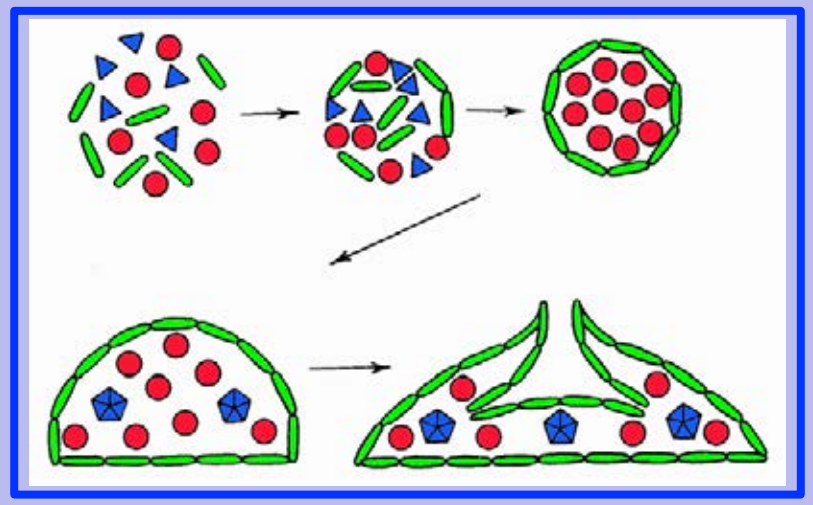
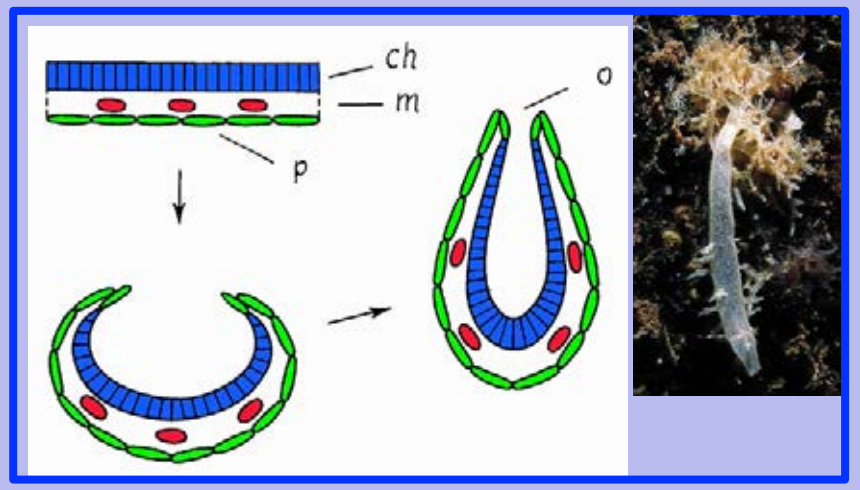
MARINE BIOLOGY, GEOLOGY, OCEANOLOGY: INTERDISCIPLINARY RESEARCH AT MARINE STATIONS

MOSCOW, 19-21 November, 2018

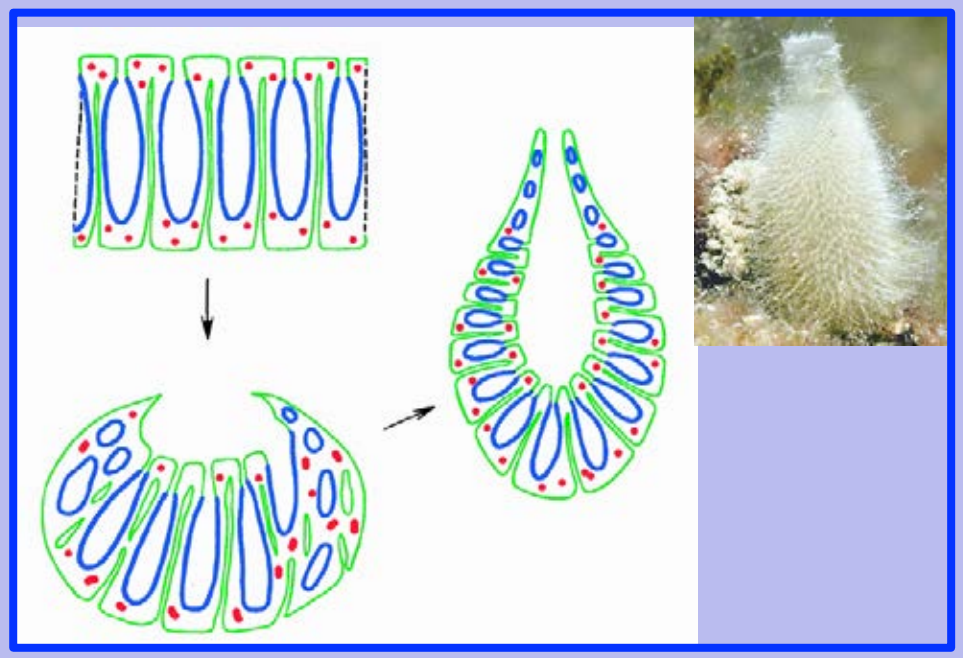
Regeneration in sponges (Porifera): comparative investigation

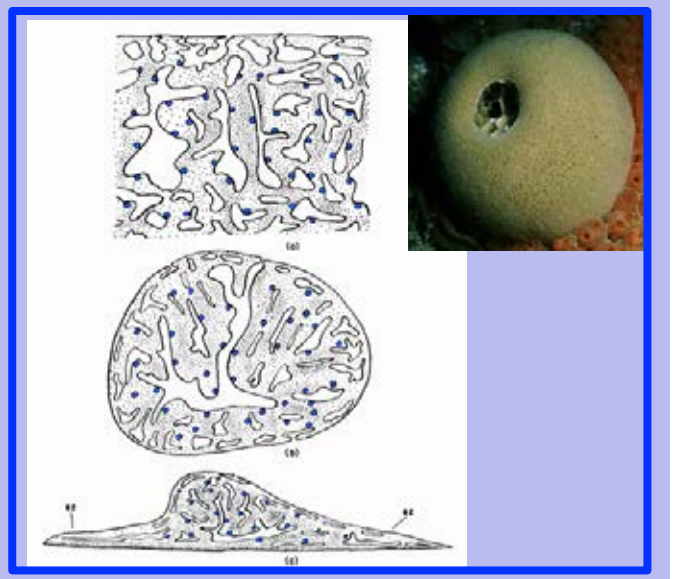
A. Ereskovsky, I.B. Borisenko, A.I. Lavrov, F.V. Bolshakov, D.B. Tokina



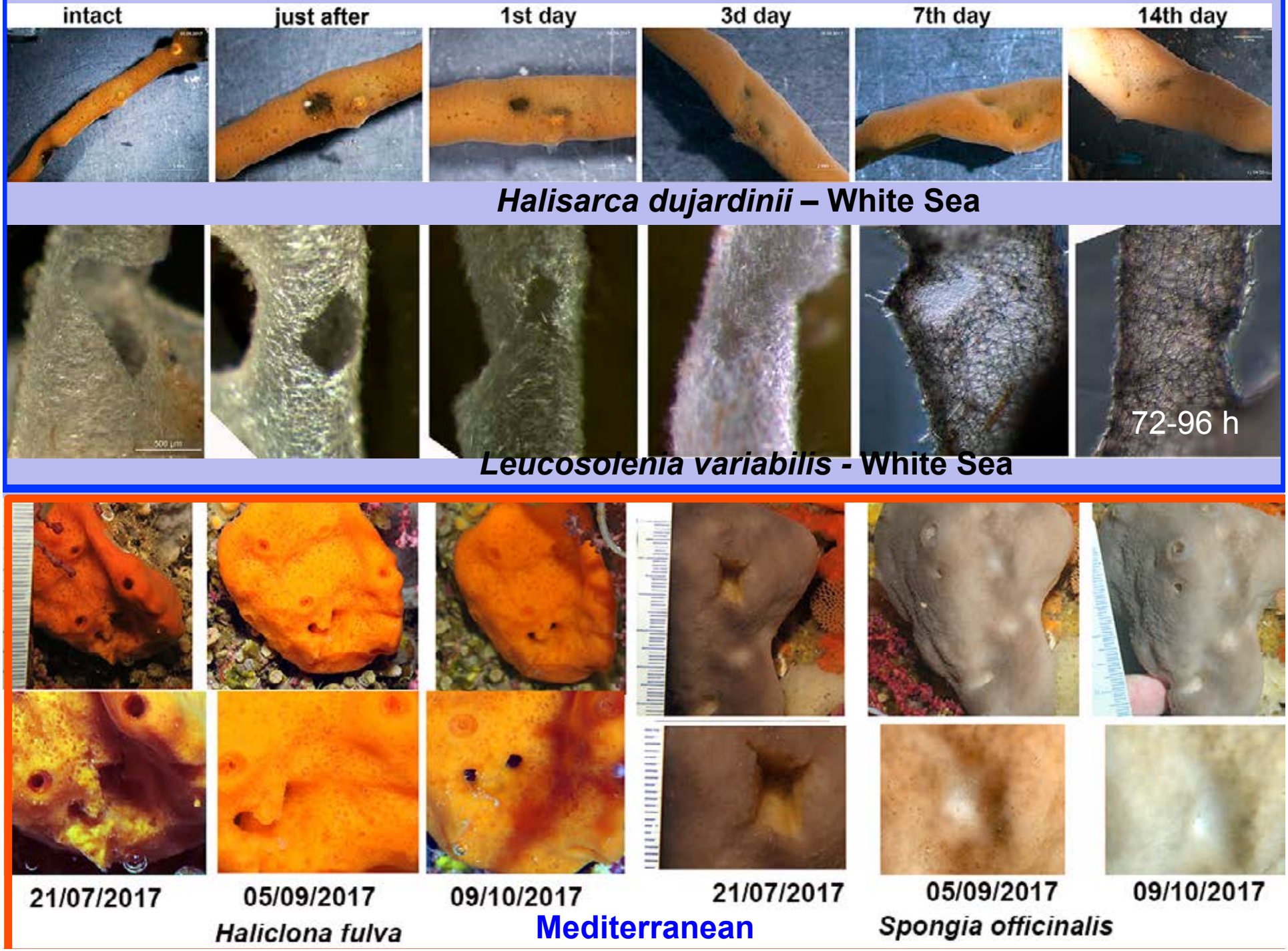


To understand evolutionary history of the diverse regeneration mechanisms, regeneration processes must be studied in early-evolved metazoans in addition to the traditional bilaterian and cnidarian models.

