Intestinal regeneration in the echinoderm *Holothuria glaberrima*

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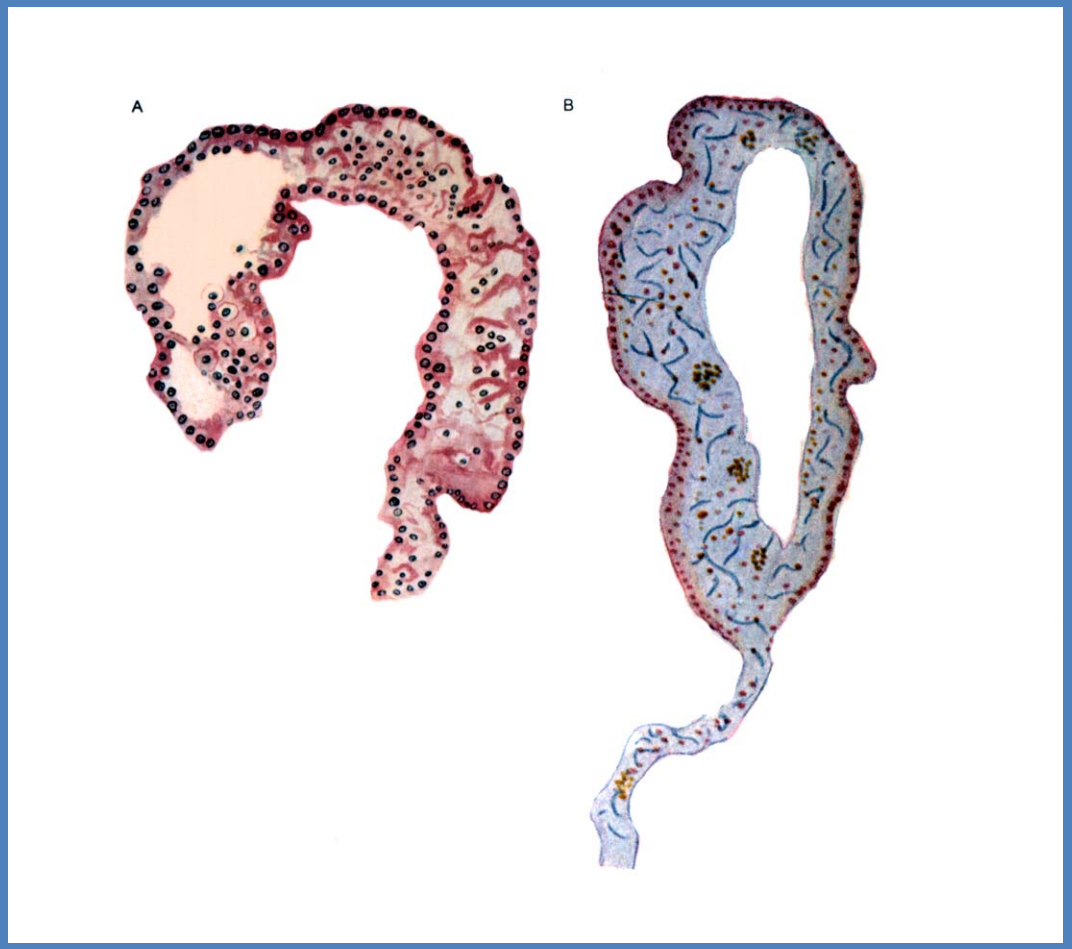
What are the ADVANTAGES and PITFALLS of the sea cucumber model system?



Bertolini 1932

The first studies on sea cucumber gut regeneration were done in the early 1900s by Fausta Bertolini at the Stazione Zoologica

Anton Dohrn in Naples

What are the ADVANTAGES and PITFALLS of the sea cucumber model system?

Funded by- NSF-IBN, NIH- NIGMS and NINDS, Whitehall Foundation and the University of Puerto Rico

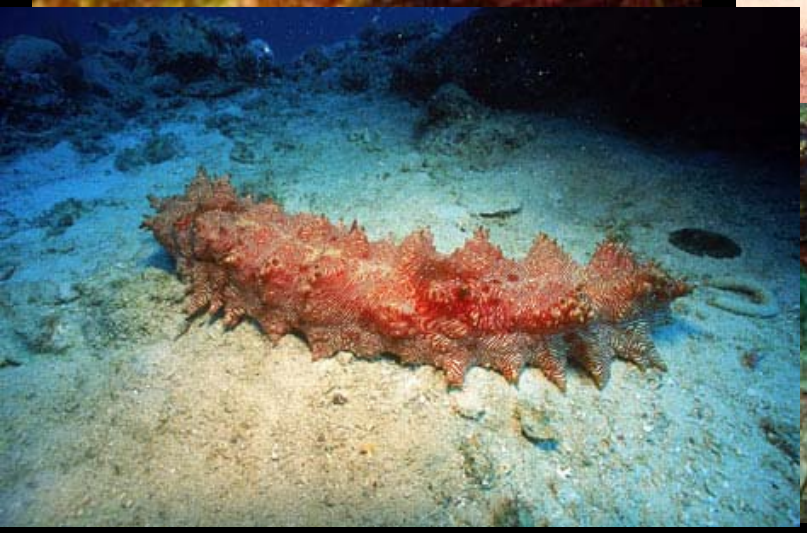
What are the ADVANTAGES and CHALLENGES of the sea cucumber model system?

Funded by- NSF-IBN, NIH- NIGMS and NINDS, Whitehall Foundation and the University of Puerto Rico

Advantage #1- Hundreds of species available for studies







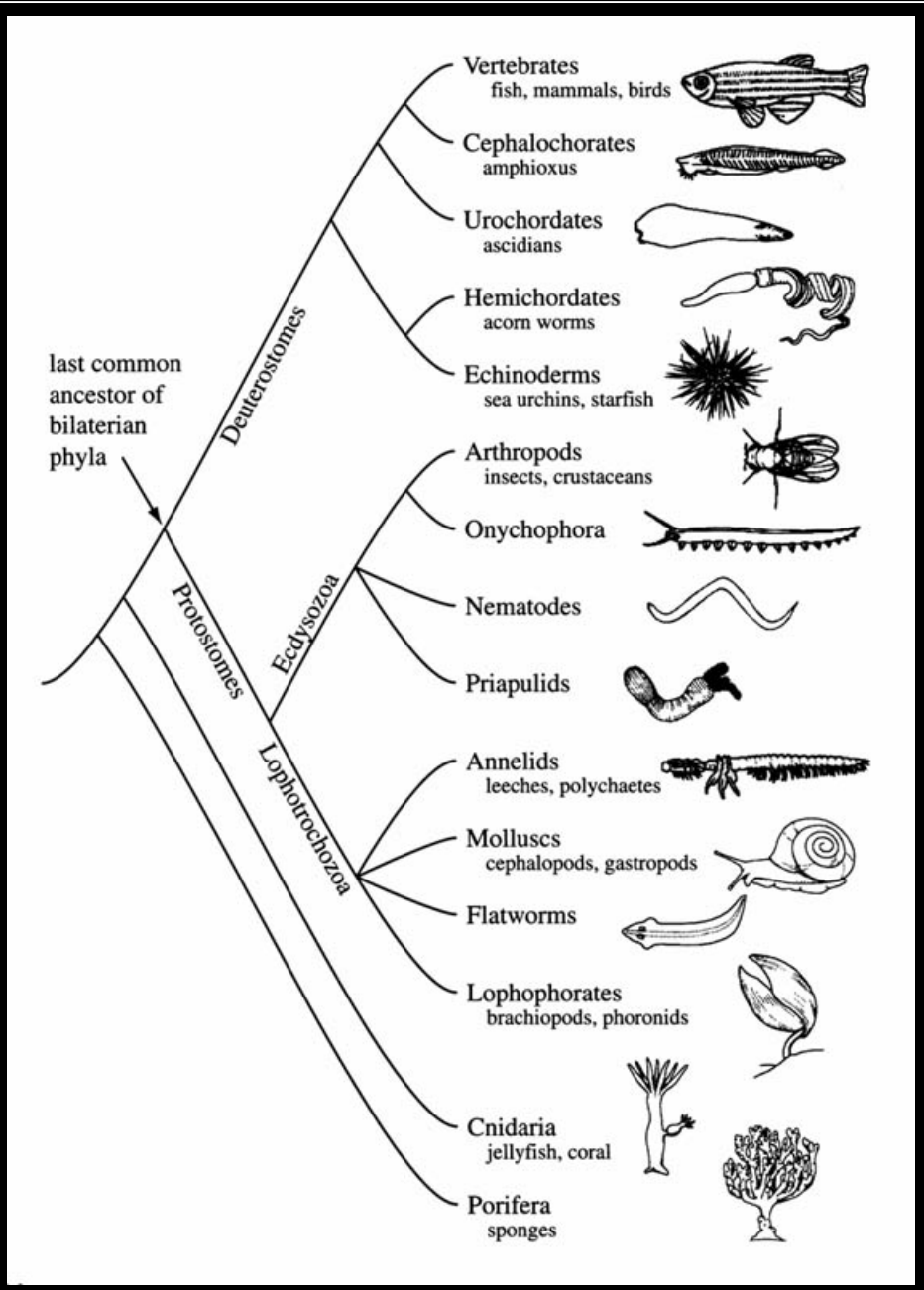
Experimental Model

the sea cucumber, *Holothuria glaberrima*

Athyonidium chilensis

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Advantage #2- Key phylogenetic position

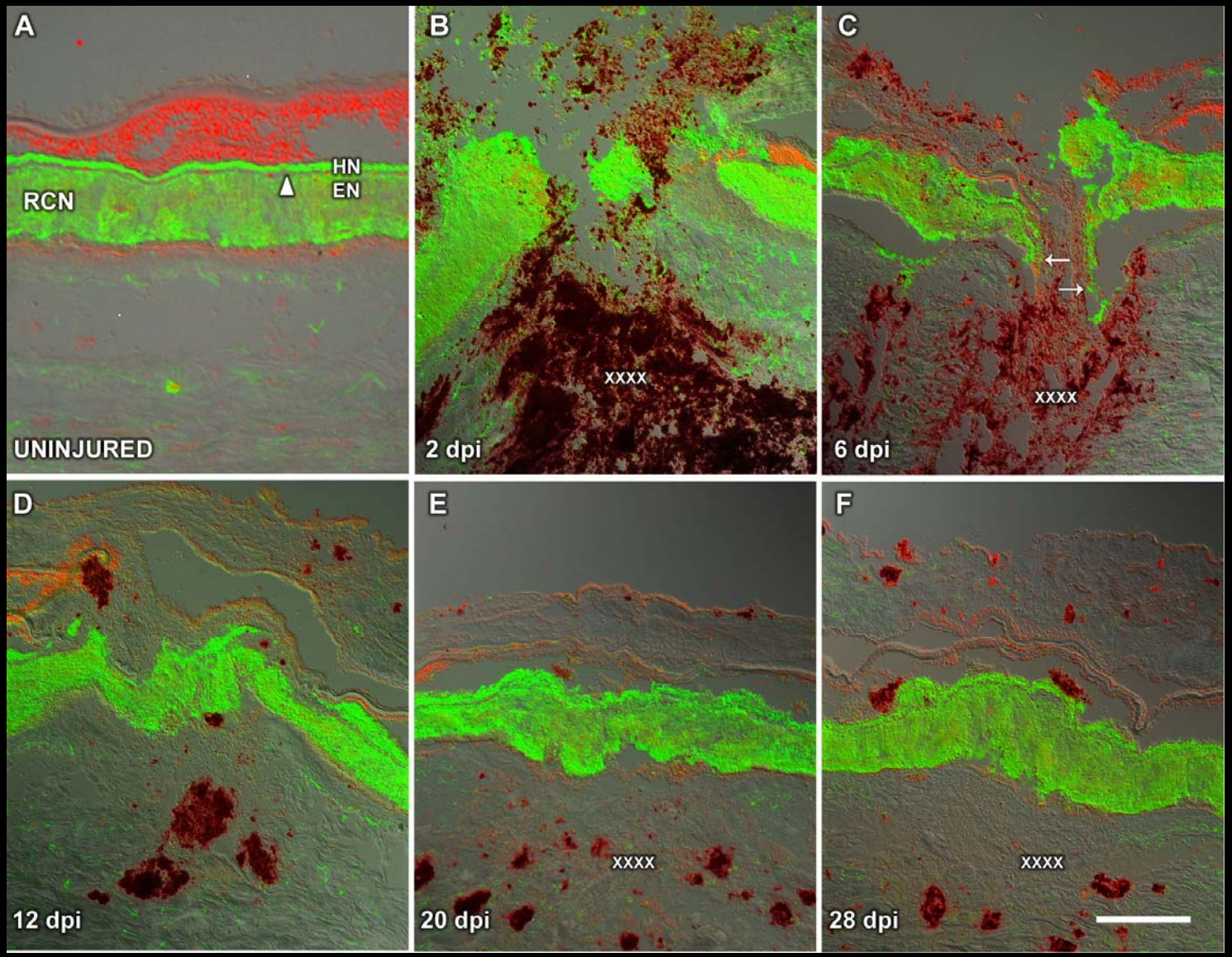


Echinoderms

Sea cucumbers are deuterostomes

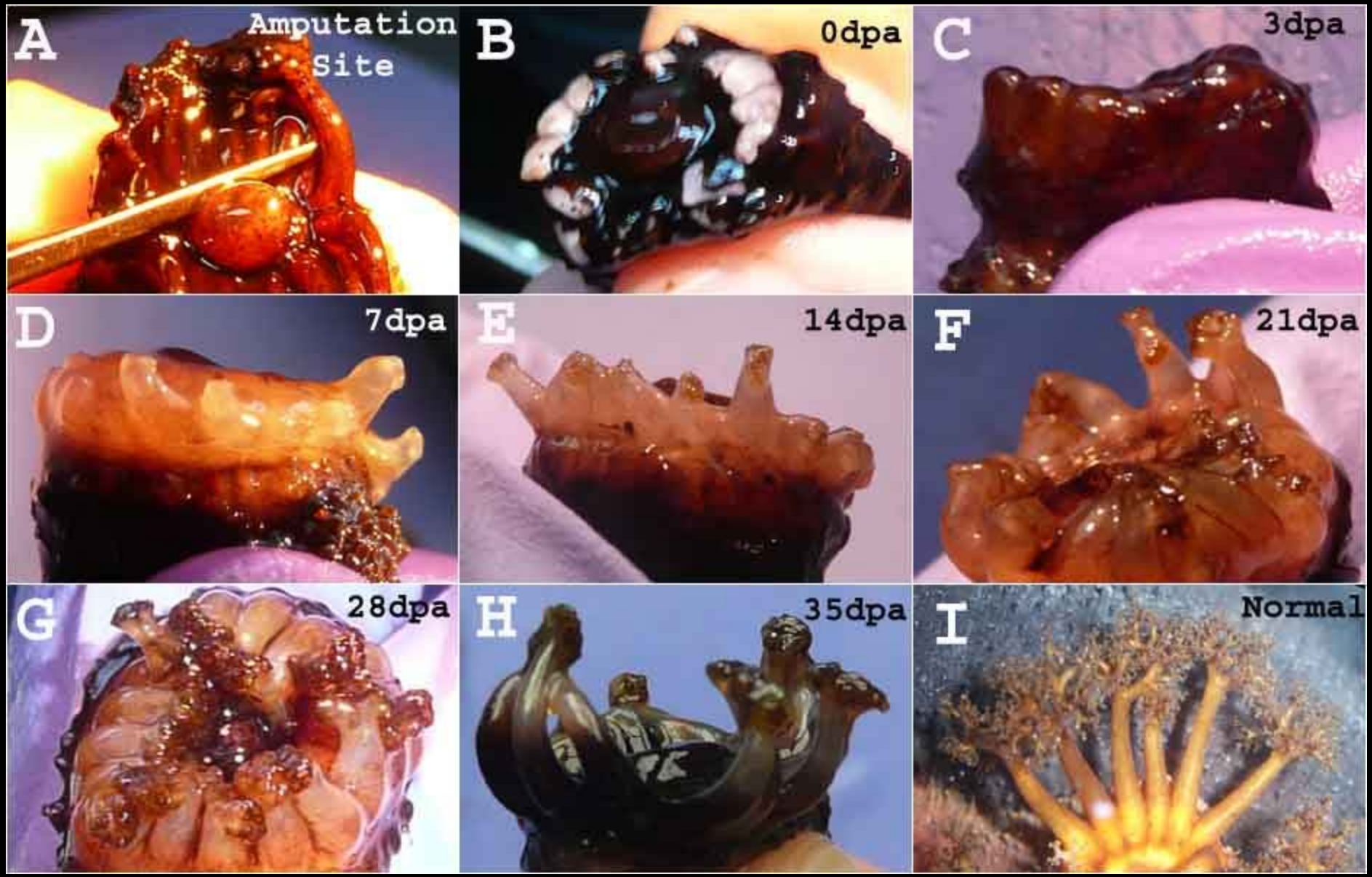
Advantage #3 - Extraordinary regenerative properties

