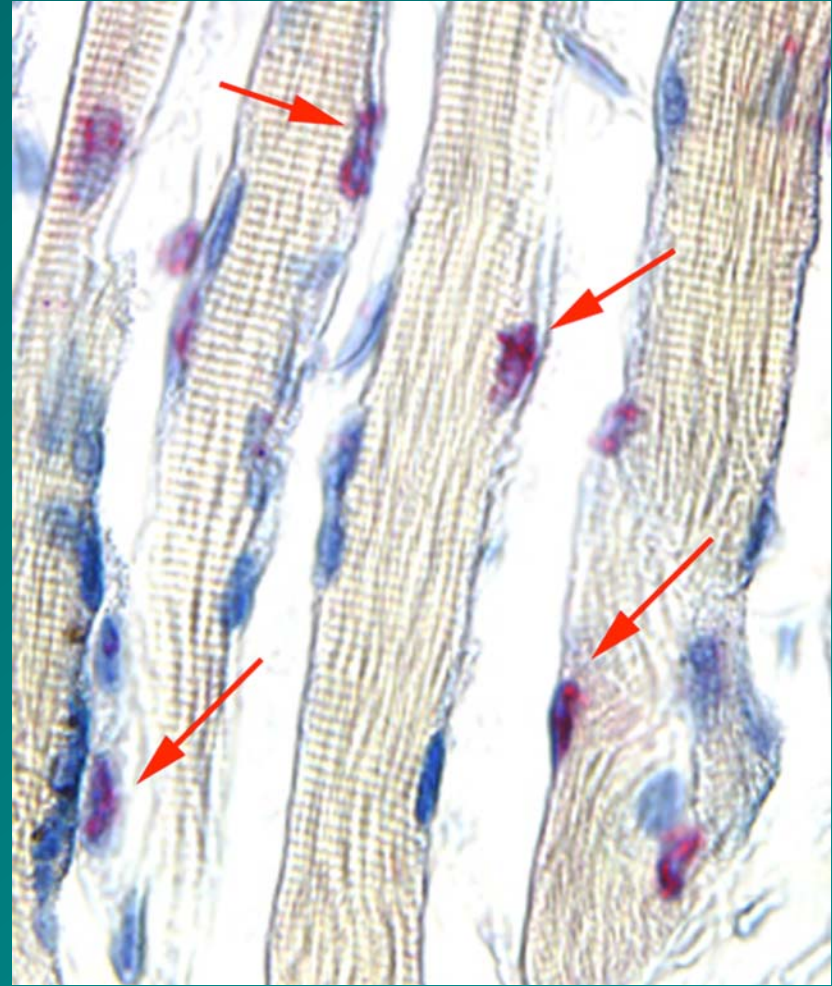


Pax7 Imm. Satellite cells

BrdU Immunostaining



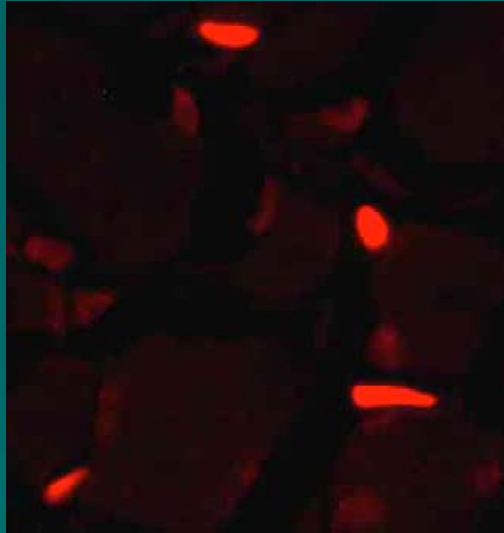
**Muscles trasversal
sections**



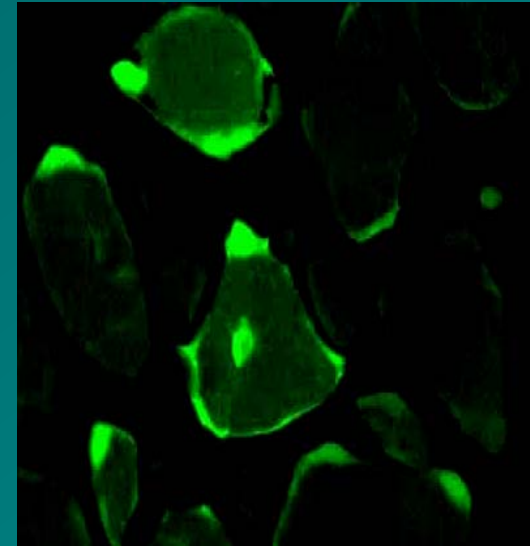
**Muscles longitudinal
sections**

Transversal muscle Immunofluorescence