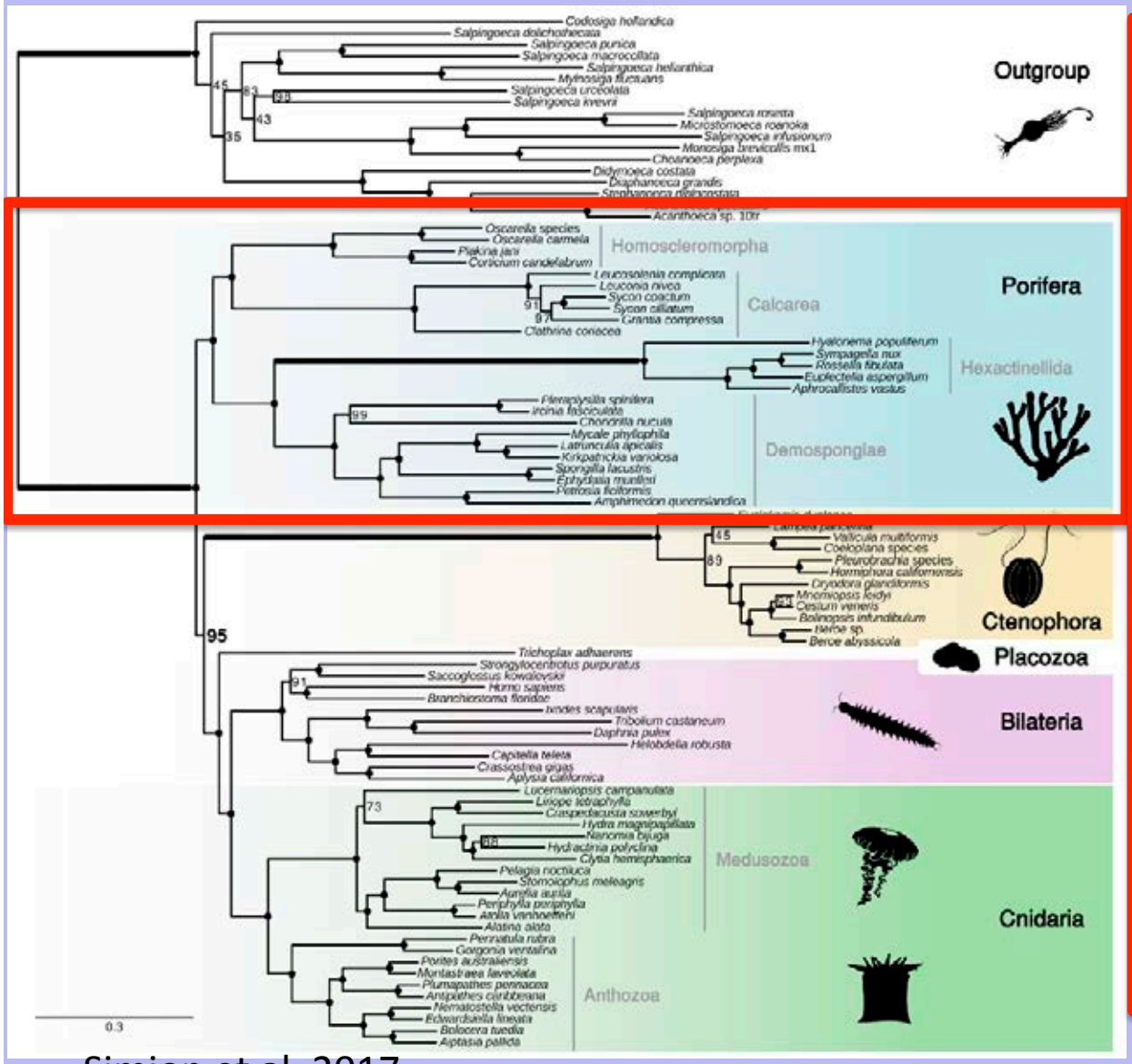


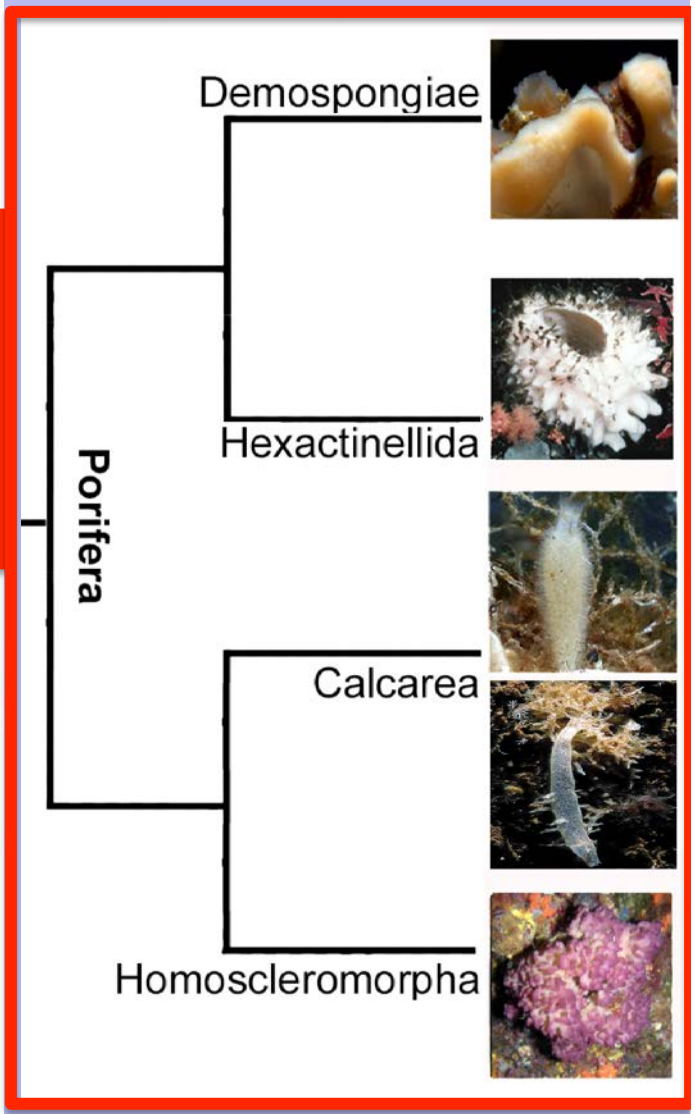


Sponges are known to possess remarkable reconstitutive and regenerative abilities


intact
just after
1st day
3d day
7th day
14th day
Halisarca dujardinii – White Sea
72-96 h
Leucosolenia variabilis - White Sea
21/07/2017
05/09/2017
09/10/2017
Haliclona fulva
21/07/2017
05/09/2017
09/10/2017
Spongia officinalis
Mediterranean

Porifera





Simion et al. 2017

The aims of this study are:

- 1) To show the variety of morphogeneses during reparative regeneration in different sponges with different organisation;
- 2) To discover the cells, involved in the regeneration;
- 3) To highlight the correlation between tissue organization and morphogenetic mechanisms involved in sponge's regeneration.

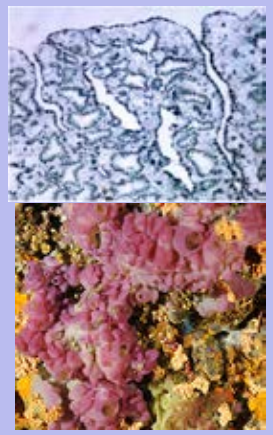
Epithelia characters in sponges

	AB polarity	Cell junctions: larvae	Cell junctions: adults	Basement membrane larvae	Basement membrane adults
Demospongiae					
Calcarea					
Homoscleromorpha					

Models

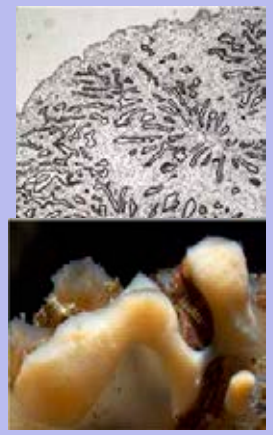
Current models

Oscarella lobularis



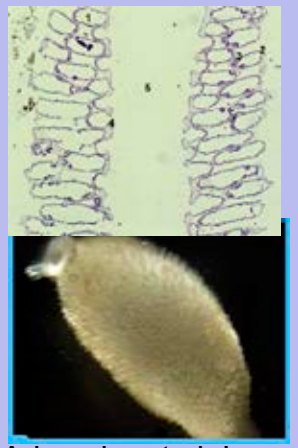
Ereskovsky et al. 2015

Halisarca dujardini



Borisenko et al. 2015, 2016

Sycon ciliatum



Adamska et al. In prep.