Nerve fiber regeneration



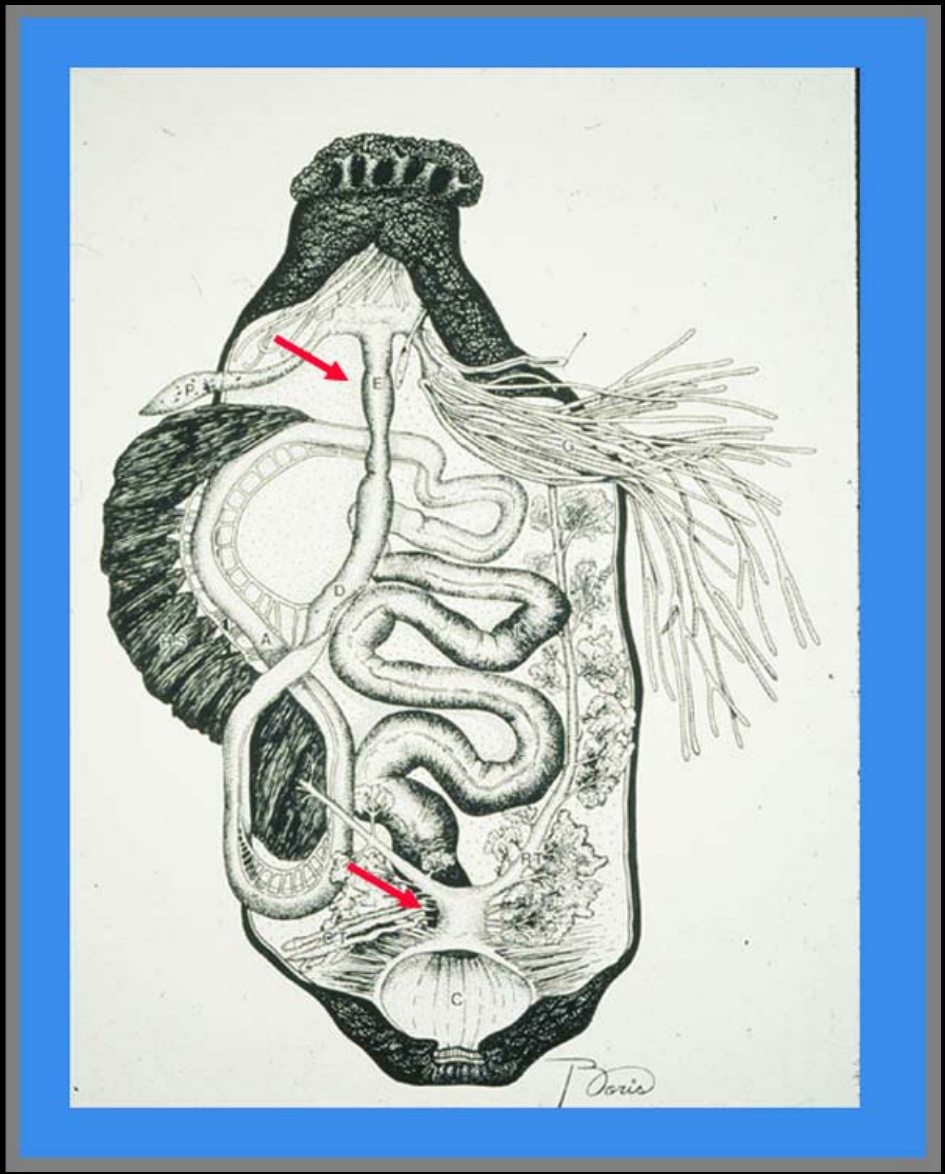
San Miguel-Ruiz et al. BMC Dev Biol 2009

Tentacle regeneration



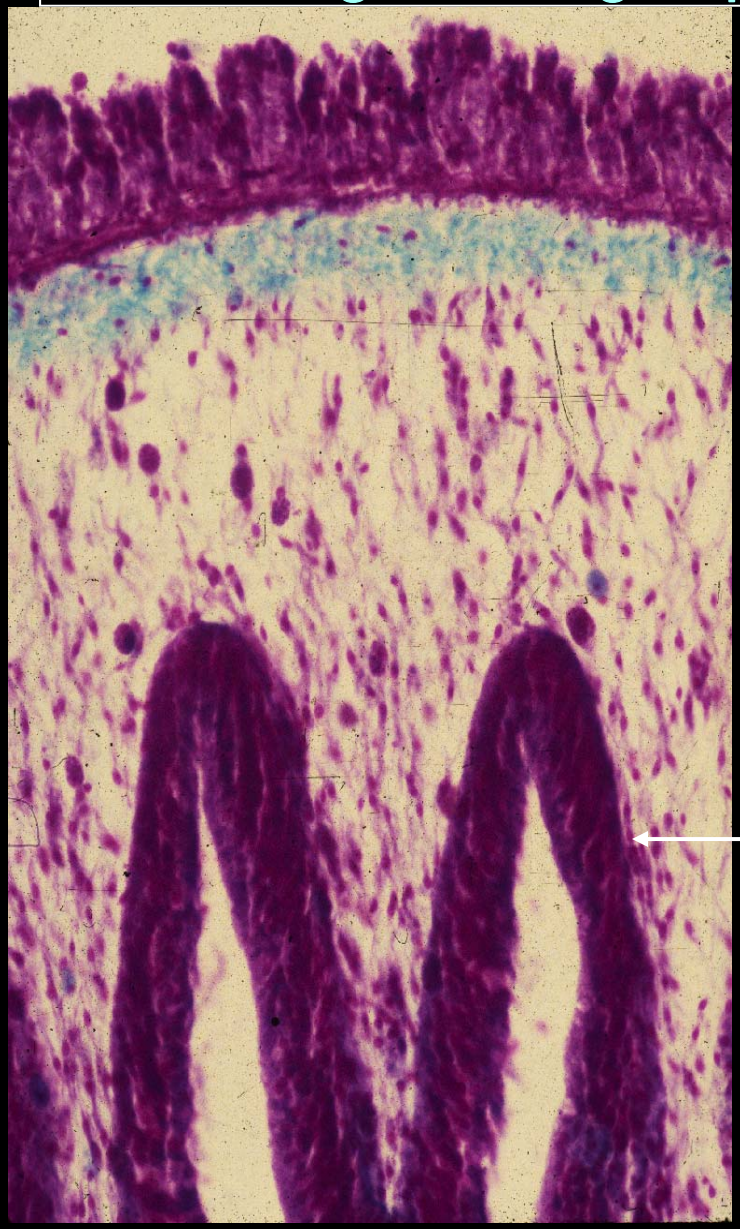
Advantage #4 - Evisceration is easily induced in lab

Advantage #5 - Evisceration follows a fixed pattern, reducing variability due to surgical manipulations

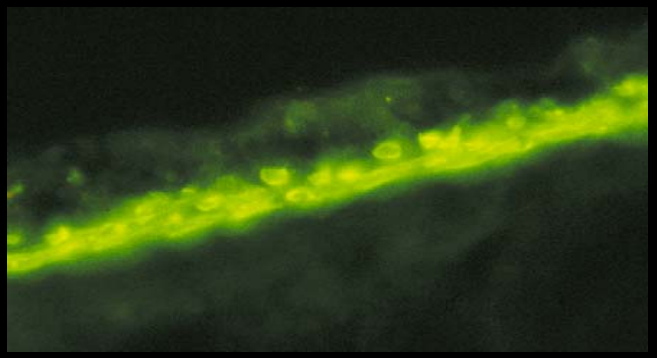


Evisceration eliminates most of the organs of the sea cucumber. In *H. glaberrima* only the left respiratory tree remains.

Advantage #6 - The digestive tract is well conserved among animal groups, particularly in deuterostomes



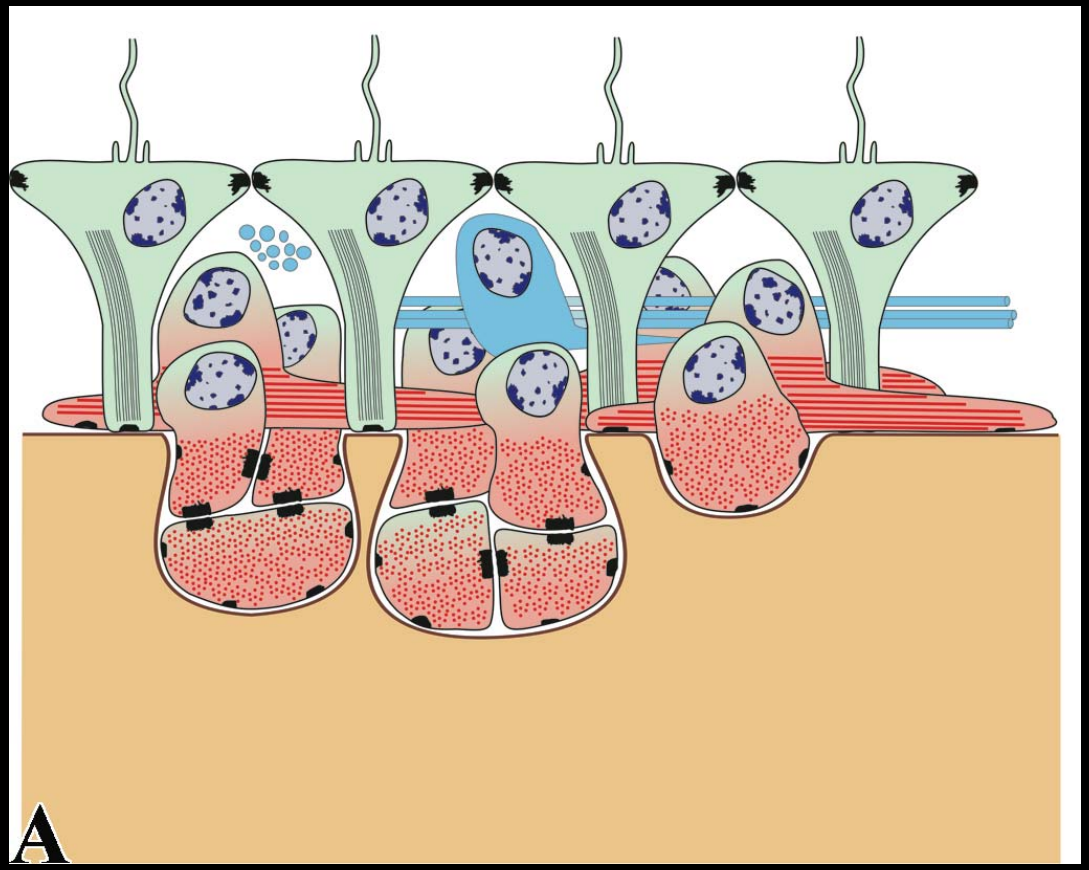
Mesothelium (includes coelomic epithelium or serosa and muscle layer)



Connective tissue layer (submucosa)

Luminal epithelium (mucosa)