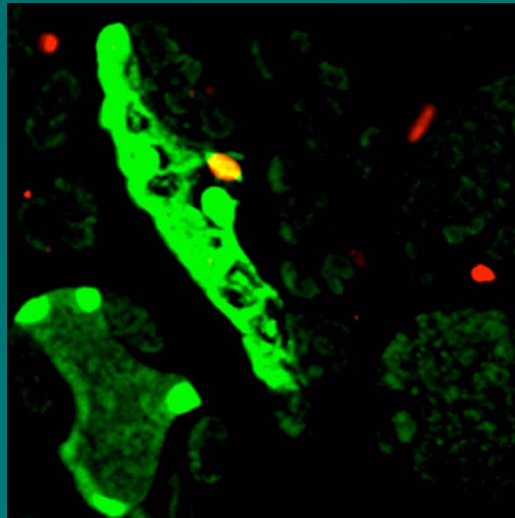
Pax7 Imm.



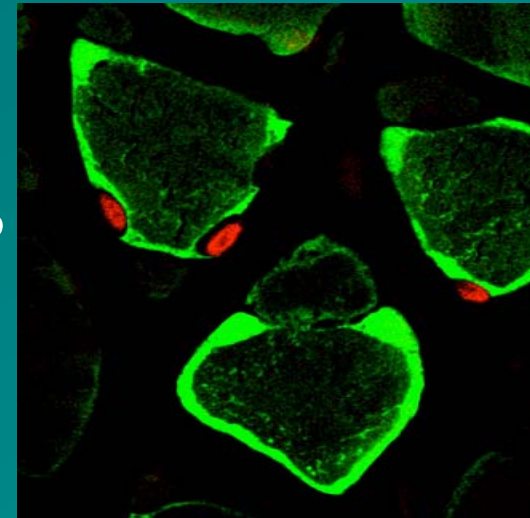
GFP Imm.

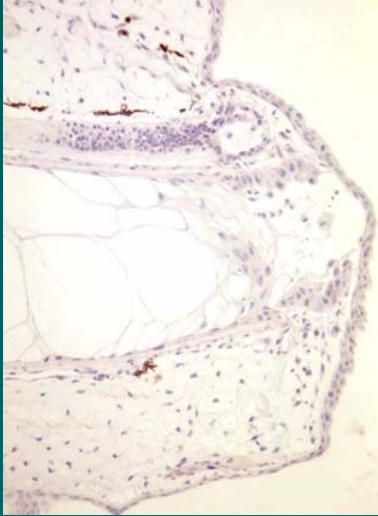


Pax7 + GFP
Imm.

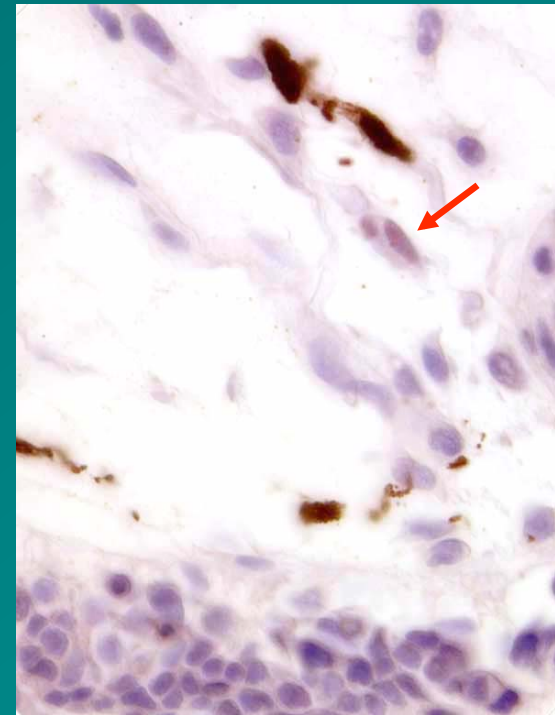
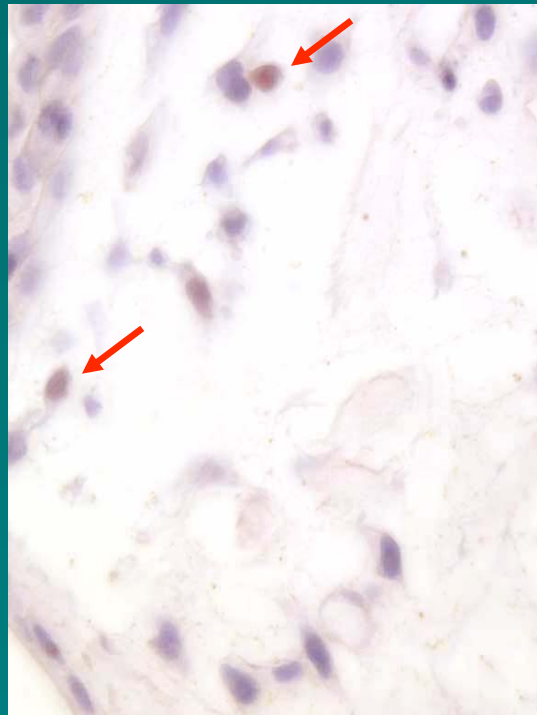
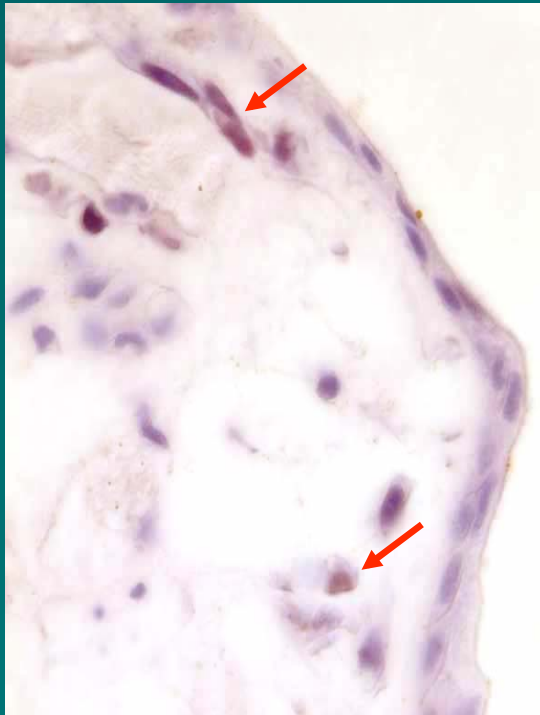