Leucosolenia variabilis

Ereskovsky et al. 2017;
Lavrovet et al. 2018

New models

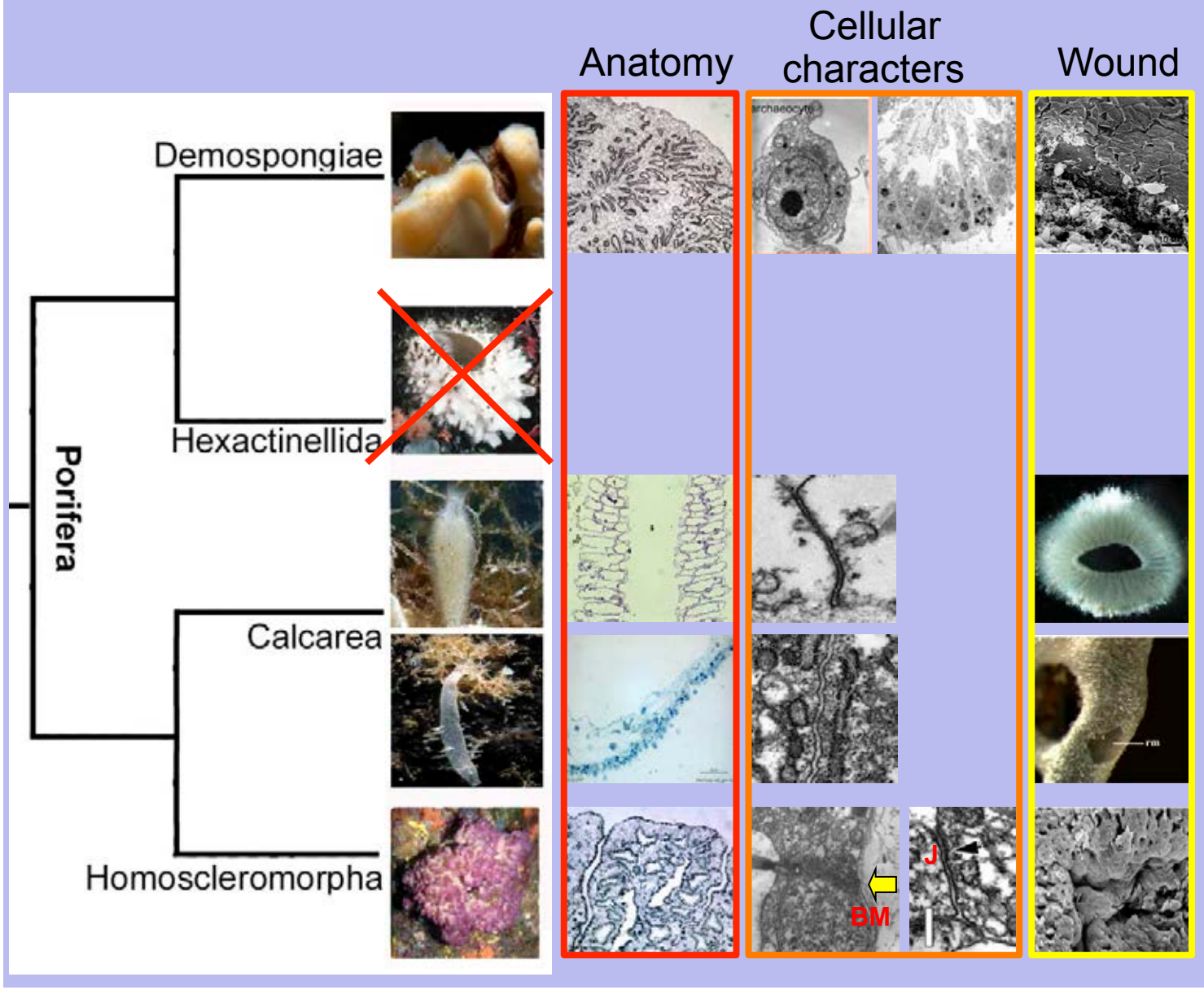
Aplysina cavernicola

Suberites domuncula

Clathrina arnesenae

Clathrina clathrus

Current models



Halisarca dujardini: no cell junctions; no basement membrane; archaeocytes

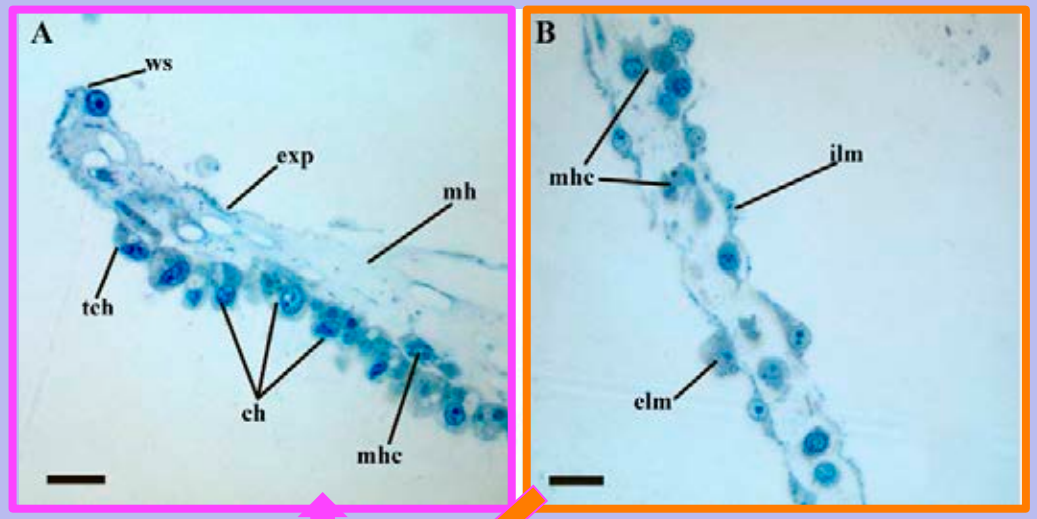
Sycon ciliatum & *Leucosolenia variabilis*: cell junctions; no basement membrane; no archaeocytes

Oscarella lobularis: true epithelium; no archaeocytes

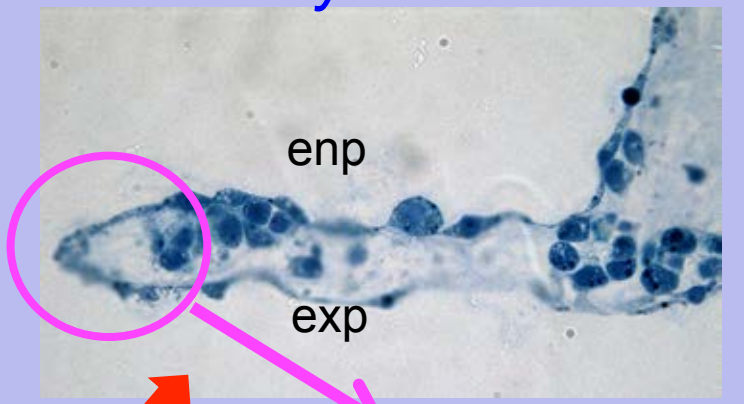
Epithelial morphogenesis and transdifferentiation