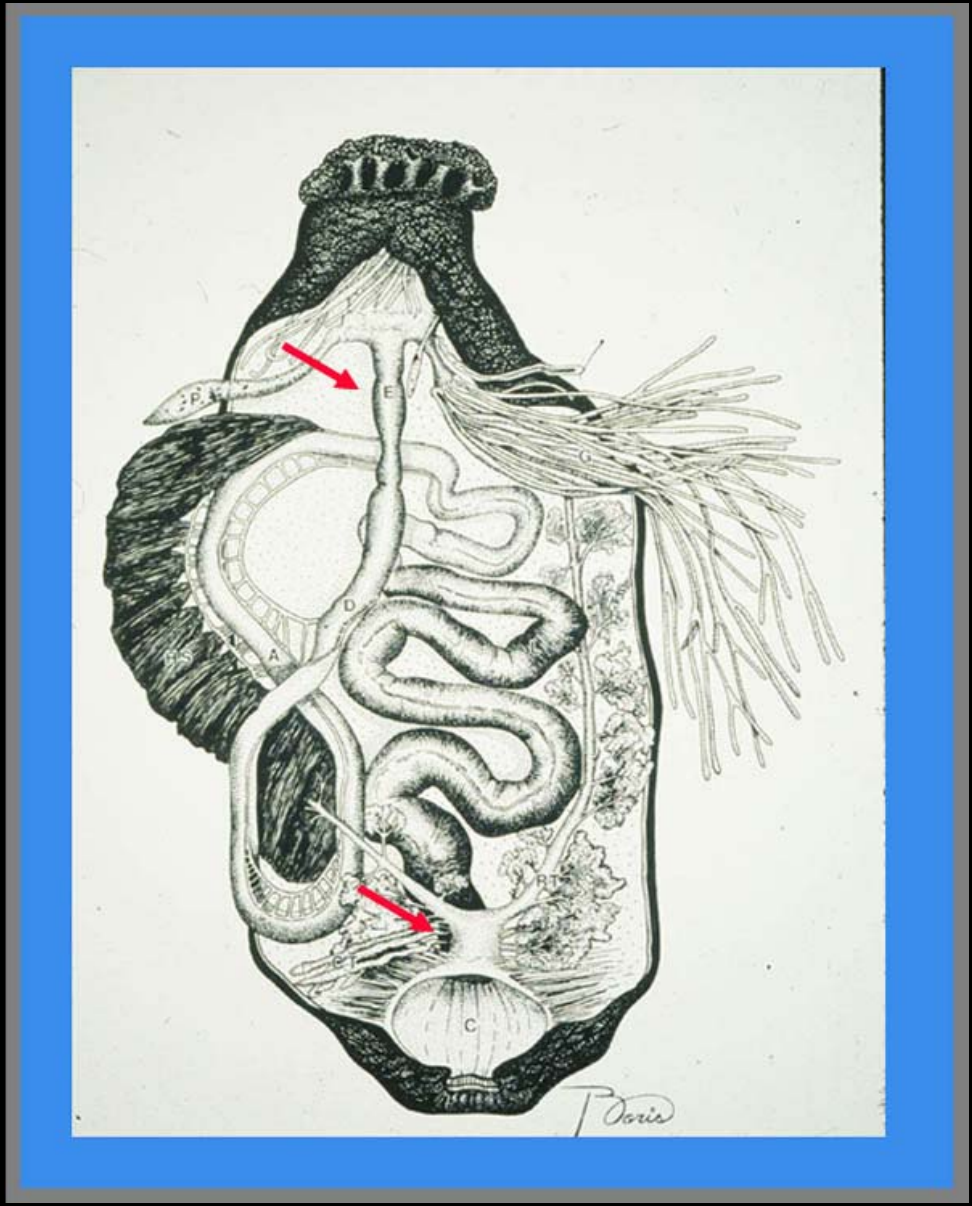
Echinoderm mesothelium

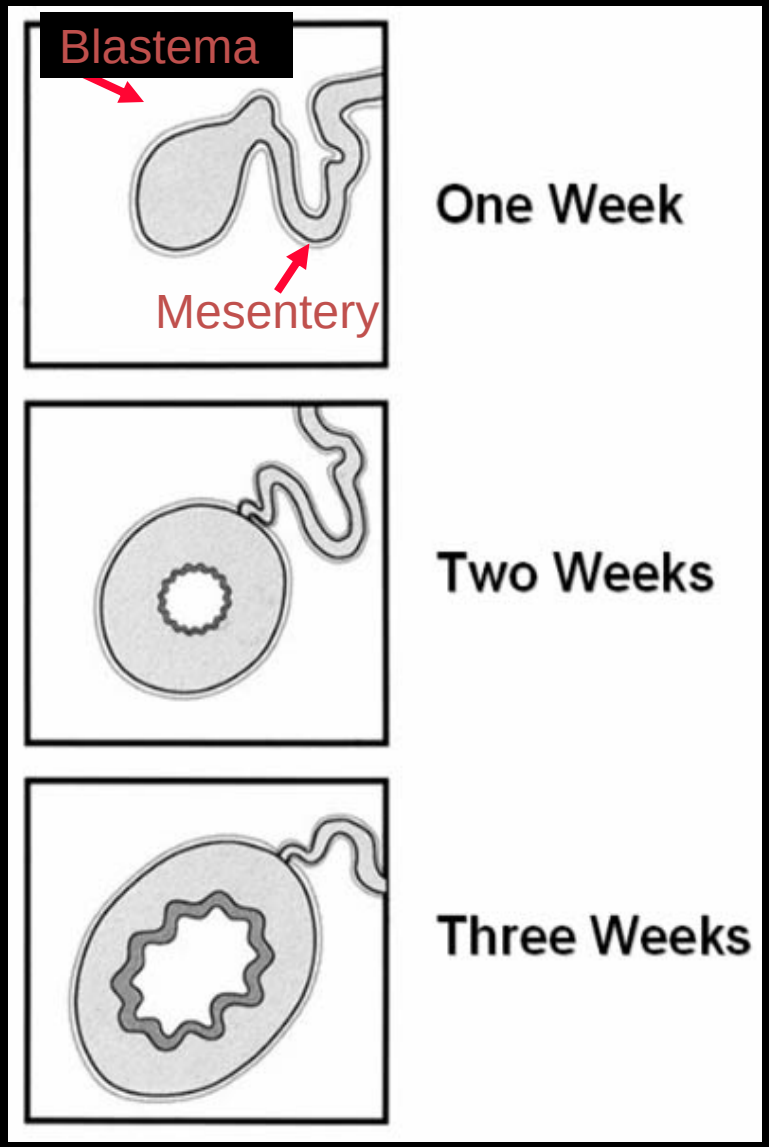


Peritoneocytes

Myocytes

Advantage #7 - Regeneration of a functional organ occurs within a month



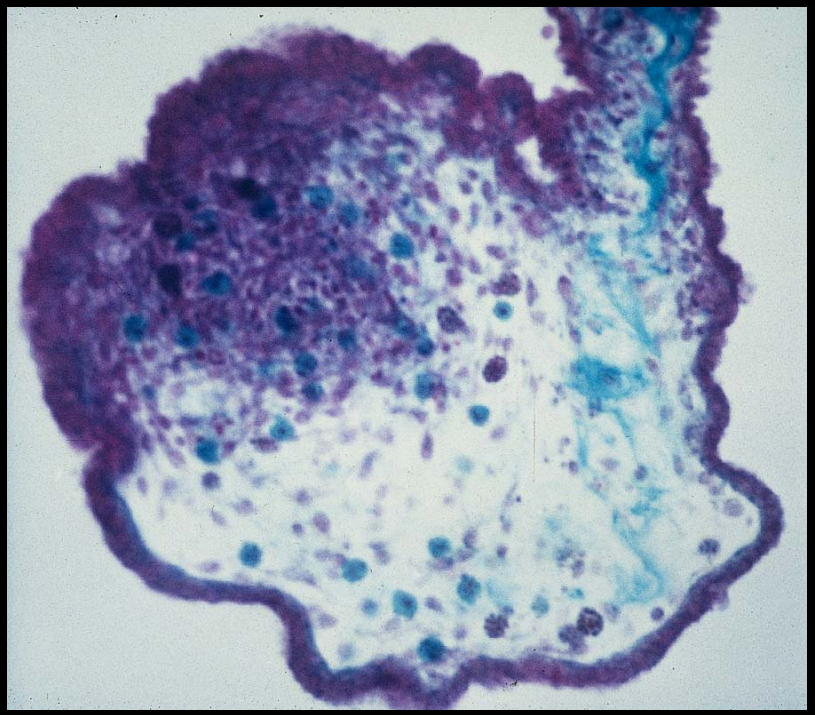


Garcia-Arraras et al. J Exp Zool 1998

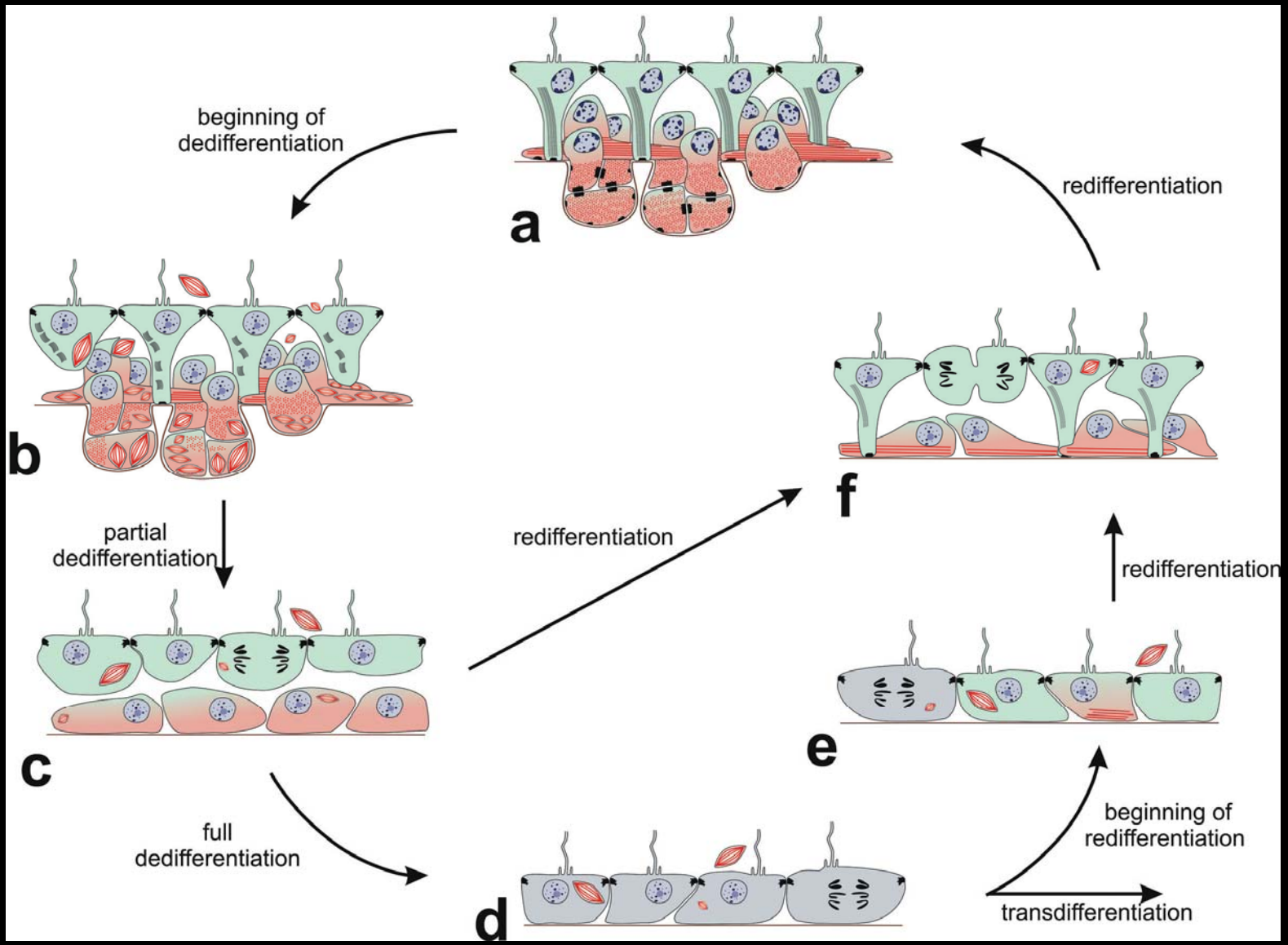
Advantage #8 - Multiple cellular events can be studied

Cellular events associated with the formation of a regeneration blastema include:

1. Cell dedifferentiation
2. Cell proliferation
3. Apoptosis
4. Epithelial to mesenchymal transition.
5. ECM remodeling
6. Cell migration
7. Cell differentiation
8. Cell-cell interactions to form a new organ

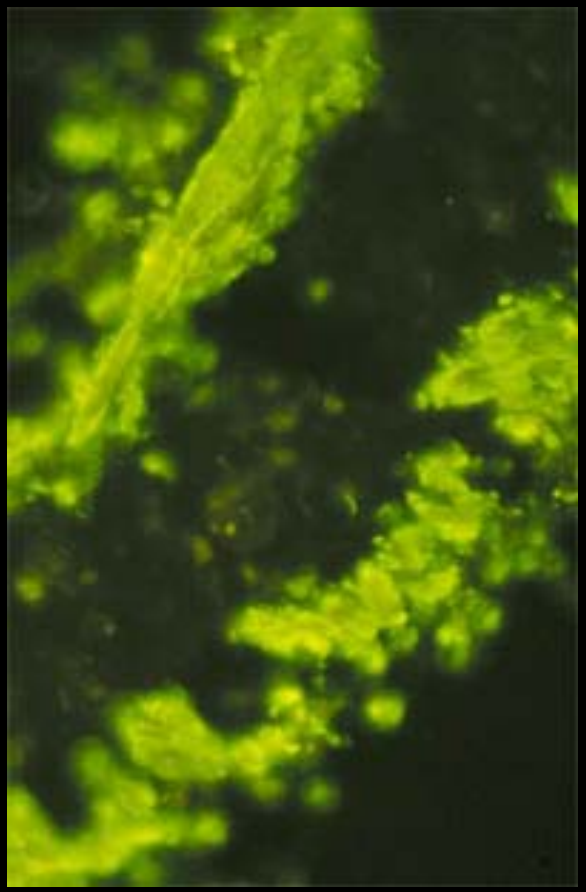


Cell Dedifferentiation

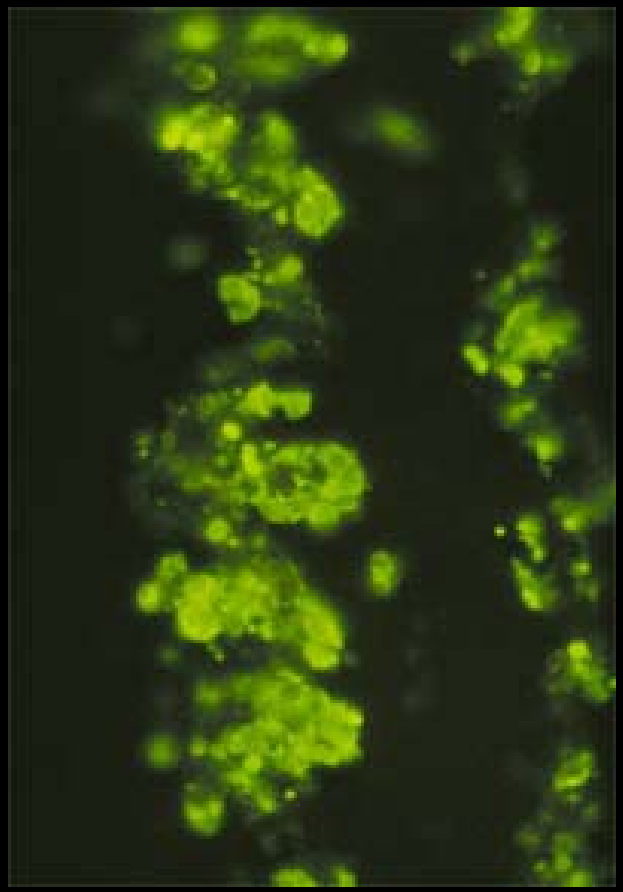


Garcia-Arraras and Dolmatov- Curr Pharm Design 2010

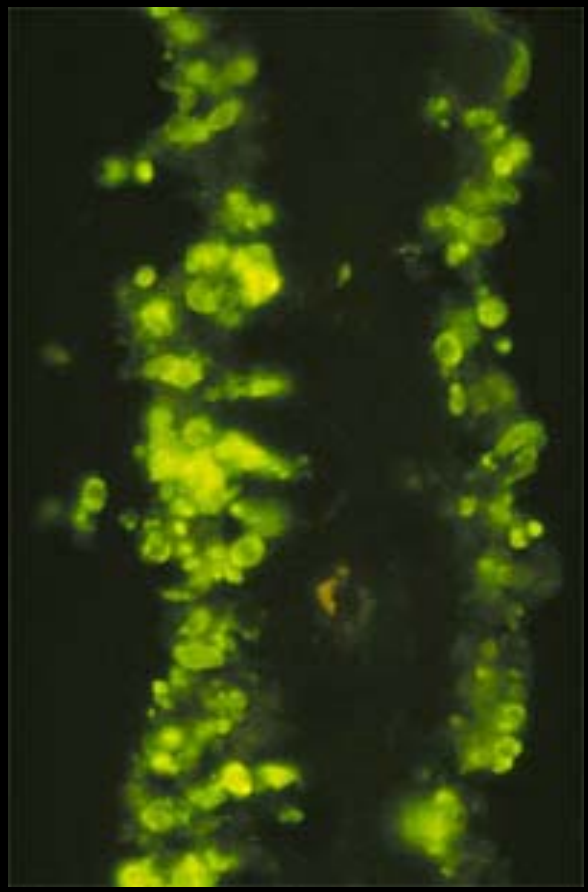
A gradient of muscle dedifferentiation can be found in the mesentery during regeneration



