Intestinal regeneration in the echinoderm *Holothuria glaberrima*

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

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

Sea cucumbers are deuterostomes

Advantage #2 - Key phylogenetic position
Advantage #3 - Extraordinary regenerative properties
Nerve fiber regeneration

San Miguel-Ruiz et al. BMC Dev Biol 2009
Tentacle regeneration

A. Amputation Site
B. 0 dpa
C. 3 dpa
D. 7 dpa
E. 14 dpa
F. 21 dpa
G. 28 dpa
H. 35 dpa
I. Normal
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.
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.
Muscle cell dedifferentiation involves the formation of spindle like structures

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

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

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

3-d 7-d 14-d
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

Ortiz-Pineda et al. In prep
Orpin shares some similarity in the calcium-binding EF hand domain with other calcium binding proteins.
RT-PCR validation of Orpin overexpression in the 3-day regenerating intestine.
Inhibitors of matrix metaloproteases inhibit intestinal regeneration

Challenge #3 - Loss of function/Transgenics

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

VERY LOW POSSIBILITY OF BEING SCOOPED
TOP ADVANTAGE -
VERY LOW POSSIBILITY OF BEING SCOOPED

THE END