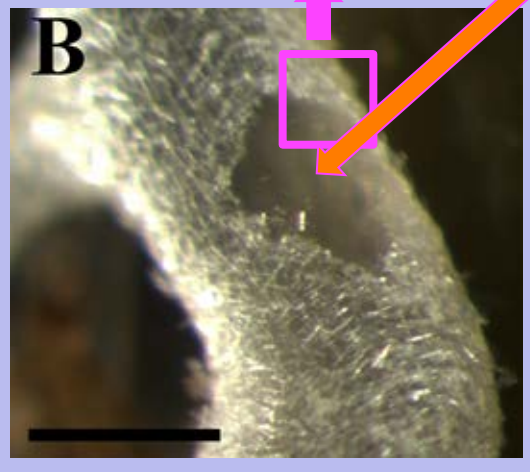
Regenerative membrane in Calcarea

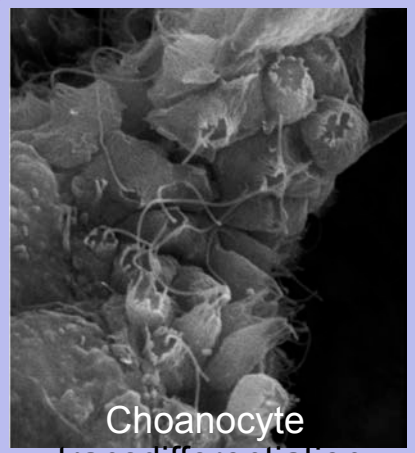
Leucosolenia

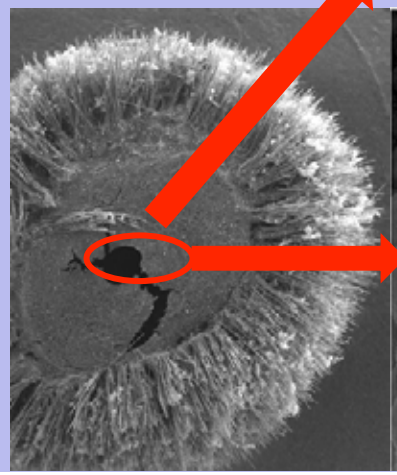


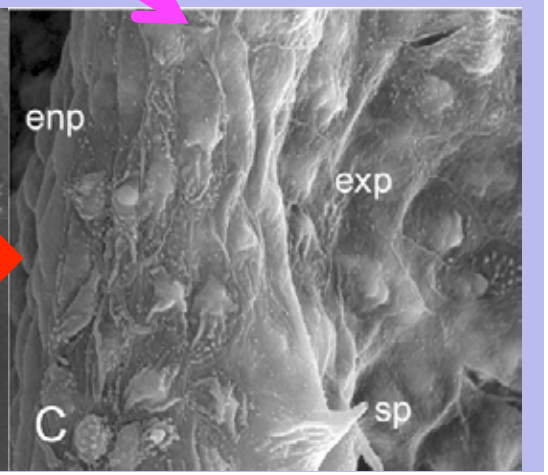
Sycon







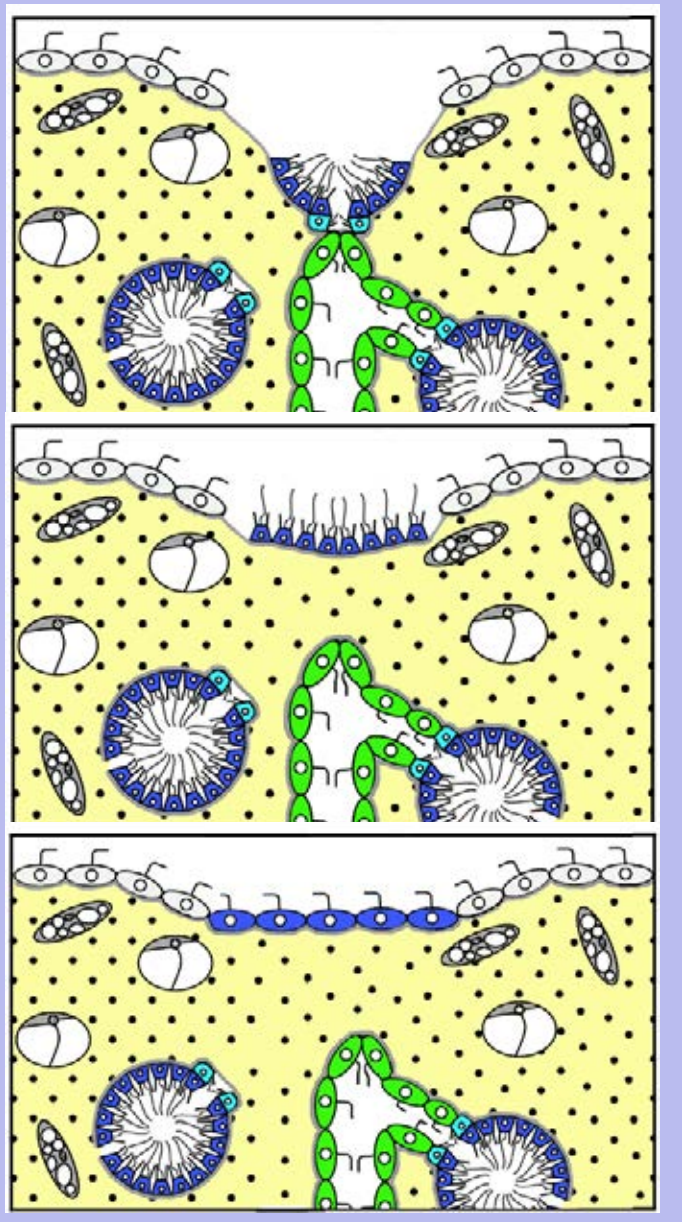




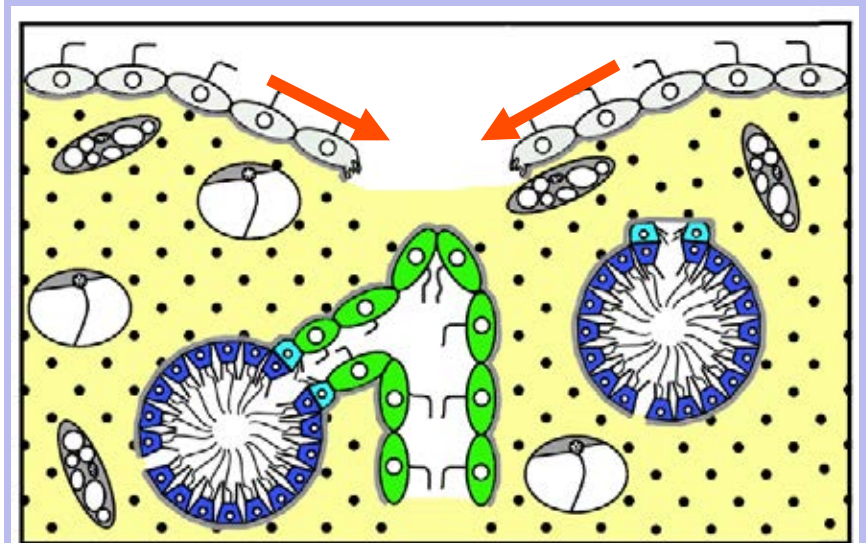
Epithelial morphogenesis and transdifferentiation

Homoscleromorpha

Choanoderm
transdifferentiation during
Oscarella regeneration

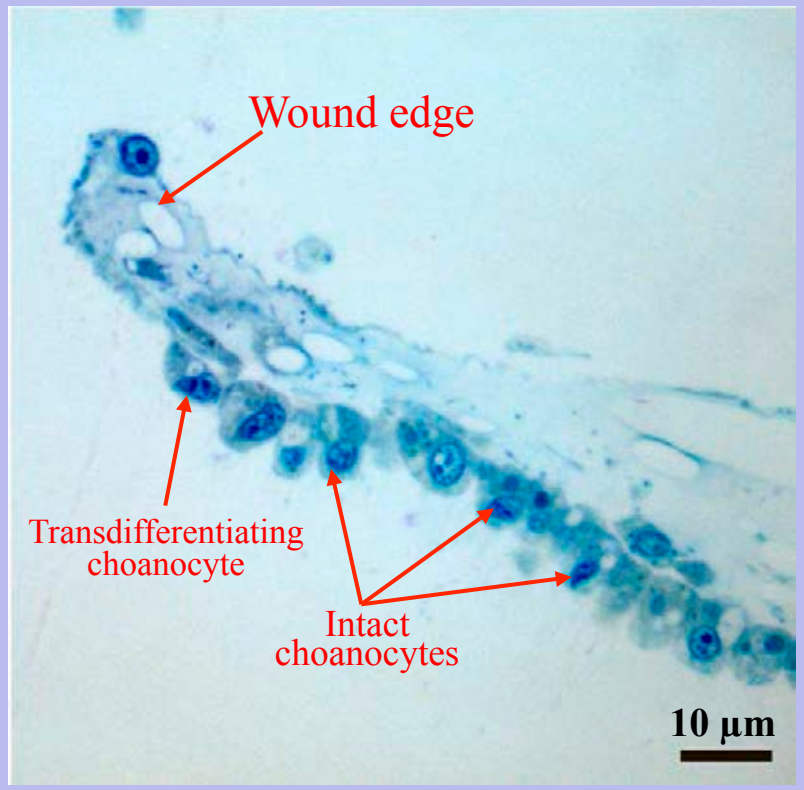


Spreading of the **pinacoderm**
sheet on the wound surface
during *Oscarella* regeneration



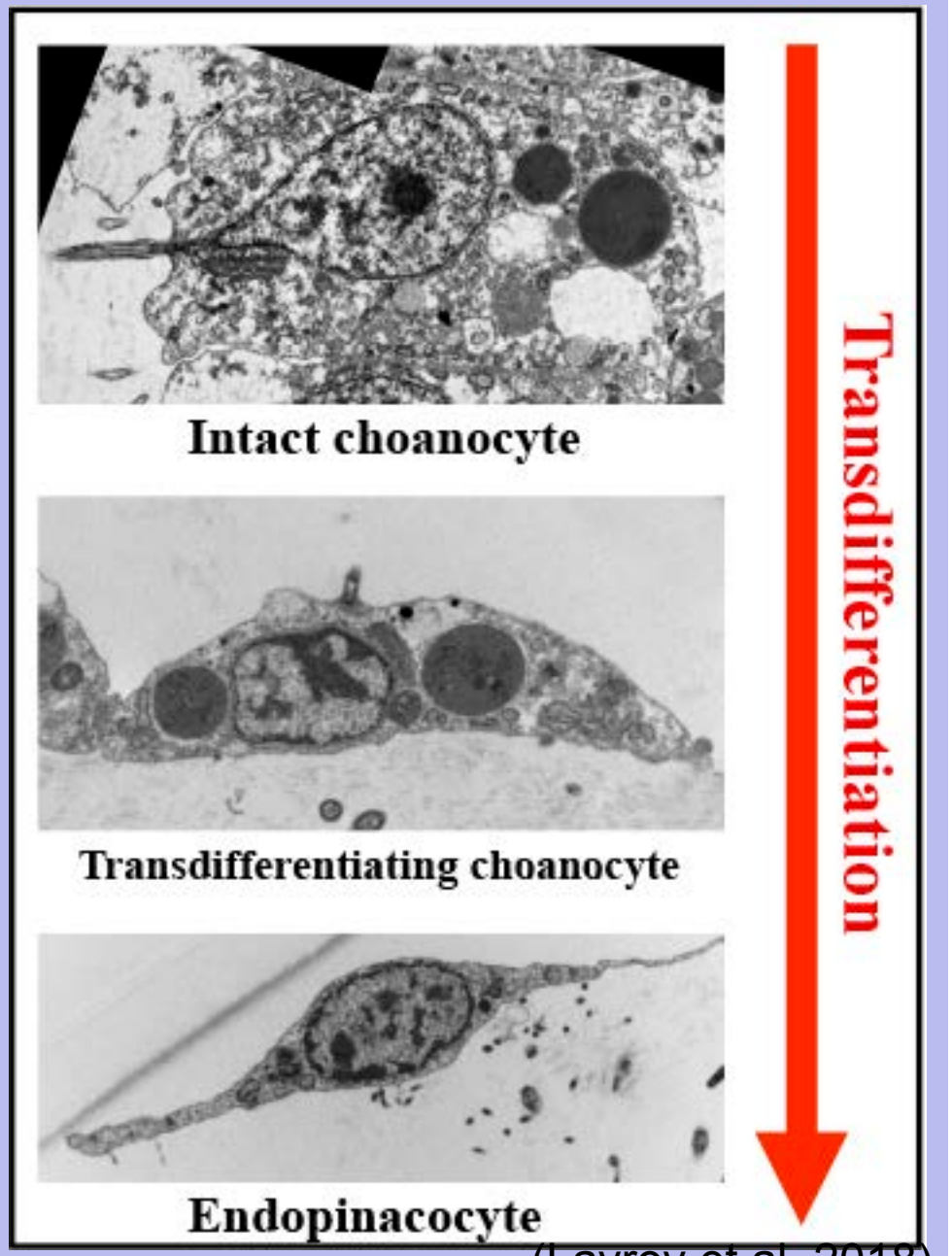
Ereskovsky et al. 2015

Cell transdifferentiation



The continuous epithelium appears on the wound edge. It forms due to the joining of the intact exopinacocytes and **endopinacocyte arising from the choanocytes through their transdifferentiation**