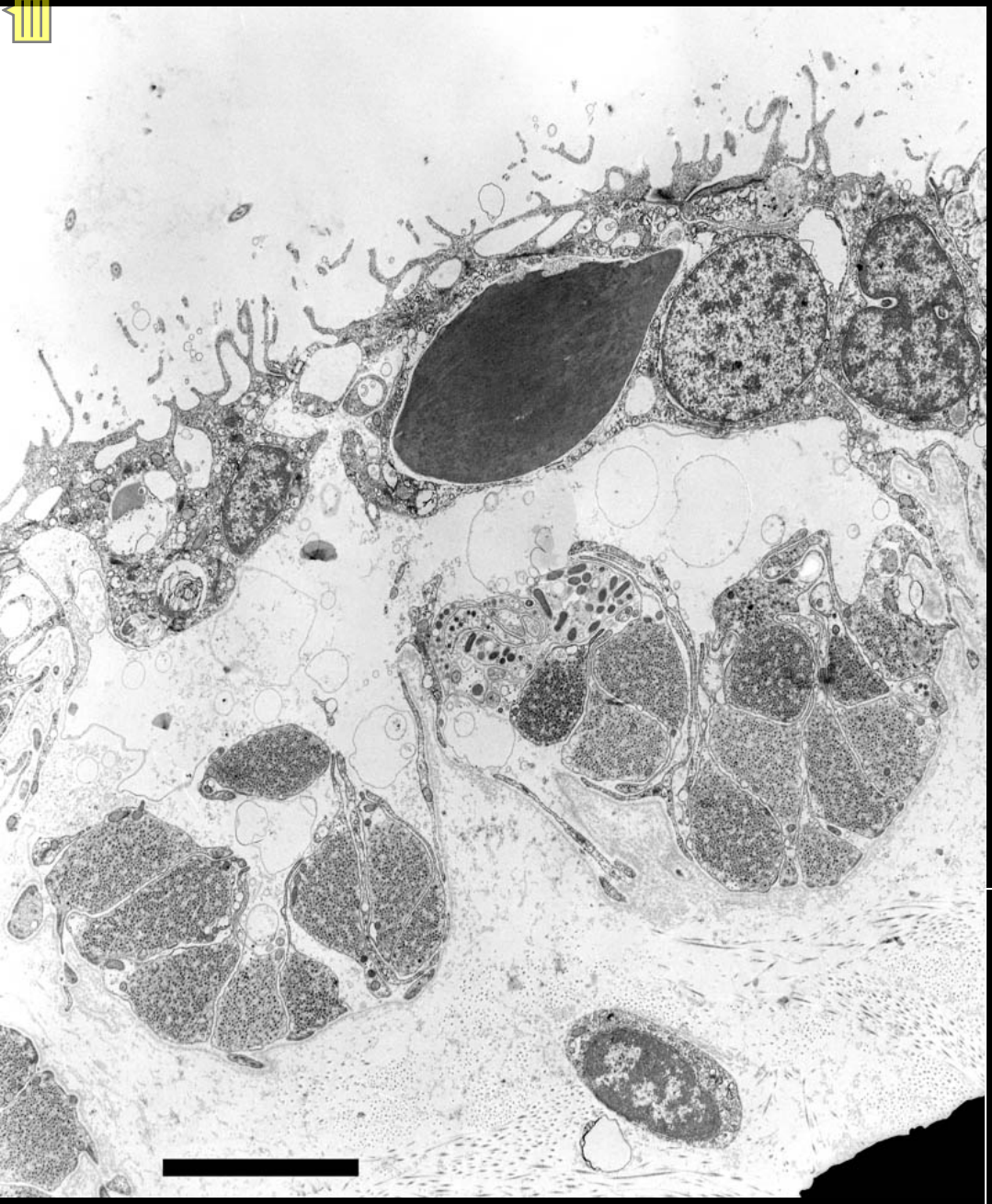
Near body wall

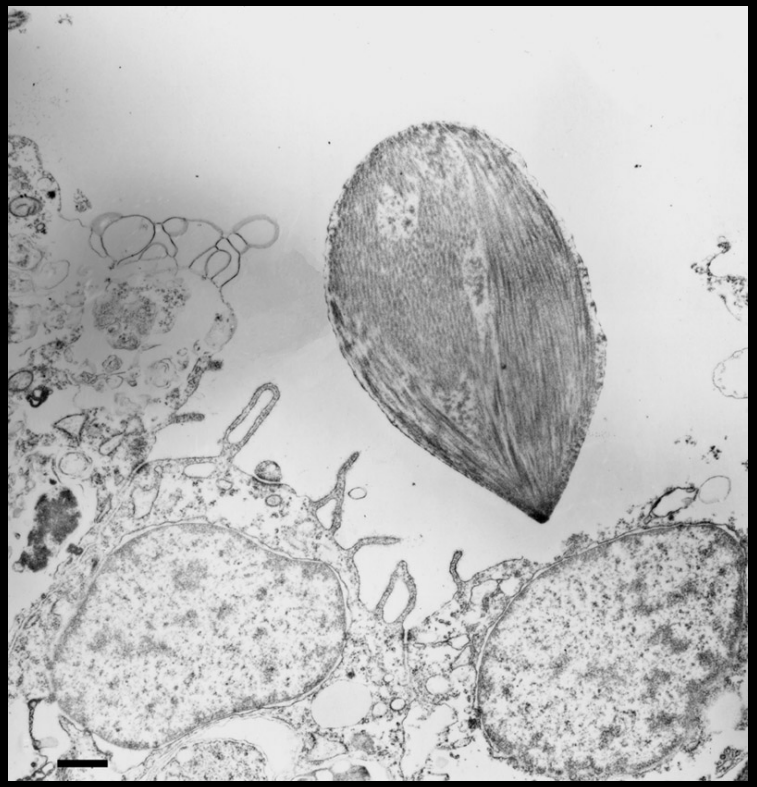


Medial



Near blastema-like structure

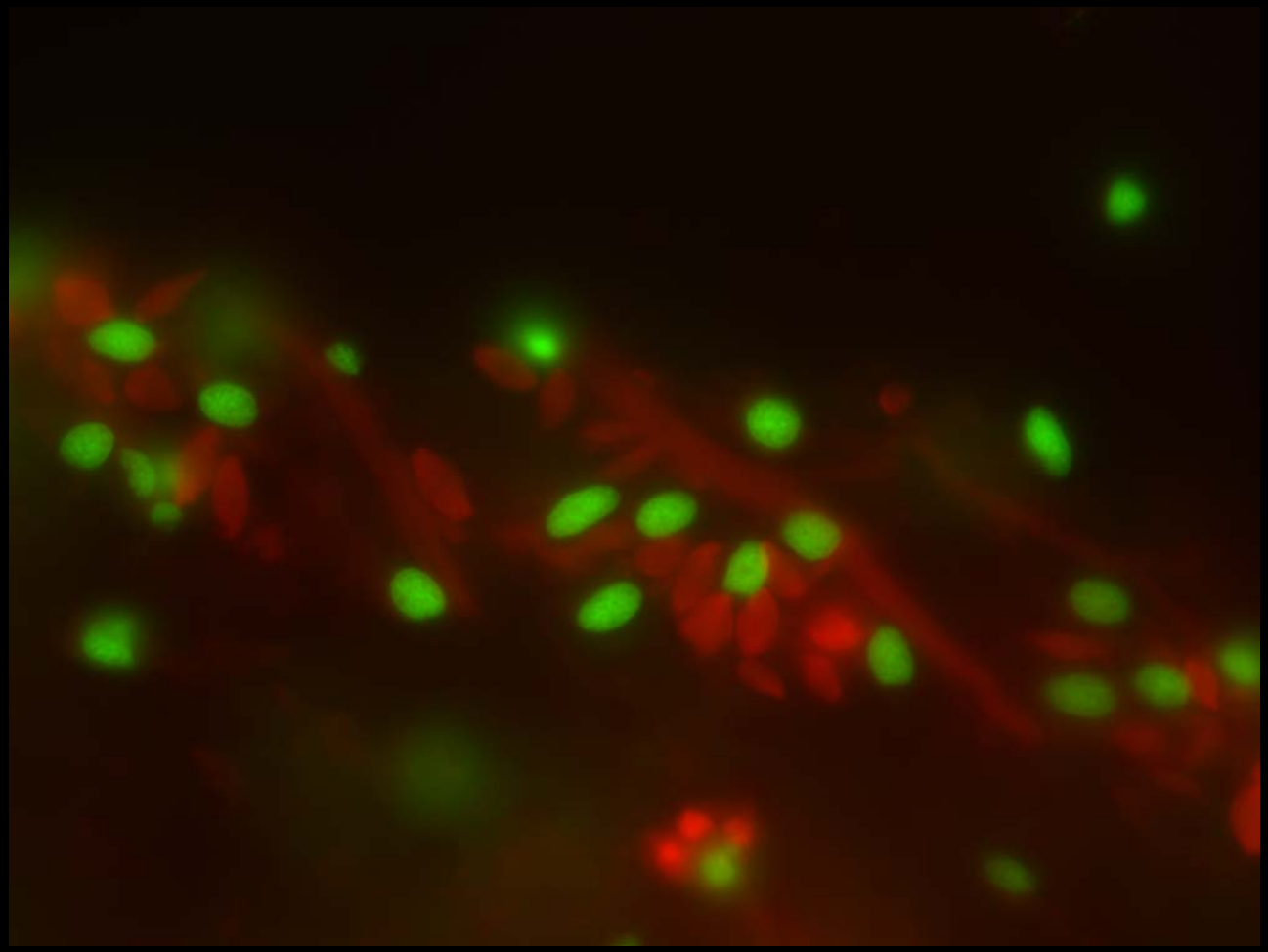




Muscle cell dedifferentiation involves the formation of spindle like structures

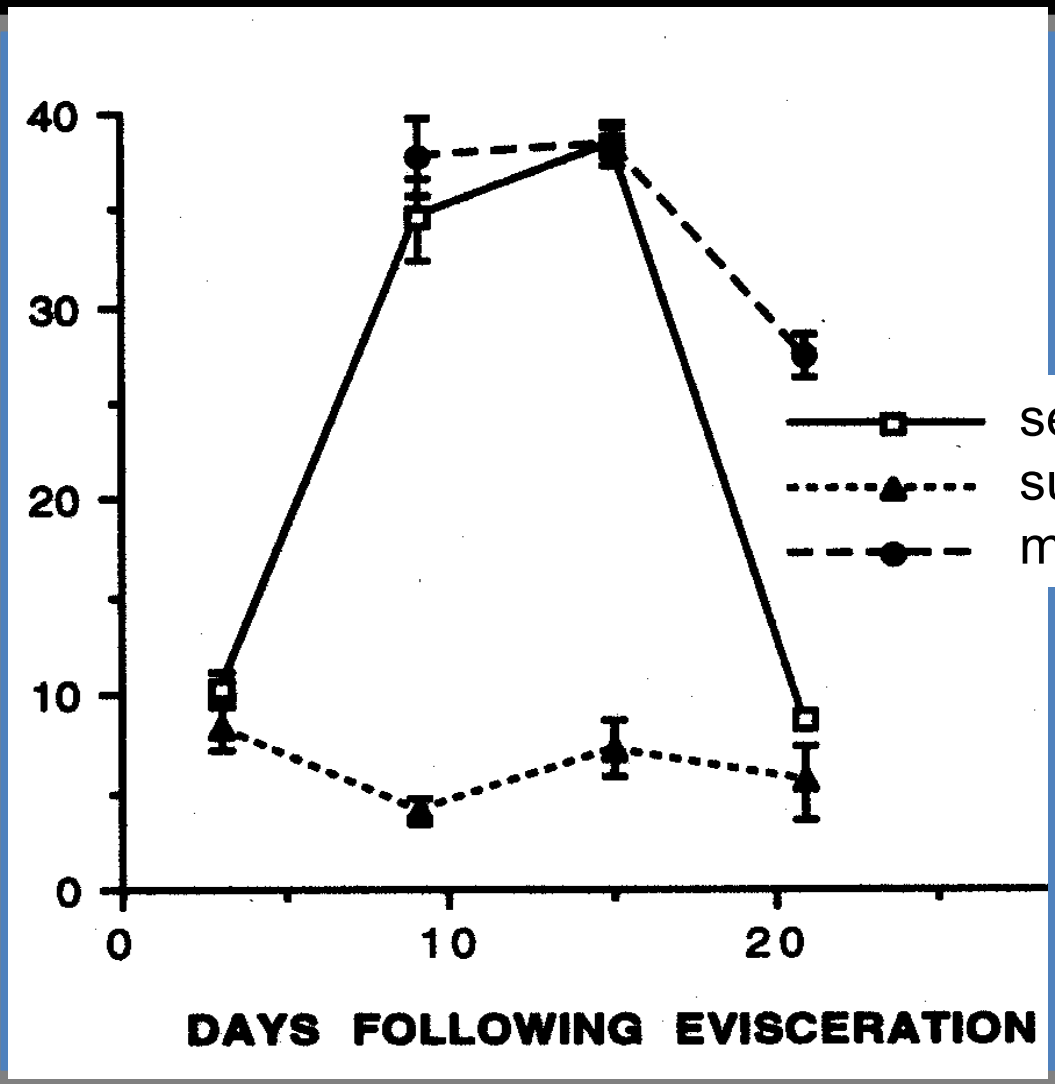
Candelaria et al. Cell Tissue Res 2006

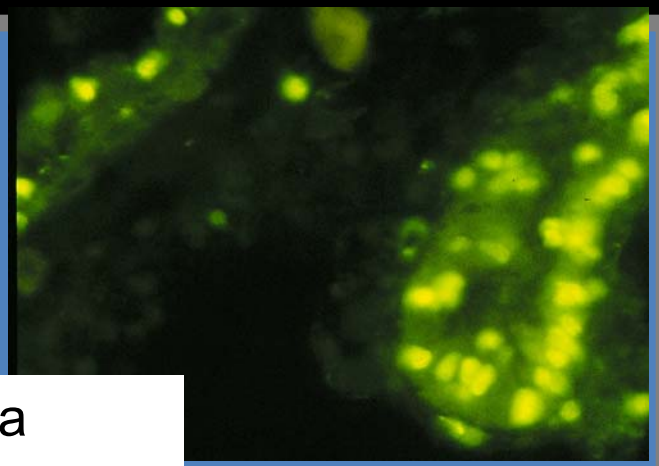
SLSs (red) are formed as cells dedifferentiate. These SLSs are not associated with cell nuclei (green)