Pax7 + GFP
Imm.





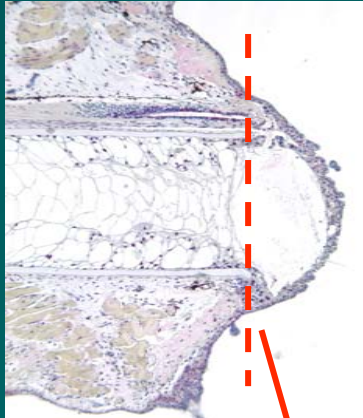
3 d. Pax7 positive blastemal cells



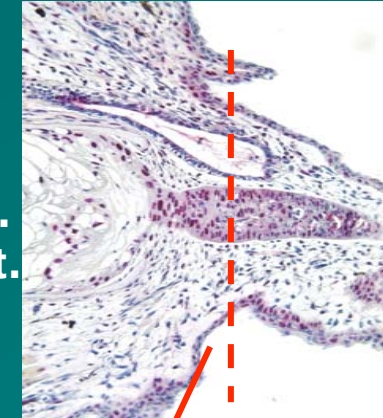
Summary

- **Host tadpoles are able to regenerate a complete and functional tail like the original**
- **Each tissue behaves independently: Notochord and Spinal Cord regenerate from the same original tissue**
- **During normal tail regeneration there is no Metaplasia**
- **The regenerating muscle fibers do not come from the stump muscle but from muscle satellite cells as the mammals. The early dorsal PSM does not have the competence to give rise to satellite cells as lateral and late PSM.**

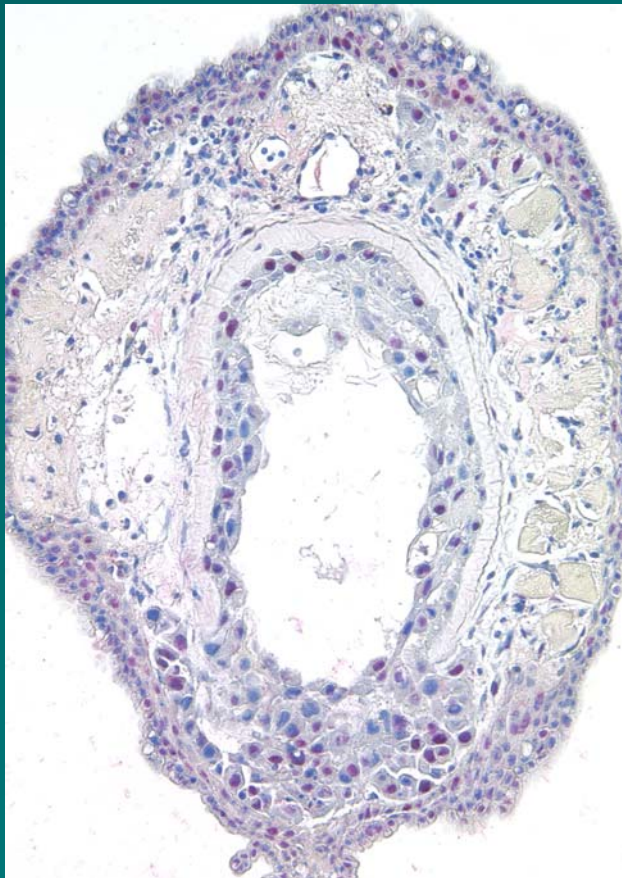
Immunostaining anti PCNA



2 d. p.amp.
Longit.sect.



5 d. p.amp.
Longit.sect.



Transverse
section