Leucosolenia variabilis

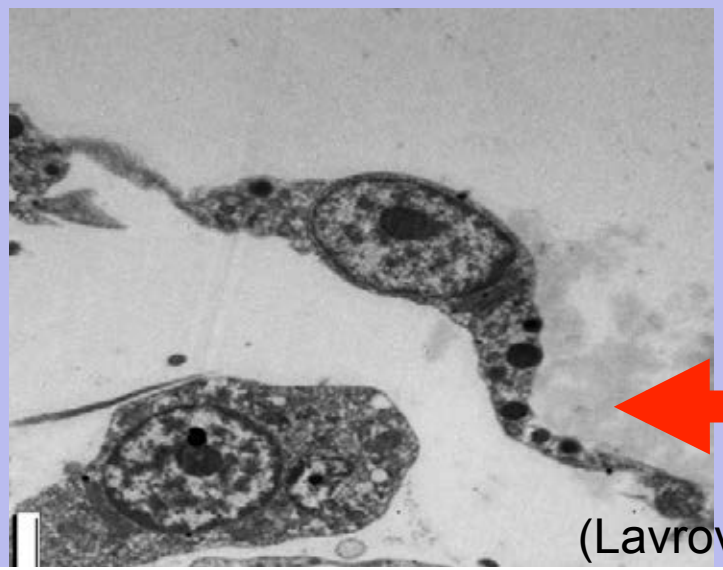


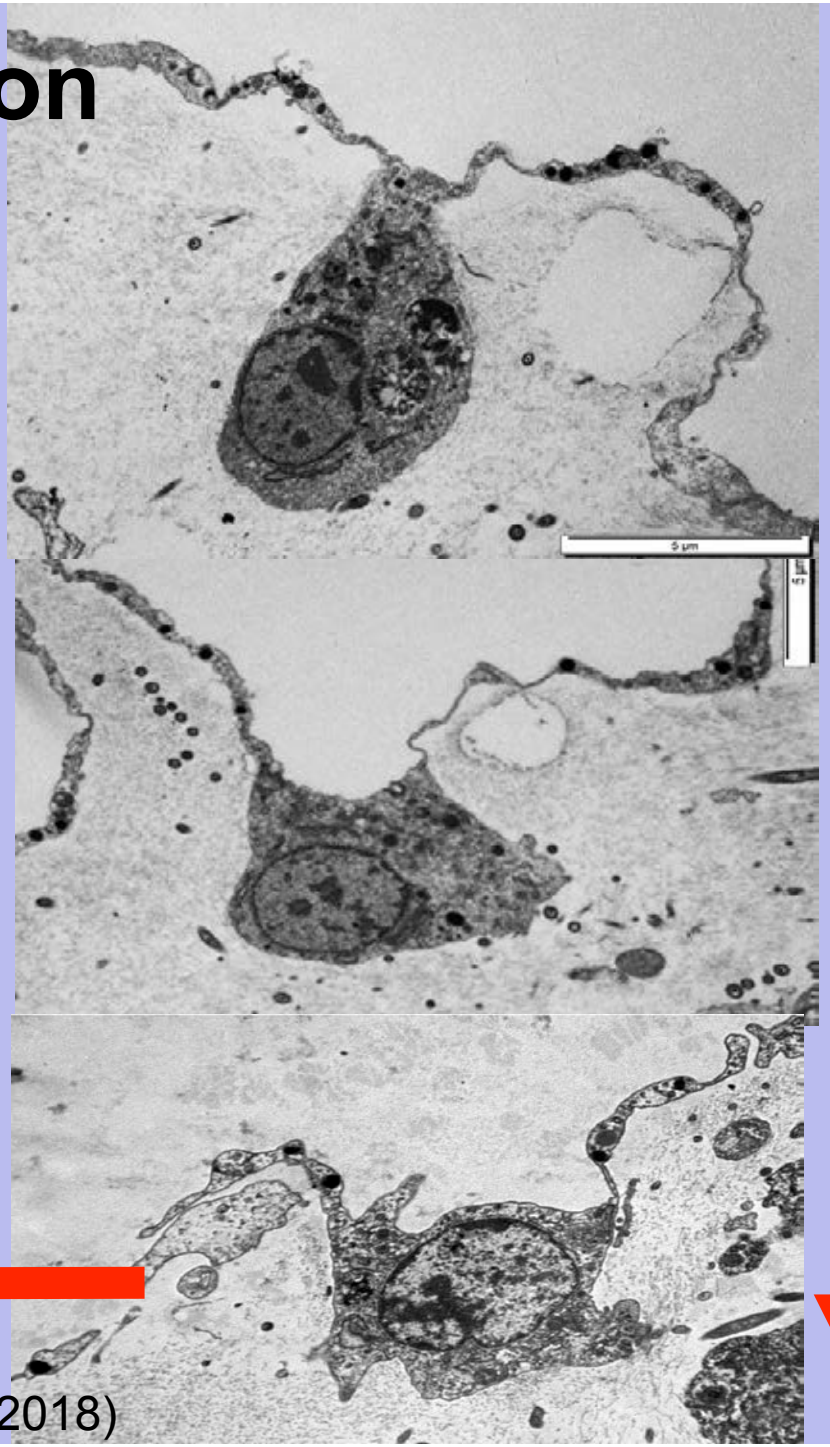
(Lavrov et al. 2018)

Cell transdifferentiation

Exopinacocytes

Leucosolenia variabilis

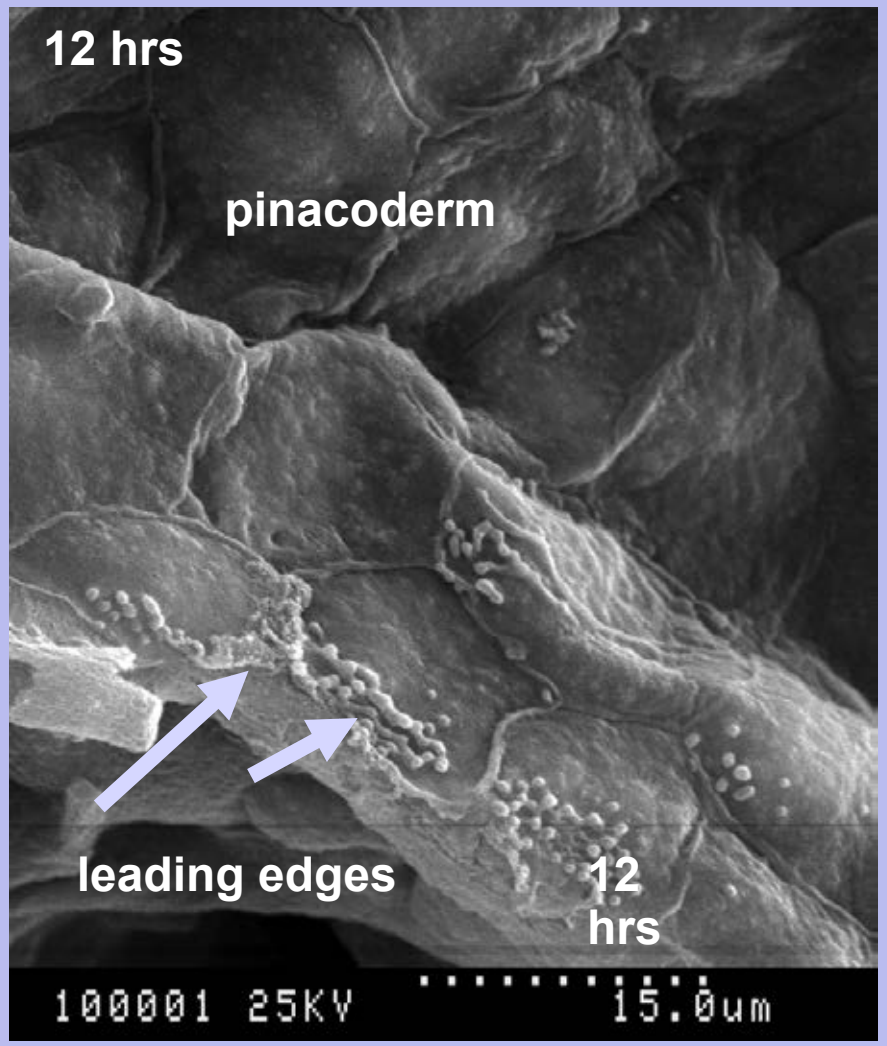


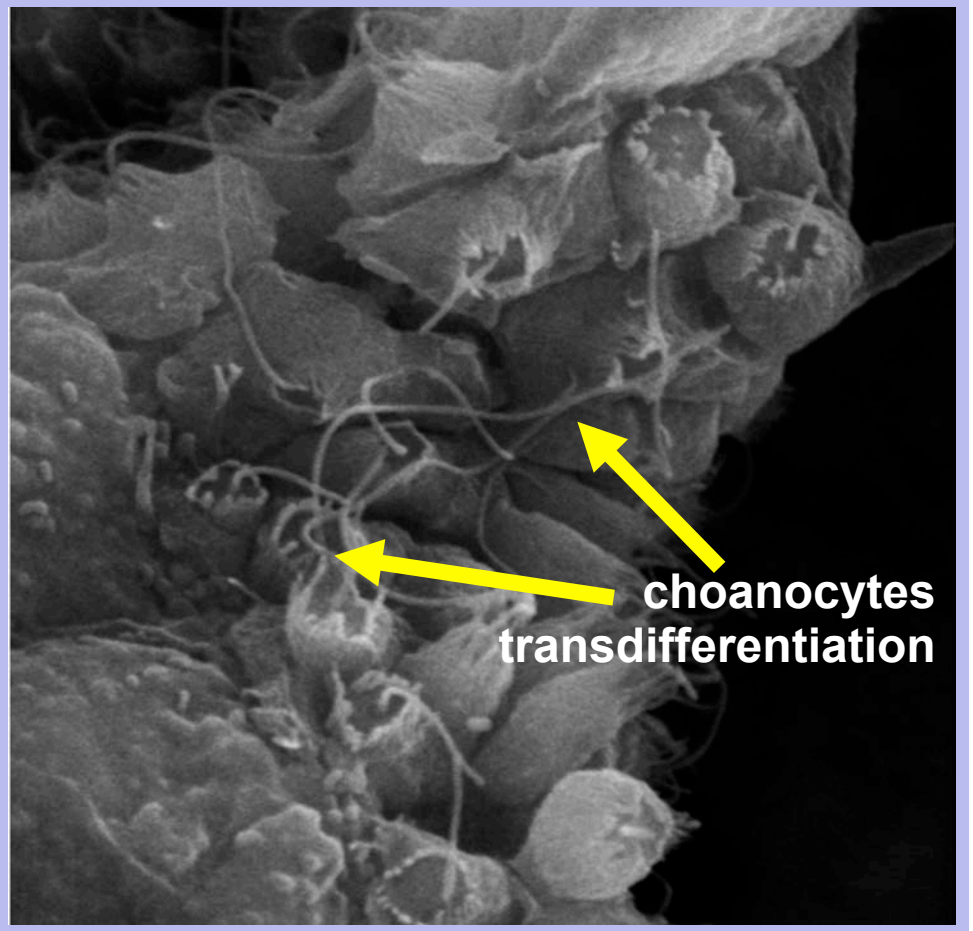


(Lavrov et al. 2018)

Regeneration in *Sycon*: cellular mechanisms

EM analysis demonstrated that at 24 hrs all exposed choanocyte chambers are covered by pinacocytes, with a combination of migration of pinacocytes and transdifferentiation of choanocytes observed within hours from the dissection.