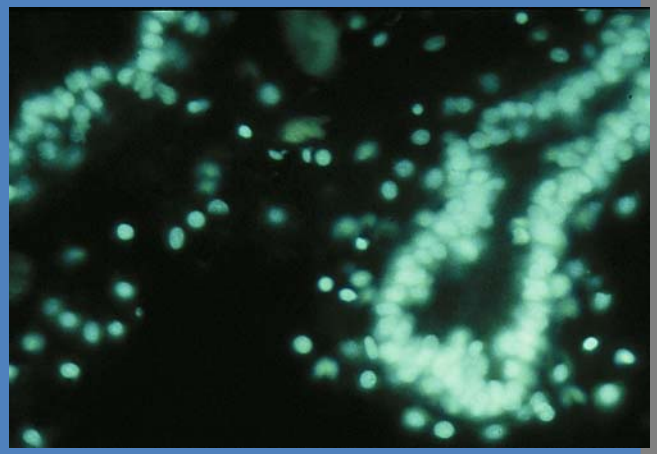


Cell Proliferation

Percentage cell division in specimens sacrificed 24 hrs following BrdU injection

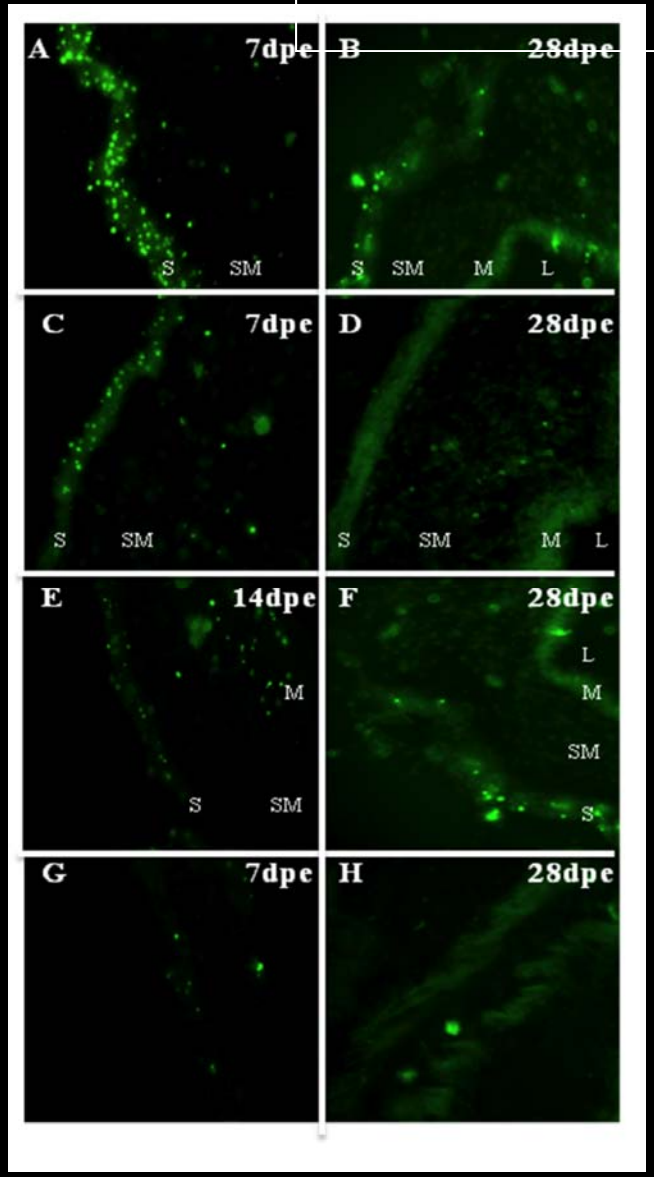


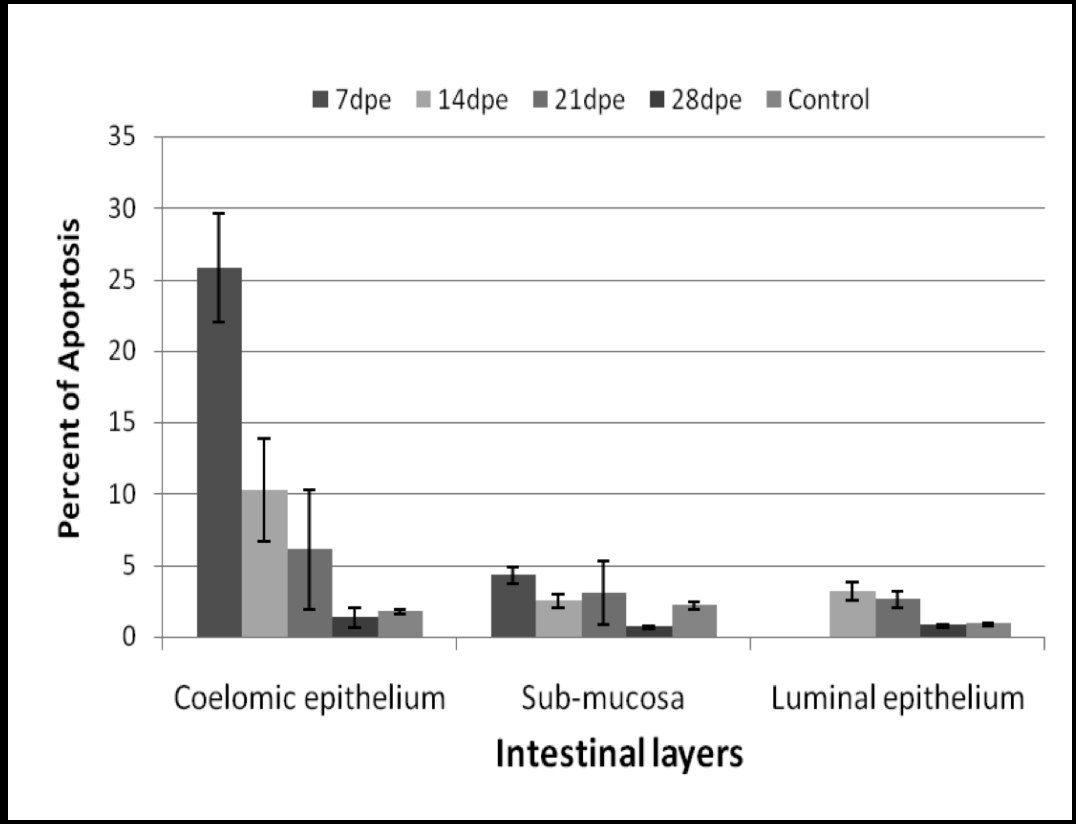




Garcia-Arraras et al. J Exp Zool 1998

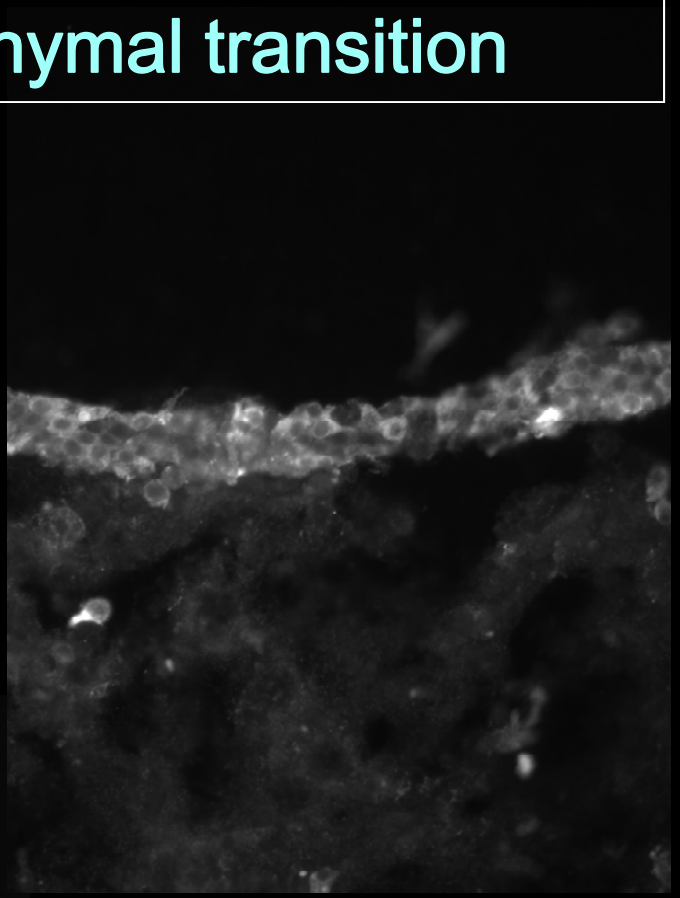
Apoptosis

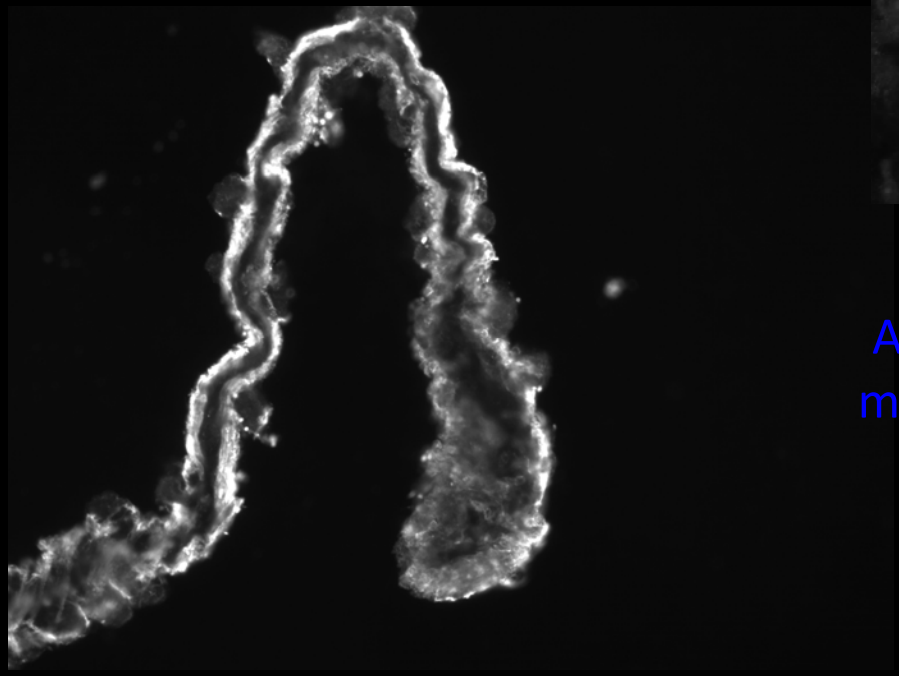




TUNEL assays show large number of apoptotic cells in the regenerating epithelia.

Epithelial to mesenchymal transition



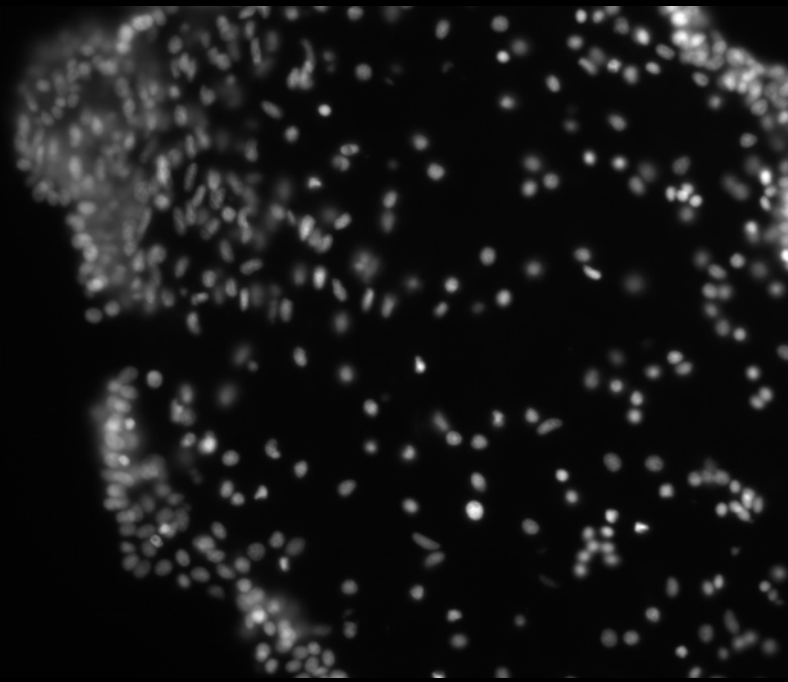


Antibody MES-1 recognizes the mesothelium and epithelial cells of the blastema.

Garcia-Arraras et al. In prep.

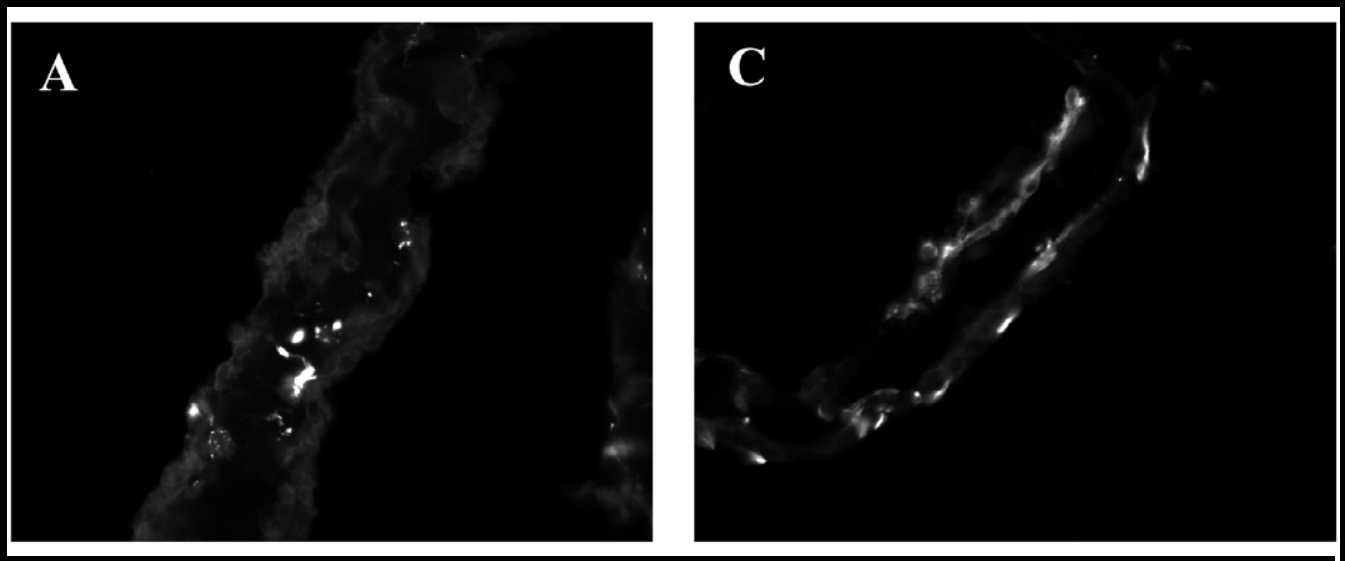
Epithelial cells at the tip of the regenerating mesentery ingress to form the underlying mesenchyme

MES-1 labeling



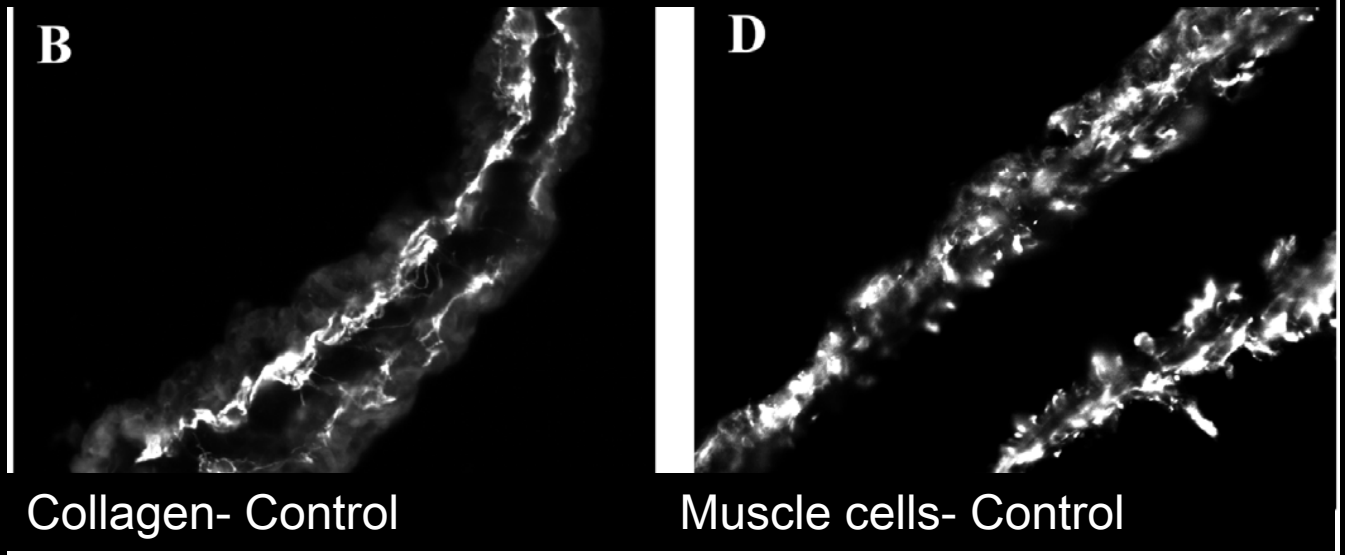
DAPI- nuclei staining

Extracellular matrix (ECM) remodeling



Collagen 7-d reg.

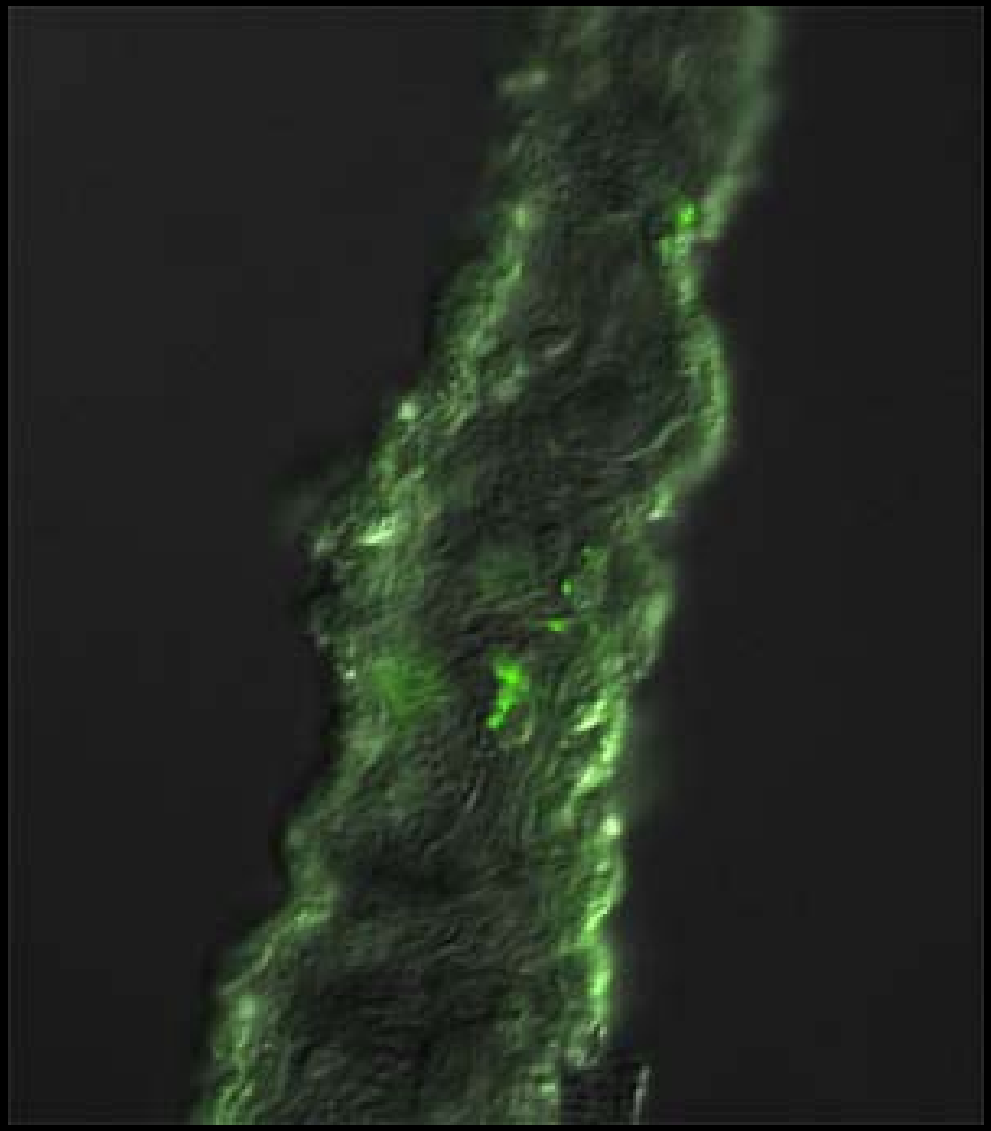
Muscle cells 7-d reg.

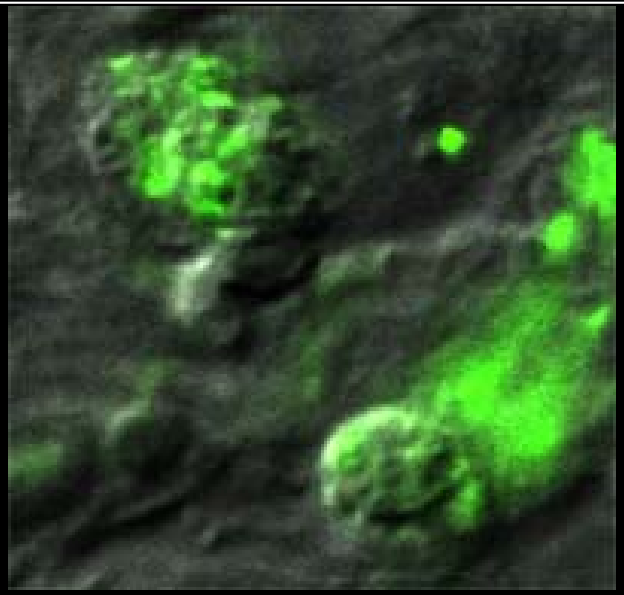


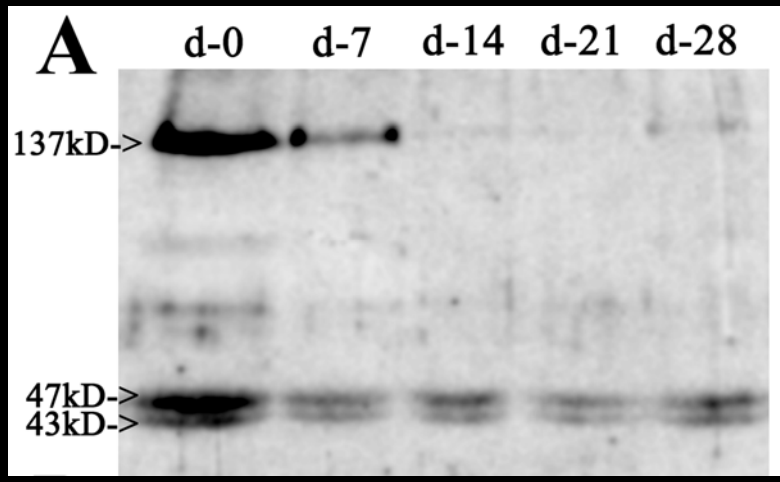
Collagen- Control

Muscle cells- Control

Collagen is degraded by phagocytic amoebocytes

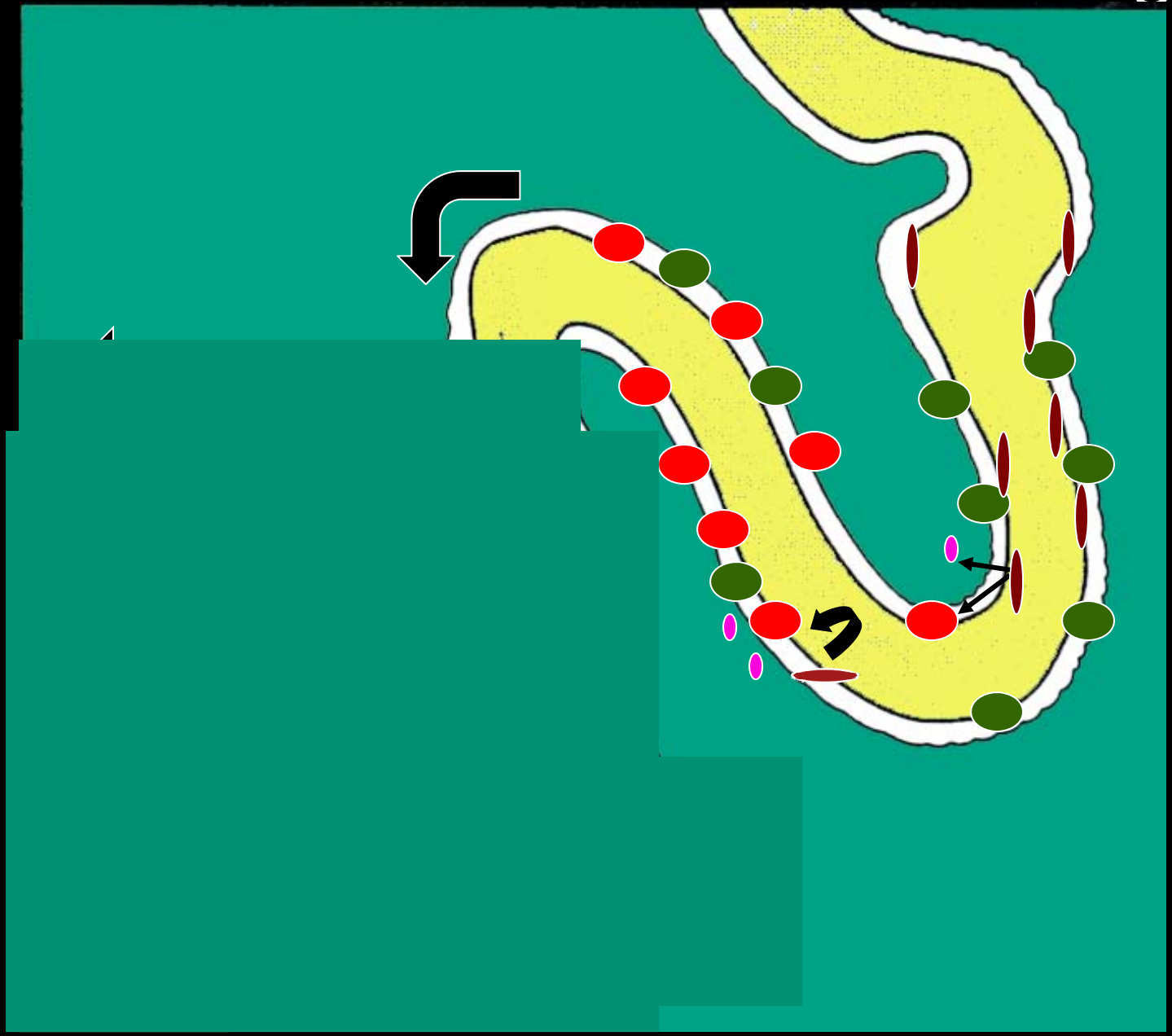




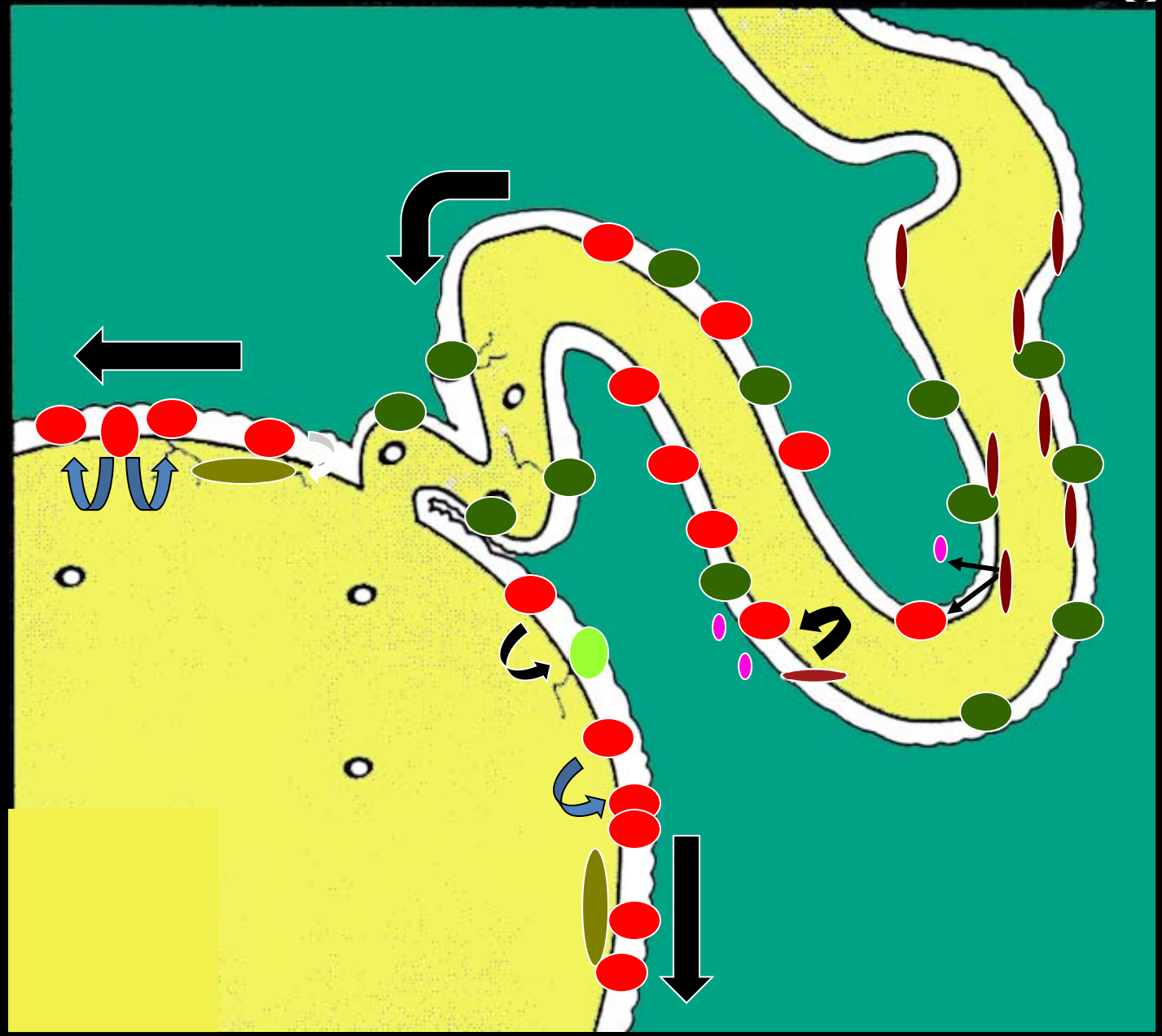


Quiñones et al. Dev Biol 2002

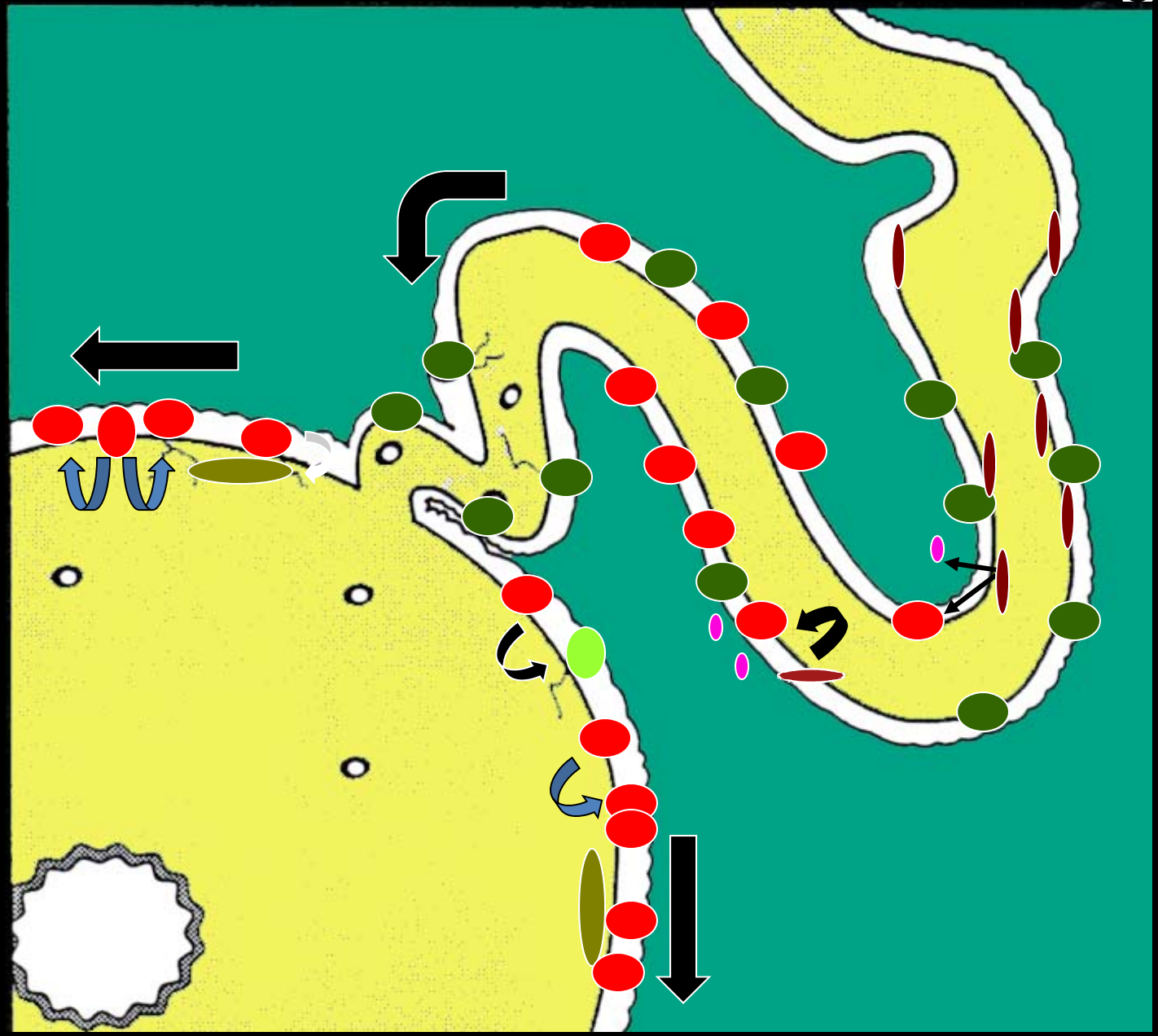
REGENERATION MODEL- cellular origins



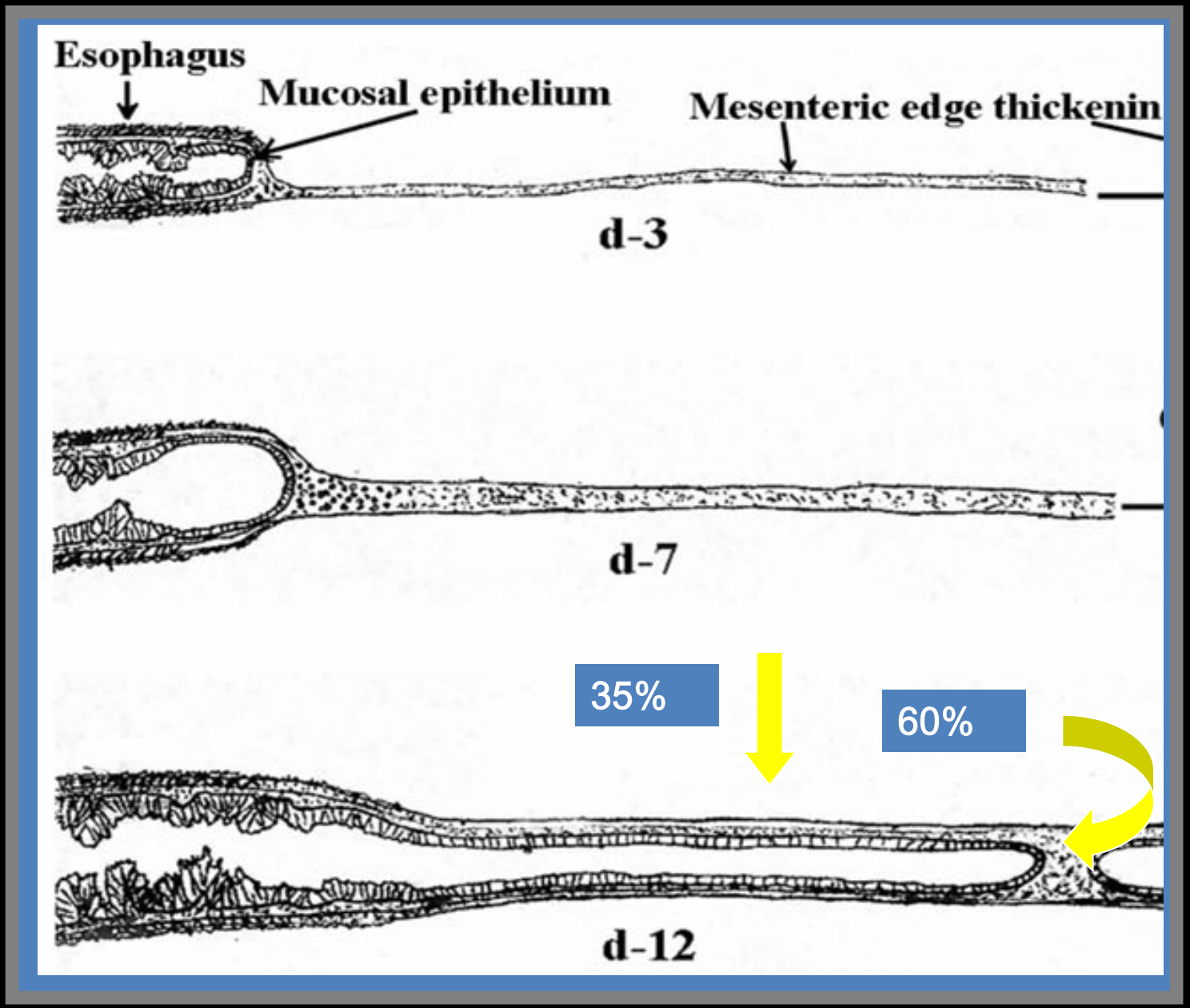
REGENERATION MODEL- cellular origins



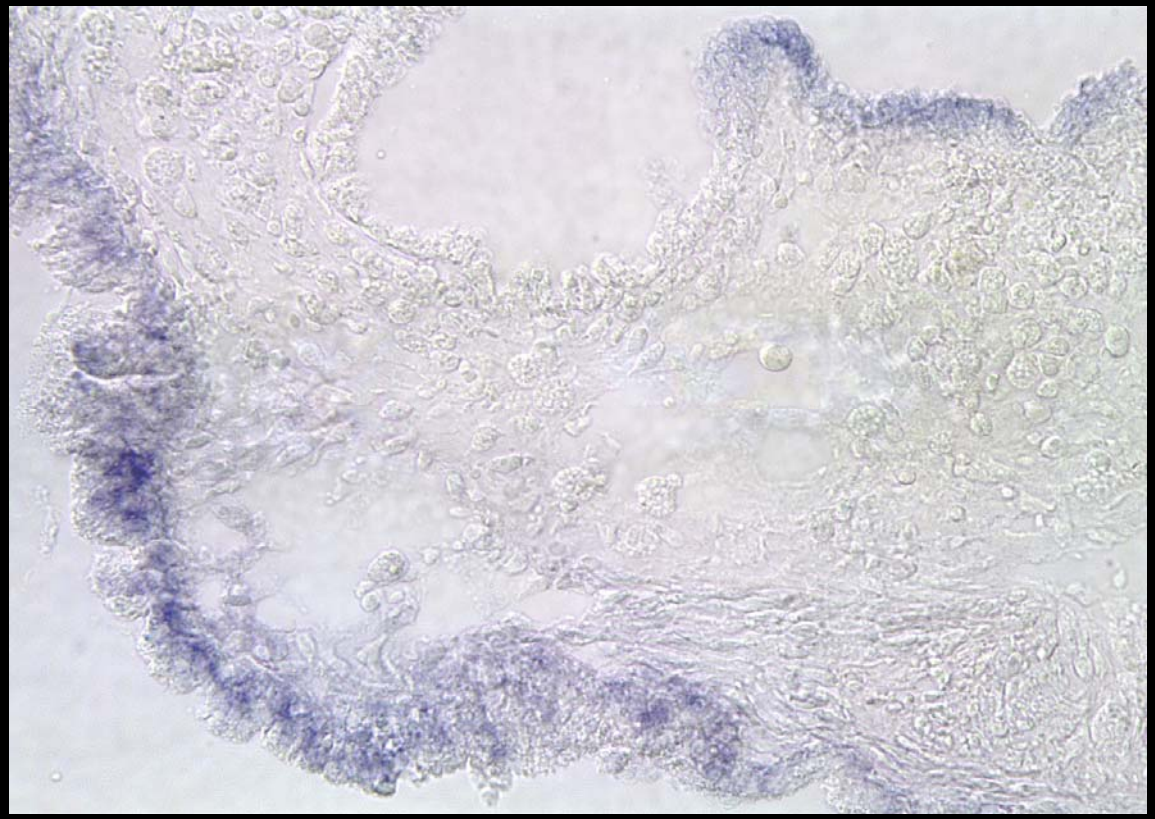
REGENERATION MODEL- cellular origins



Cell Migration



Challenge #1 - Limited molecular tools



In situ hybridization for Survivin mRNA
in 10-day regenerating animal

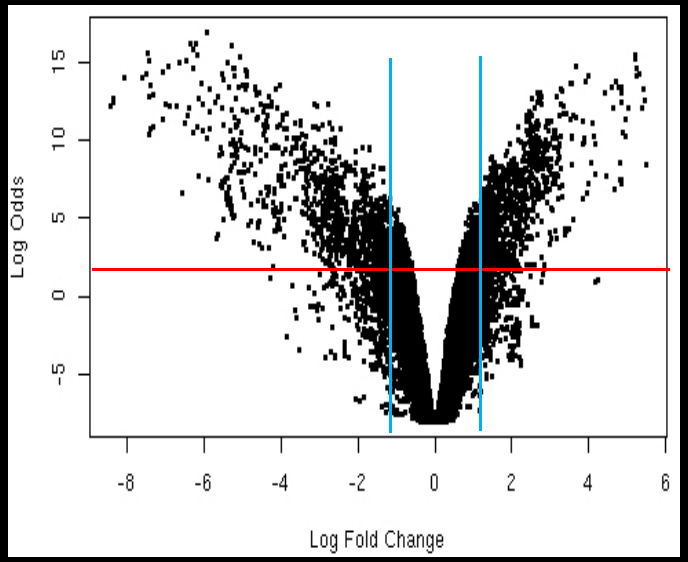
Mashanov et al. In prep.

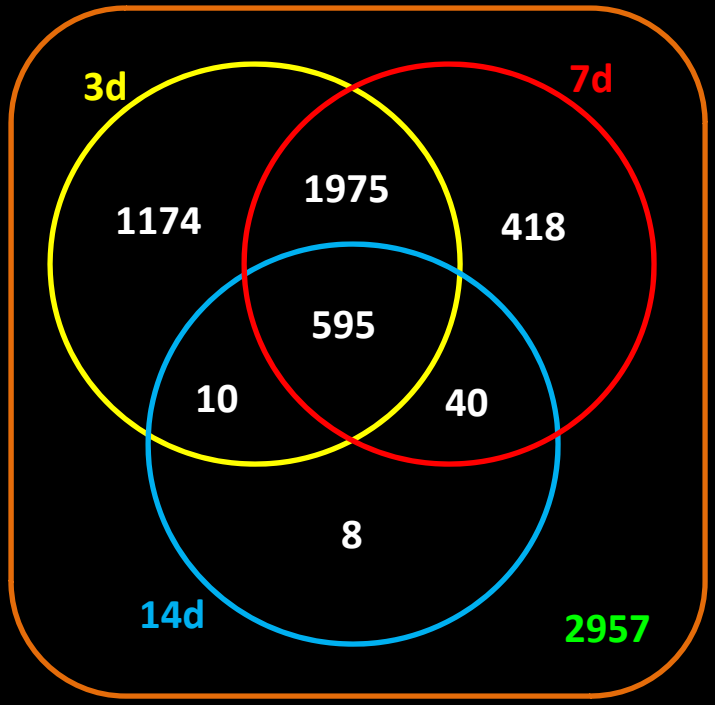
Challenge #2 - Genomics

-We have a databank with over 7000 ESTs from 3 cDNA libraries of normal and regenerating intestines

- Microarrays were done with custom made microchips with over 7000 H. glaberrima ESTs.

- A large number of ESTs are differentially expressed in regenerating animals