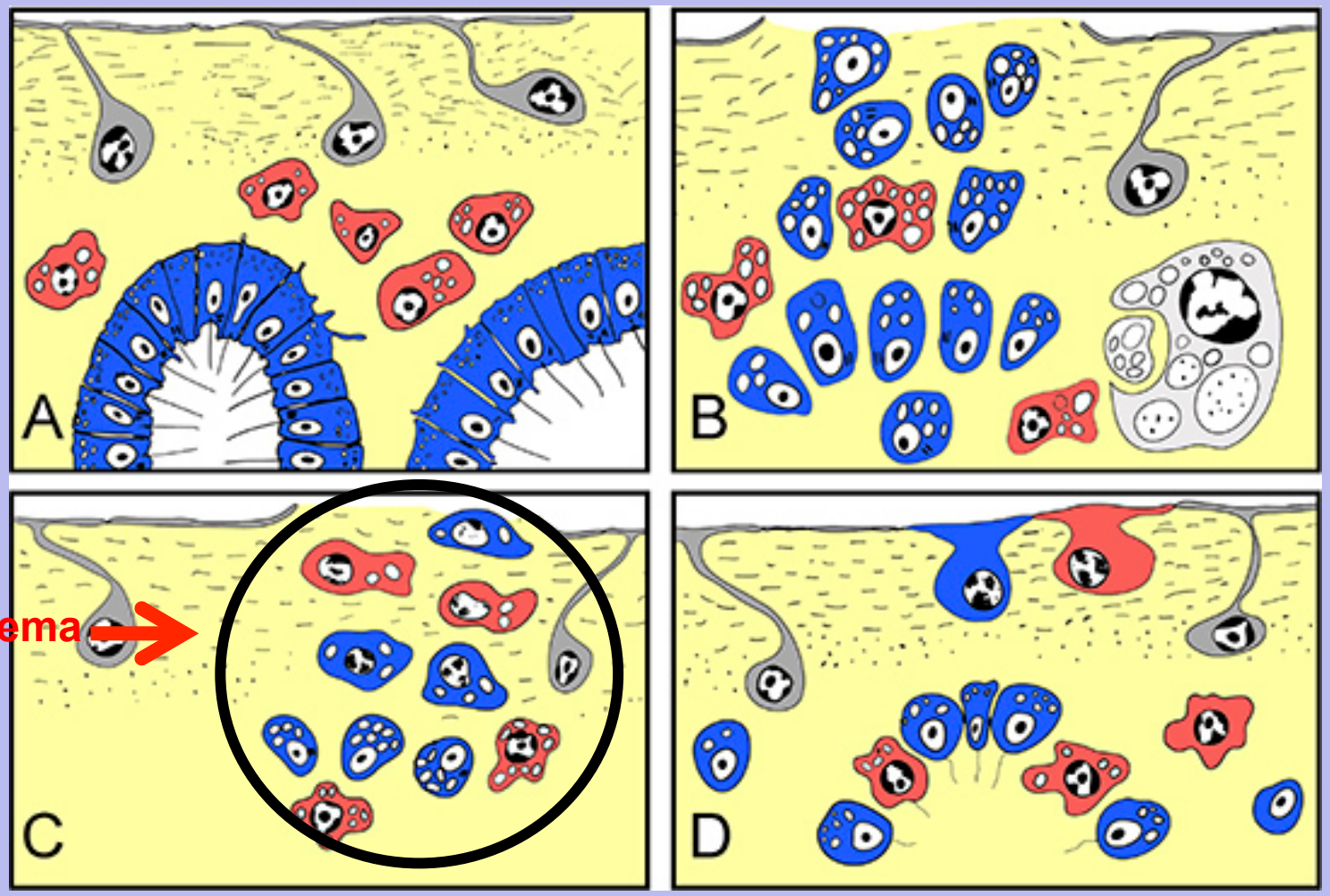




(Adamska et al in prep.)

Mesenchymal-epithelial transformations

Demospongiae

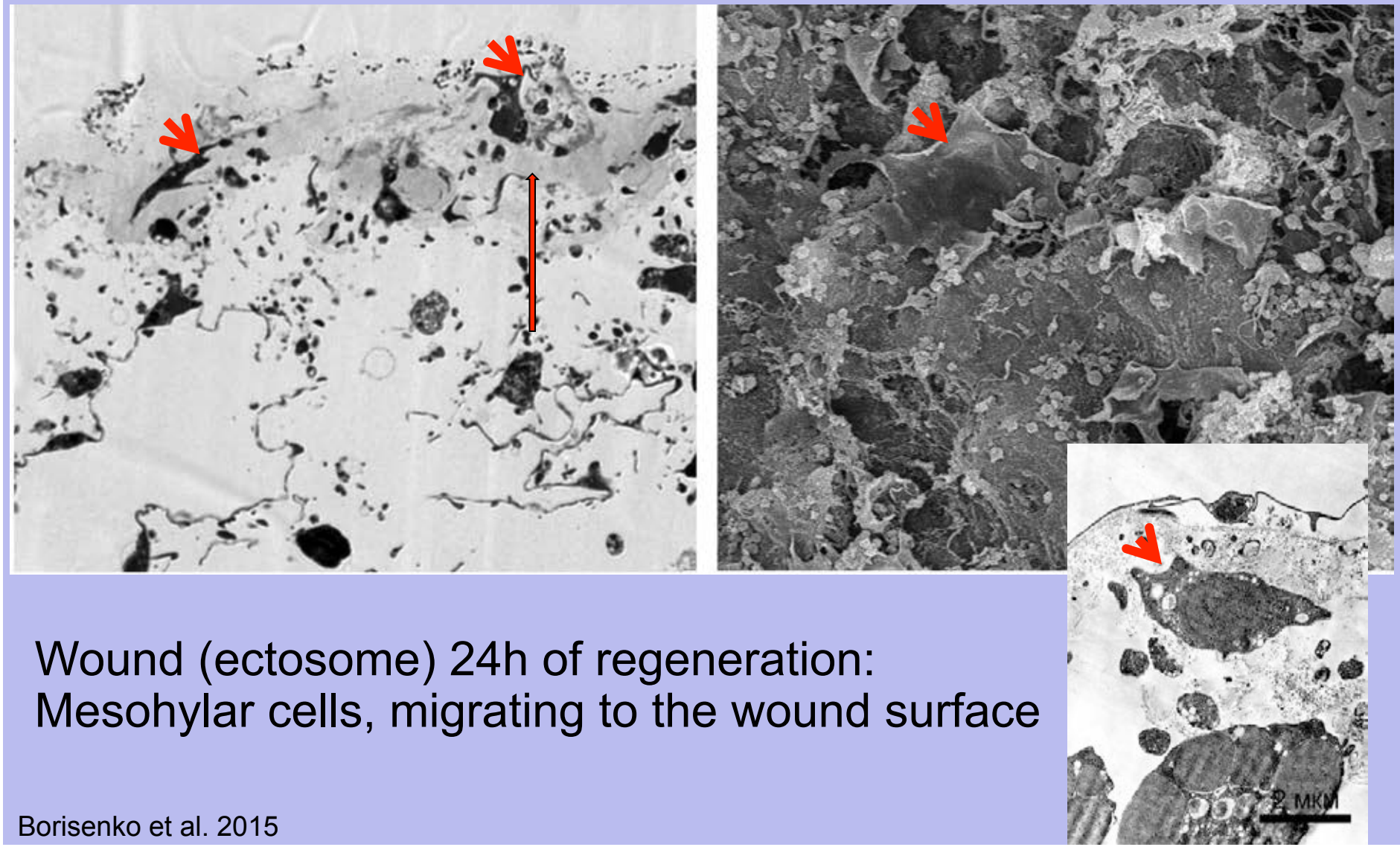


Halisarca dujardini regeneration and the origin of new exopinacocytes and choanocytes. (A) Intact sponge. (B) I stage of regeneration: formation of “regenerative plug”. (C) II stage of regeneration: wound healing and formation of a “blastema”. (D) III stage of regeneration: restoration of ectosome and choanosome. Grey—exopinacocytes, blue—choanocytes, red—archaeocytes.

(Borisenko et al. 2015)

Mesenchymal-epithelial transformations

Demospongiae*: Halisarca dujardini*

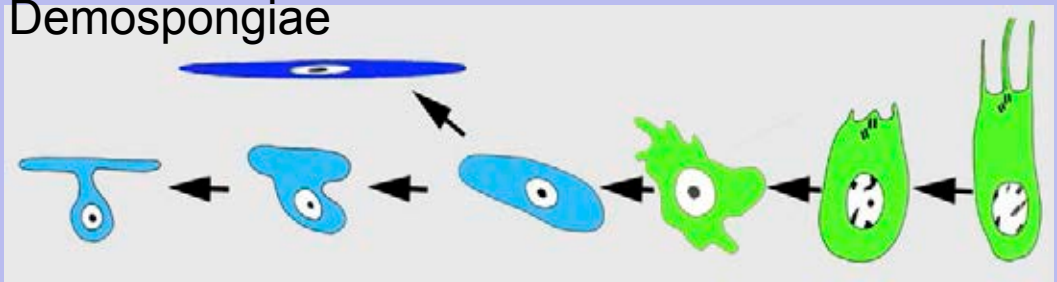


Wound (ectosome) 24h of regeneration:
Mesohylar cells, migrating to the wound surface