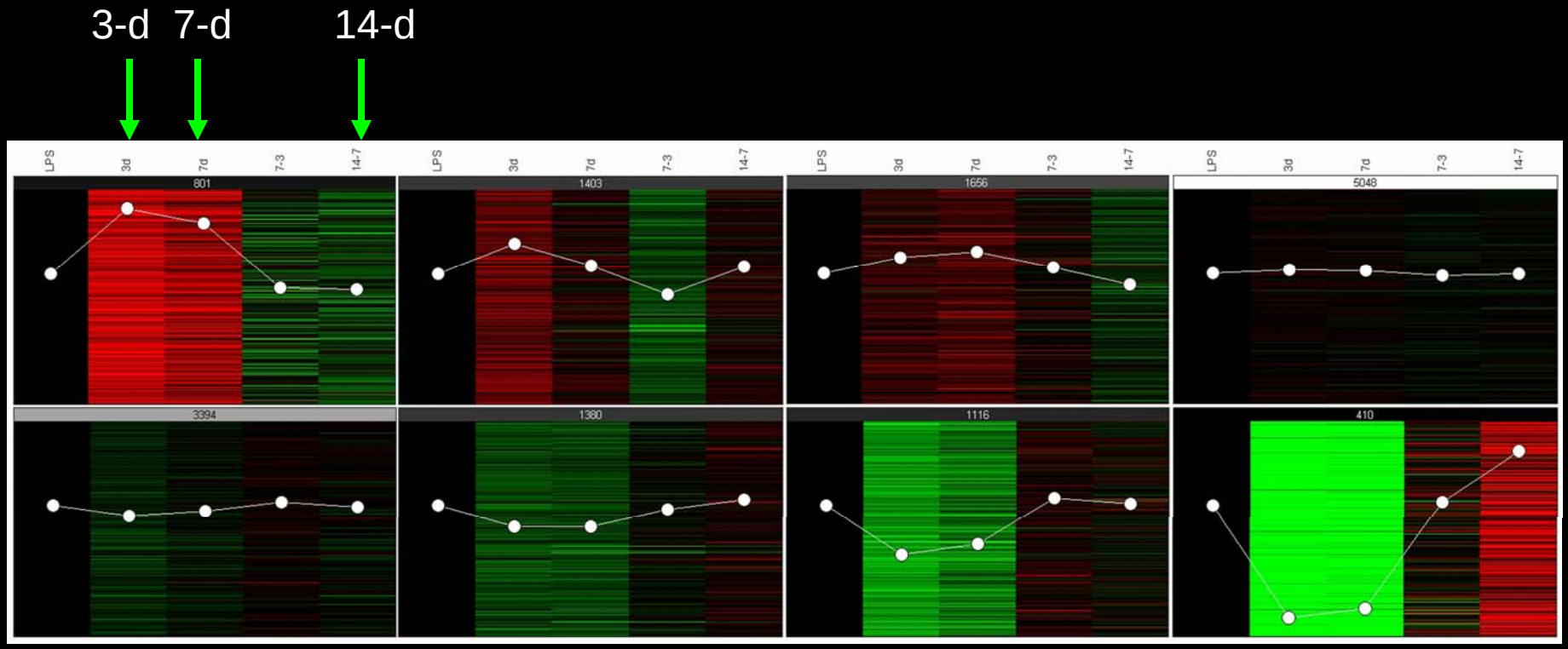
p<0.05 -- 73%
p<0.01 -- 58%
P<0.001-- 39%

Reference = Normal (non-eviscerated)
n= 7166 p<0.01

Rojas-Cartagena et al. Physiol Genomics 2007

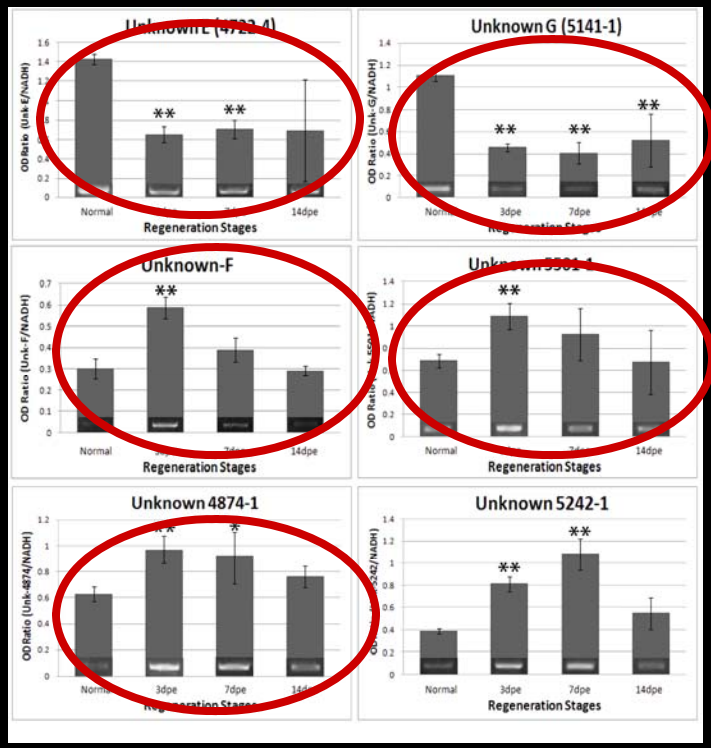
Ortiz-Pineda et al. BMC Genomics 2009

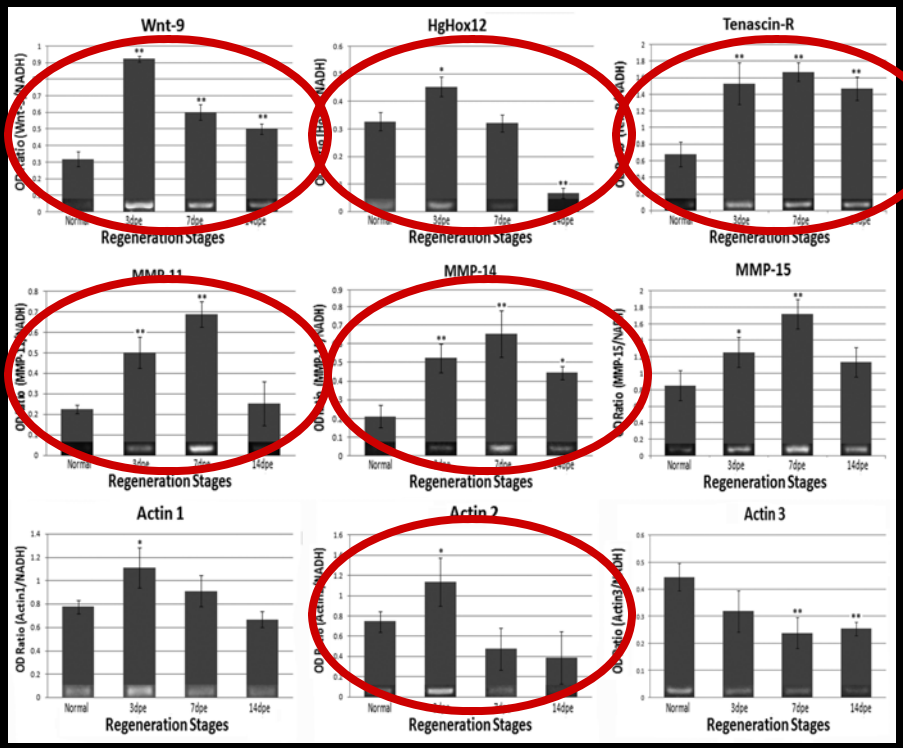
Clusters of gene expression



Over 85% of genes validated with PCR showed the same level of significant differences as the microarray.

Studies can focus on selected candidates or novel genes.





Ramirez-Gomez et al. PLOS One 2009

Otiz-Pineda et al. BMC Genomics 2009

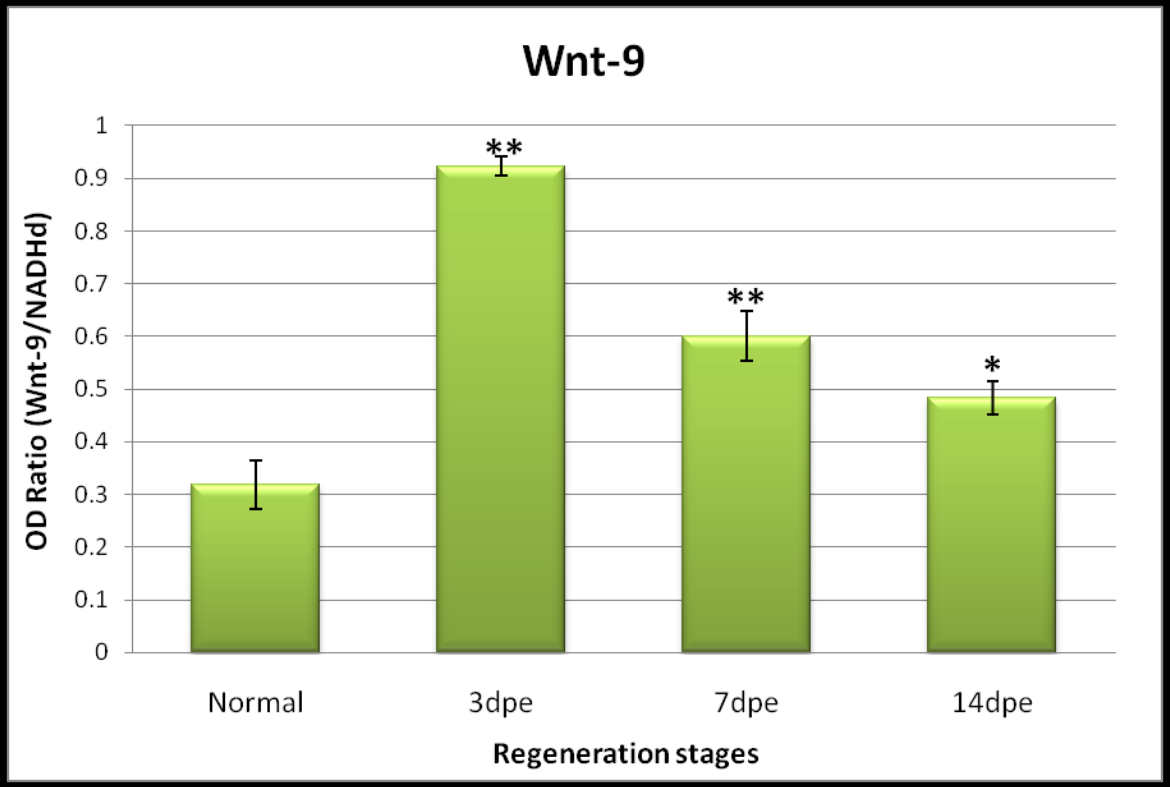
Wnt- candidate gene

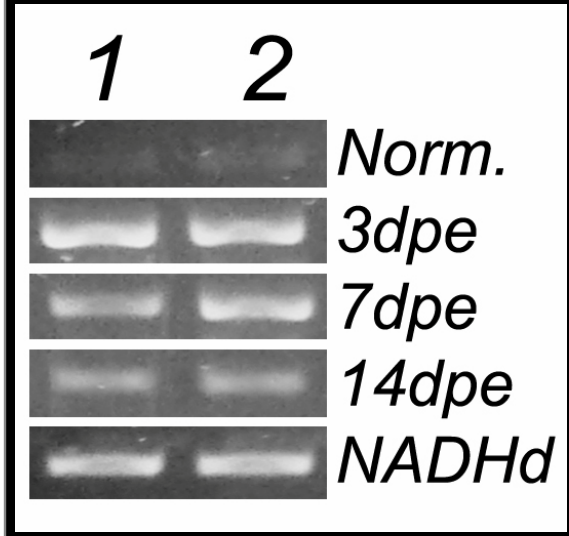
- Gene family of secreted factors with important roles as regulators of embryonic development

- Important role in maintenance and the activation of proliferation of stem cells

- Associated with regeneration processes in various animal models

Wnt-9 is over-expressed during intestinal regeneration showing the highest values in the 3-day intestine





Del Valle et al. In prep.