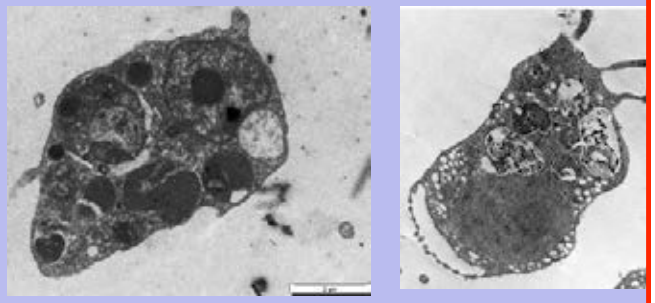
Borisenko et al. 2015

Choanocytes transdifferentiation during regeneration

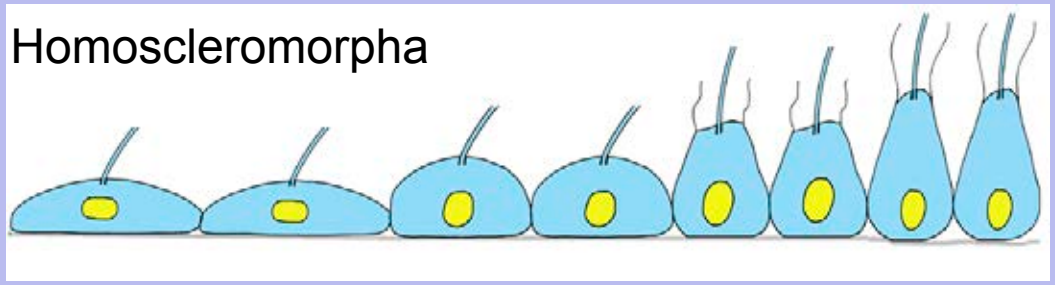
Demospongiae

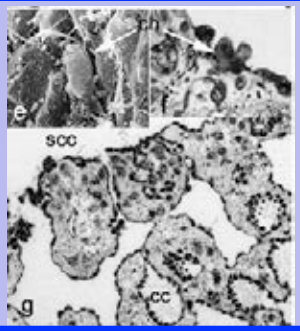


Halisarca



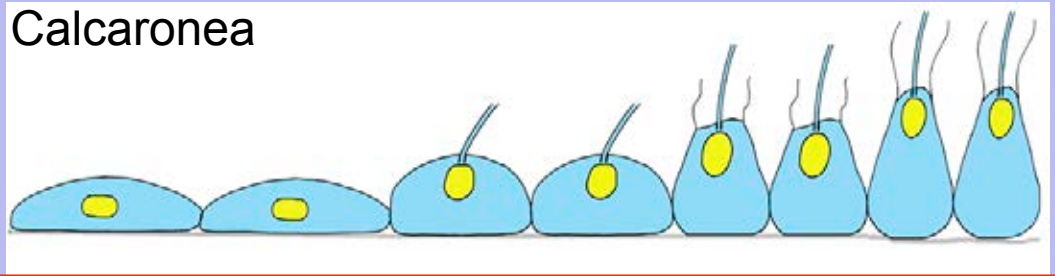
Homoscleromorpha

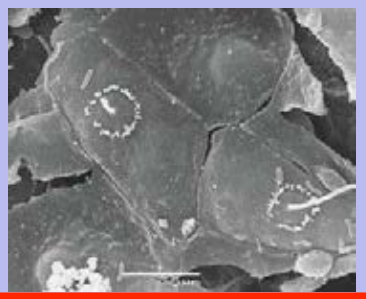


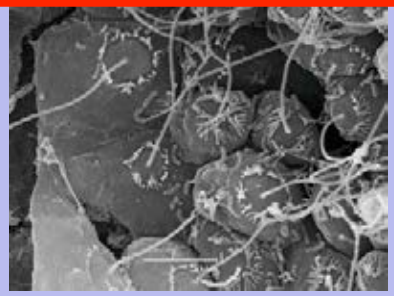


Oscarella

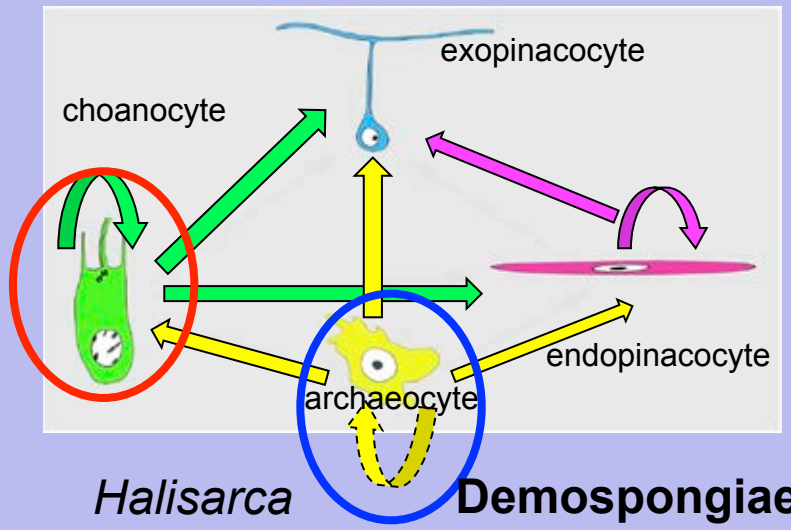
Calcaronea





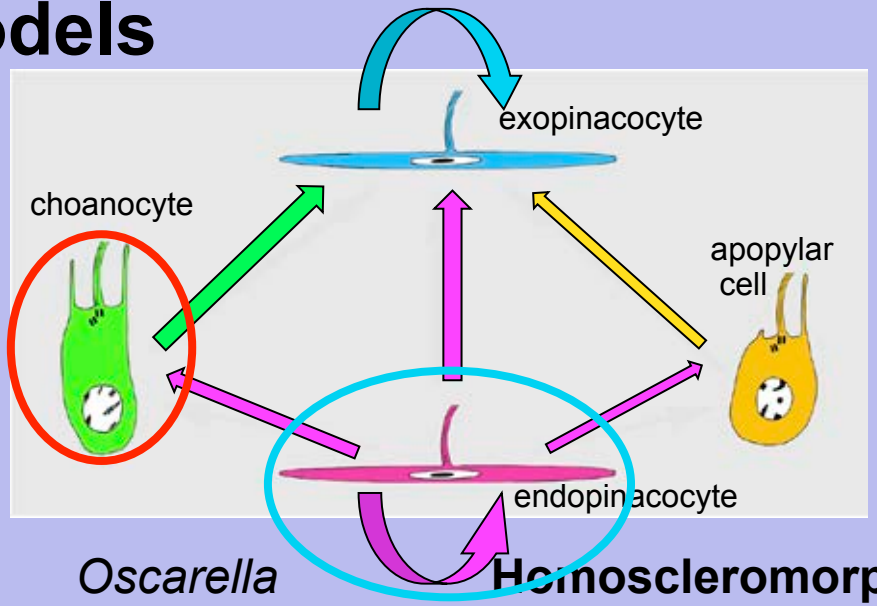


Different cells fate and the main sources of new exopinacoderm during a regeneration sponge models



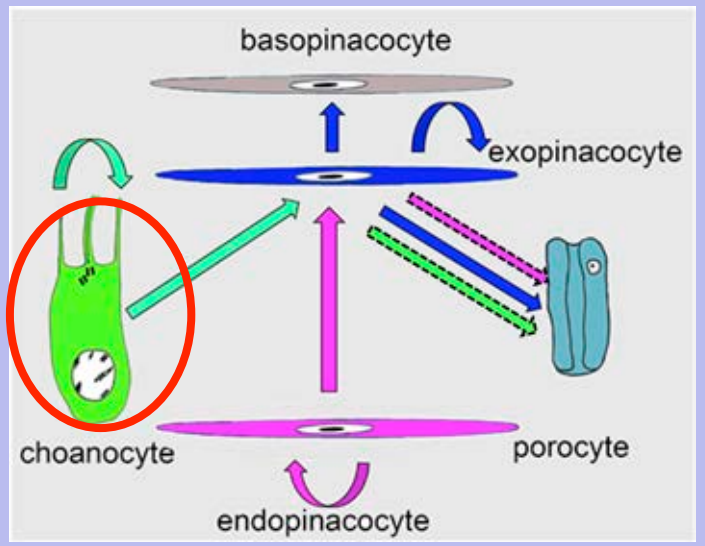
Halisarca

Demospongiae



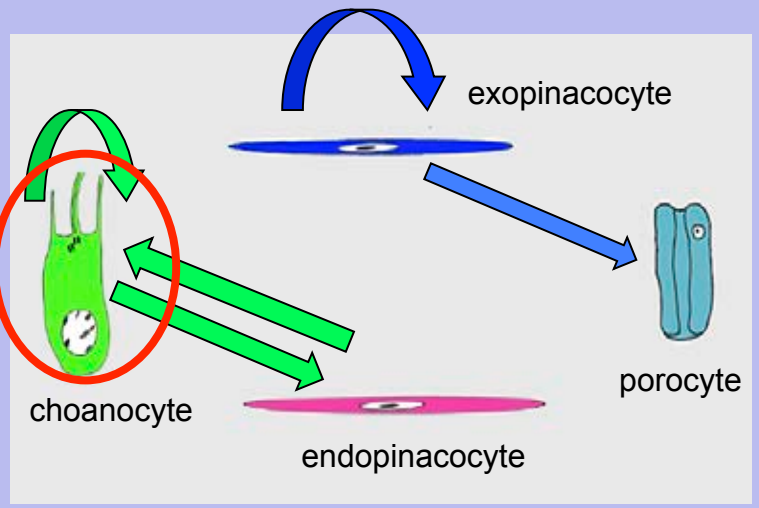
Oscarella

Homoscleromorpha



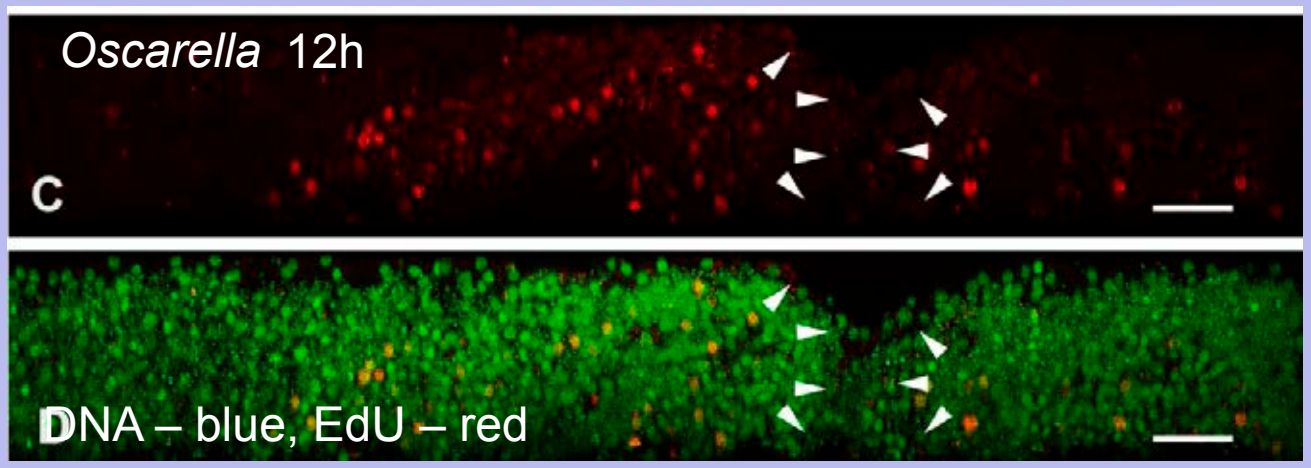
Sycon

Calcarea

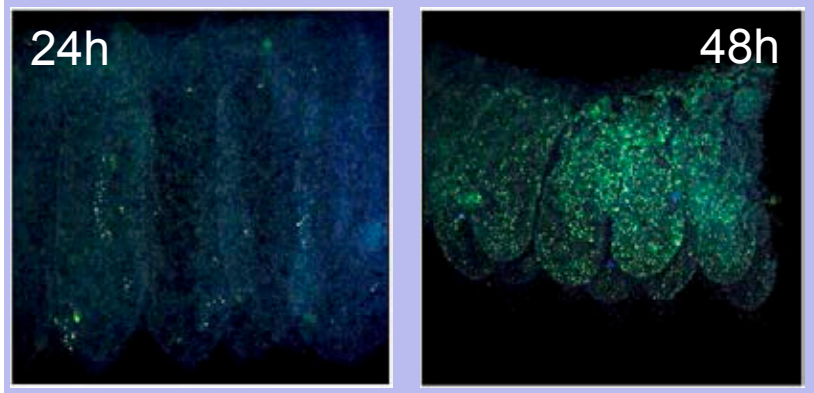


Leucosolenia

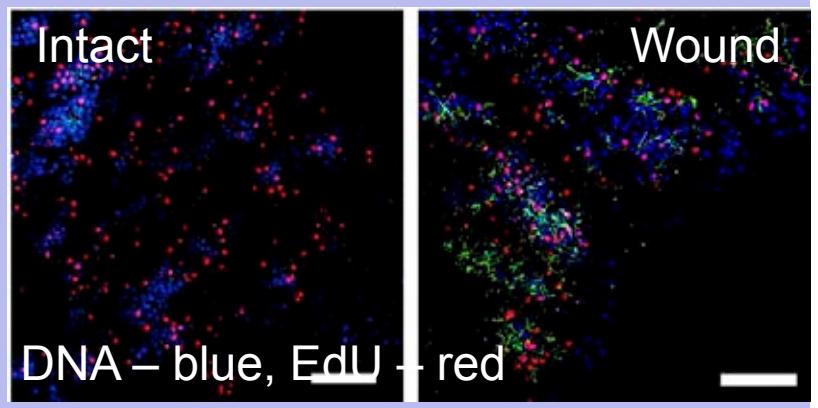
Cell proliferation during regeneration



Sycon



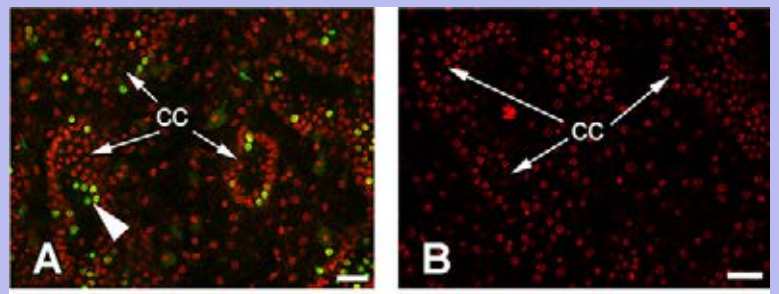
Leucosolenia 24h



Oscarella, Sycon, Leucosolenia: We did not detect any changes in cell proliferation neither in the wound nor in the adjacent intact areas. *Sycon, Leucosolenia:* **Proliferation is virtually absent from the forming regenerative membrane and is not limited to its vicinity.**