Orpin- novel gene

- Large number of ESTs in regenerating cDNA libraries that assemble into one contig
- Over-expressed at 3-days of regeneration in the microarray
- No similarity to genes in database

Orpin

```
1   aaaacttctgcagaatcagttgttaagaattgtacacaggccgaaactagtcgttctaaca
62  tcgcaactcctccagaatcatccagaacaagcatttacatcaatcatggctagacttctt
                                                  M  A  R  L  L
122 gtactcgtcgctacacttgctttggtcgctgtcattagtgaagcgtgcacctcatcatct
    V  L  V  A  T  L  A  L  V  A  V  I  S  E  A  C  T  S  S  S
182 gggcgcacgccatcttgggacgttggcgactccactgaaatctaccccacttacgatcct
    G  R  T  P  S  W  D  V  G  D  S  T  E  I  Y  P  T  Y  D  P
242 gctaccgaatcagttggagtcggagtcactgttaggtttaaaagagaaaccgacgatagt
    A  T  E  S  V  G  V  G  V  T  V  R  F  K  R  E  T  D  D  S
302 aaactgcaggtcttcaaattaattgacgcatctggtgacggttacattgacgcttgcgag
    K  L  Q  V  F  K  L  I  D  A  S  G  D  G  Y  I  D  A  C  E
362 tggttaatagagggcggtattgtcaaaaactttgtccagttcctgacagacgatgatgtc
    W  L  I  E  G  G  I  V  K  N  F  V  Q  F  L  T  D  D  D  V
422 gatggtgatgagaagatttcttggaatgagttccagaaagtgtcagtcgcatagtagata
    D  G  D  E  K  I  S  W  N  E  F  Q  K  V  S  V  A  *
482 gaaatagcgccccagttccagttatttaatatagtctattttagagacctgtagggtaa
542 ggaacatttacttcaaagatgacagtctttccaaacctttcttcaaaagctcaacgaact
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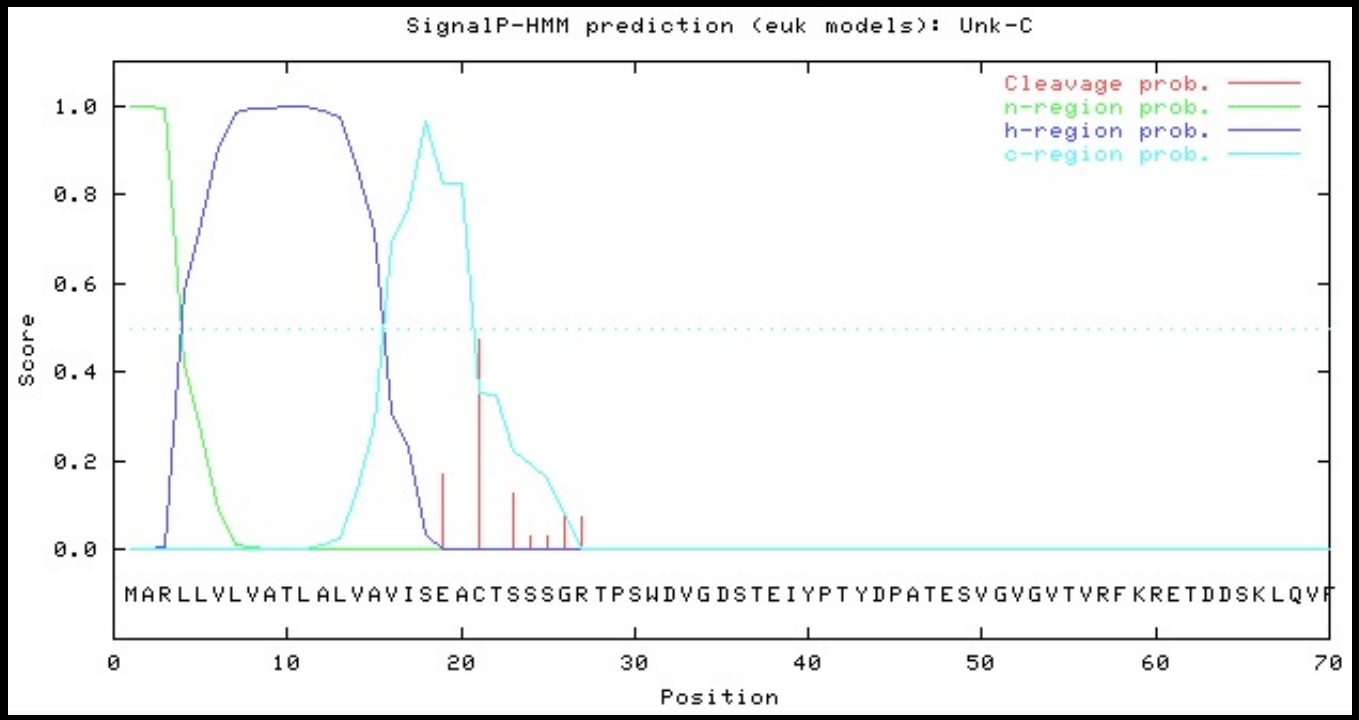
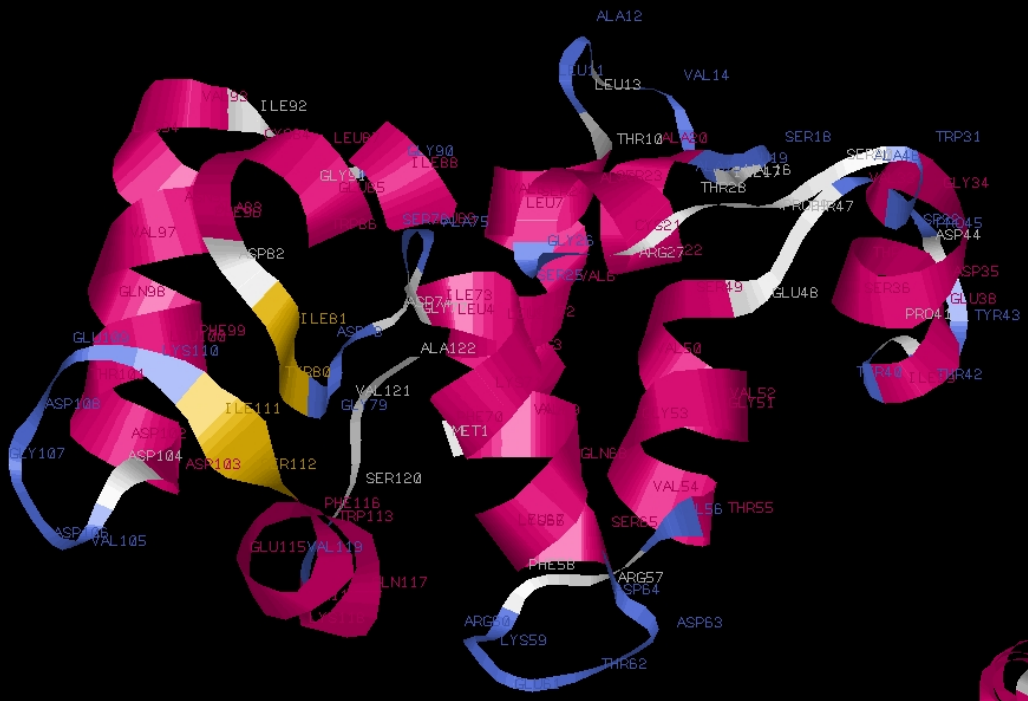


Ortiz-Pineda et al. In prep

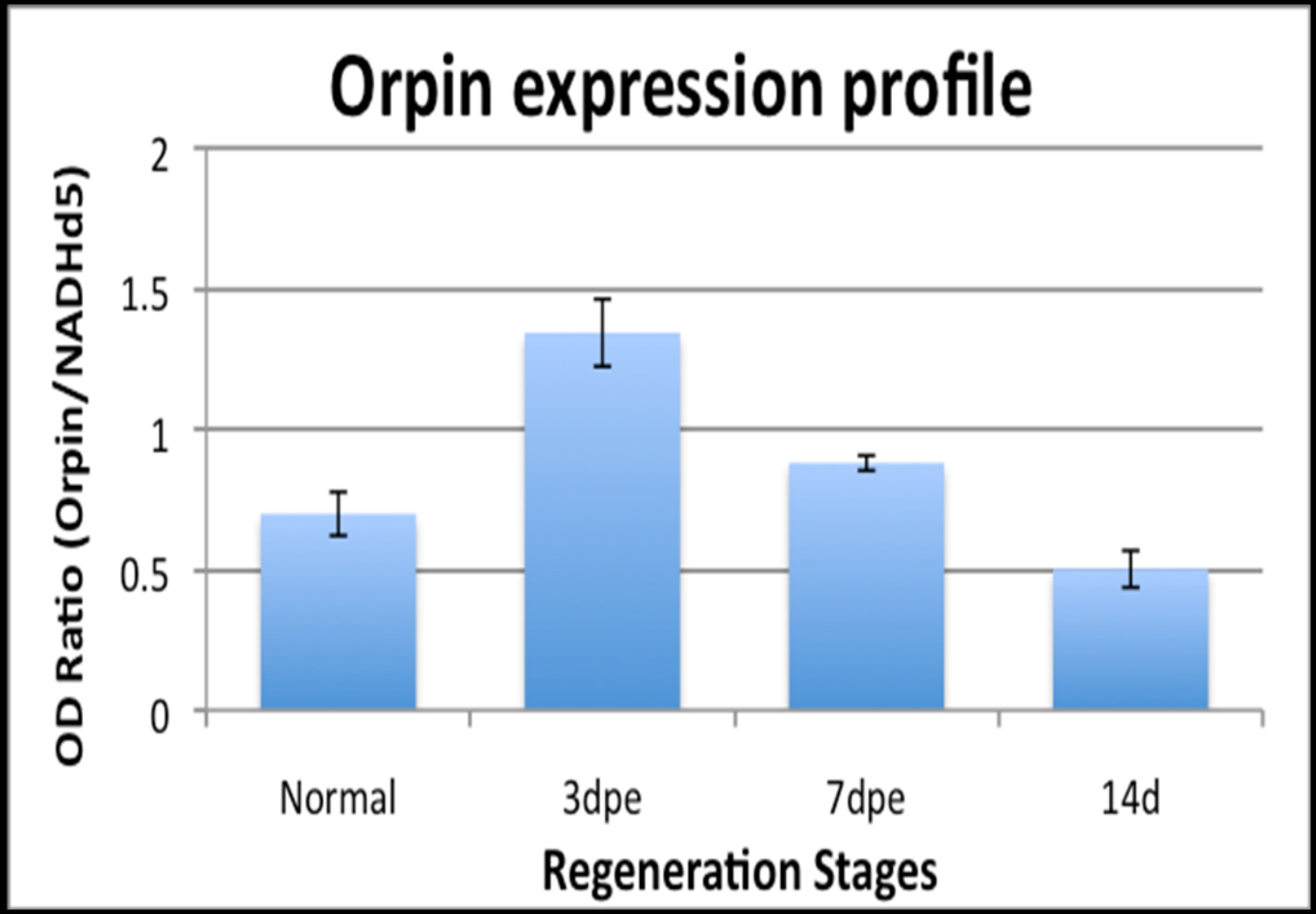
Orpin shares some similarity in the calcium-binding EF hand domain with other calcium binding proteins



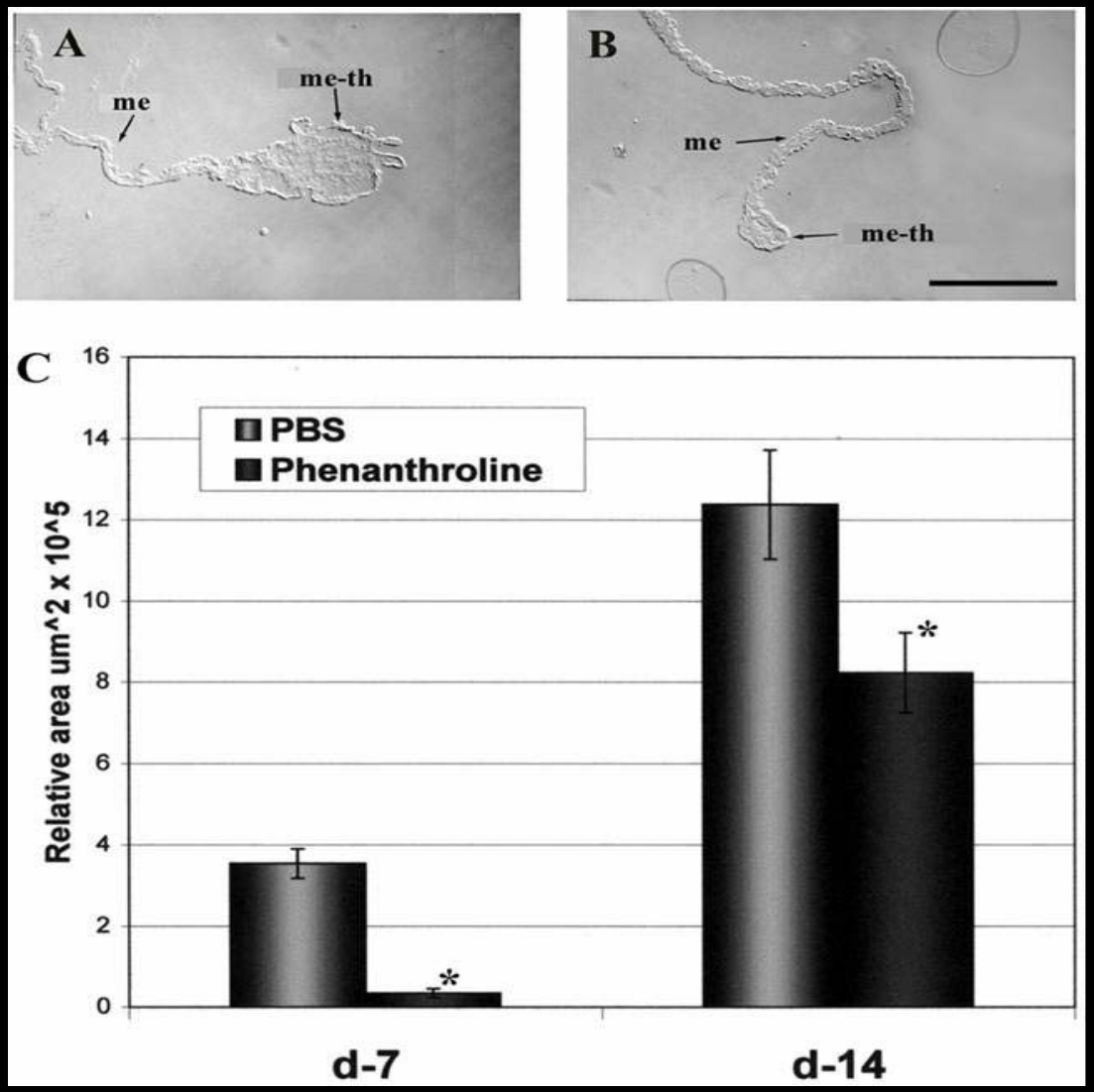
Orpin

Calmodulin

RT-PCR validation of Orpin overexpression in the 3-day regenerating intestine.



Challenge #3 - Loss of function/Transgenics



Inhibitors of matrix metaloproteases inhibit intestinal regeneration

Use of pharmacological tools. For example, MMP inhibitors, apoptosis inhibitors, Wnt pathway activators.

Missing

RNAi

Transfections

Genetics

TOP ADVANTAGE -

VERY LOW POSSIBILITY OF BEING SCOOPED

TOP ADVANTAGE -

VERY LOW POSSIBILITY OF BEING SCOOPED

THE END