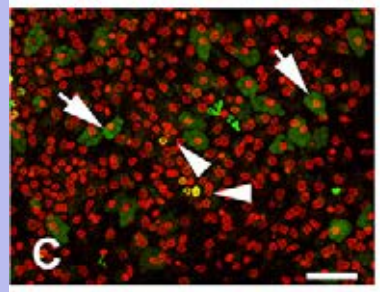
Regeneration in *Halisarca*: cell proliferation

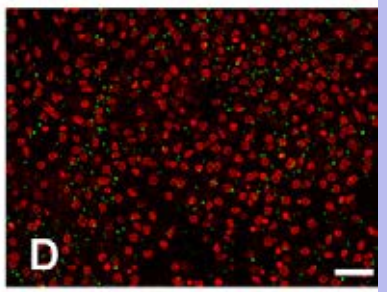
Unwounded sponge after 6 h incubation with EdU



Negative control for A without EdU

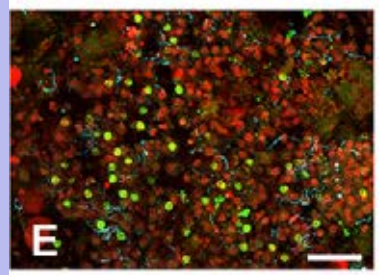
EdU incorporation after 24 h incubation

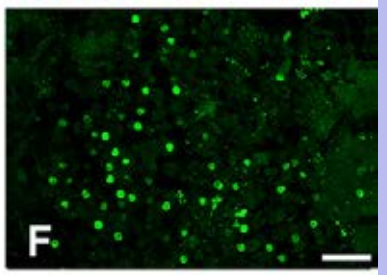




Negative control for C

Wound surface after 12 h of regeneration

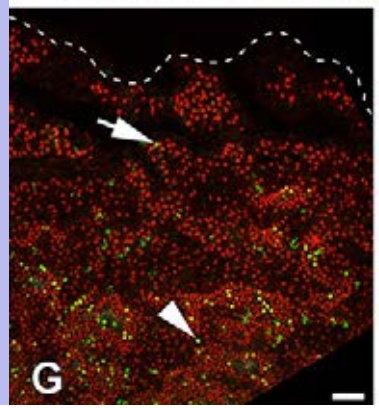


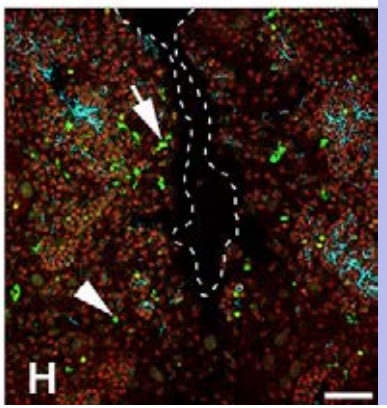


EdU only

Red—DNA, green —EdU

Wound surface at 24 h of regeneration Parallel section



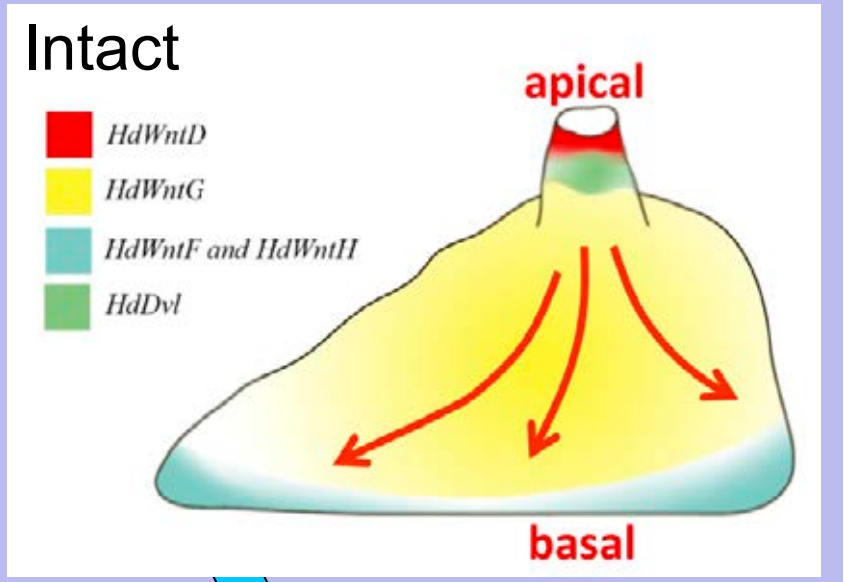


Perpendicular section of Wound surface at 24 h of regeneration

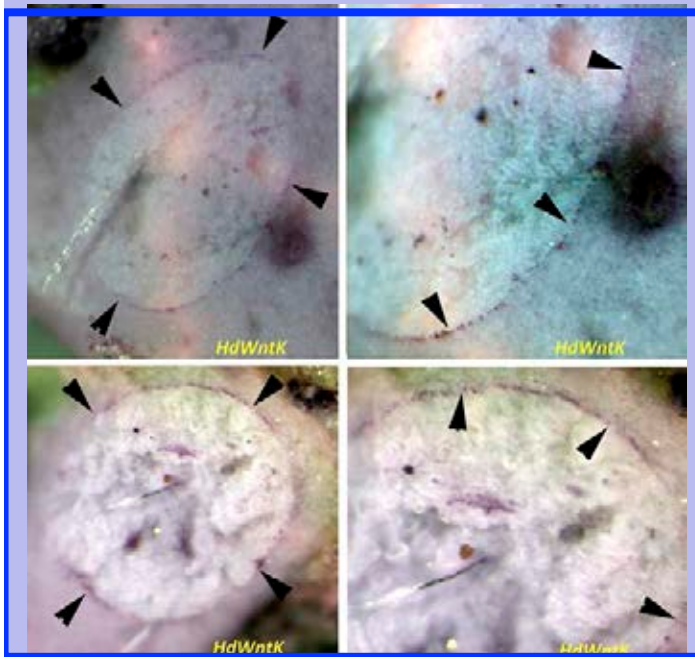
Borisenko et al. 2015

Wnt pathway is implicated in axial patterning and regeneration in the demosponge *Halisarca dujardini*

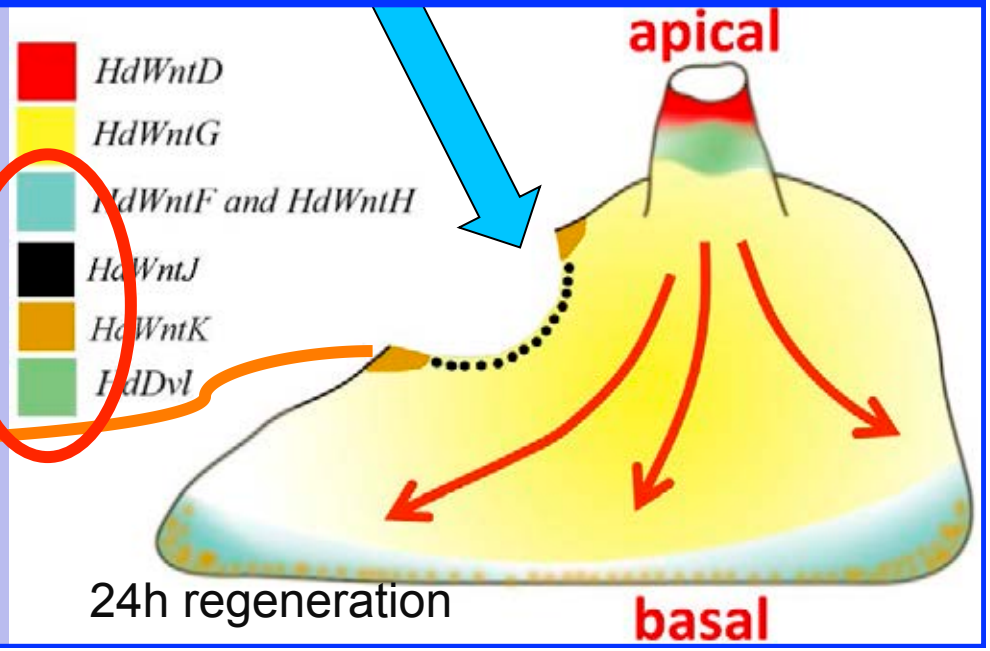
Multiple Wnt pathway components were identified, including 10 *Wnt* and 5 f*rizzled* genes, in addition to single *disheveled* and *beta-catenin* genes.



HdWntK at the wound border



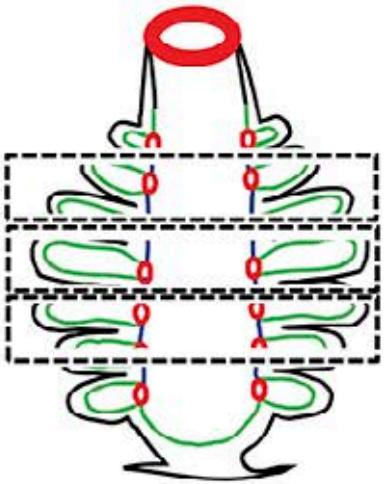
Borisenko et al. 2016

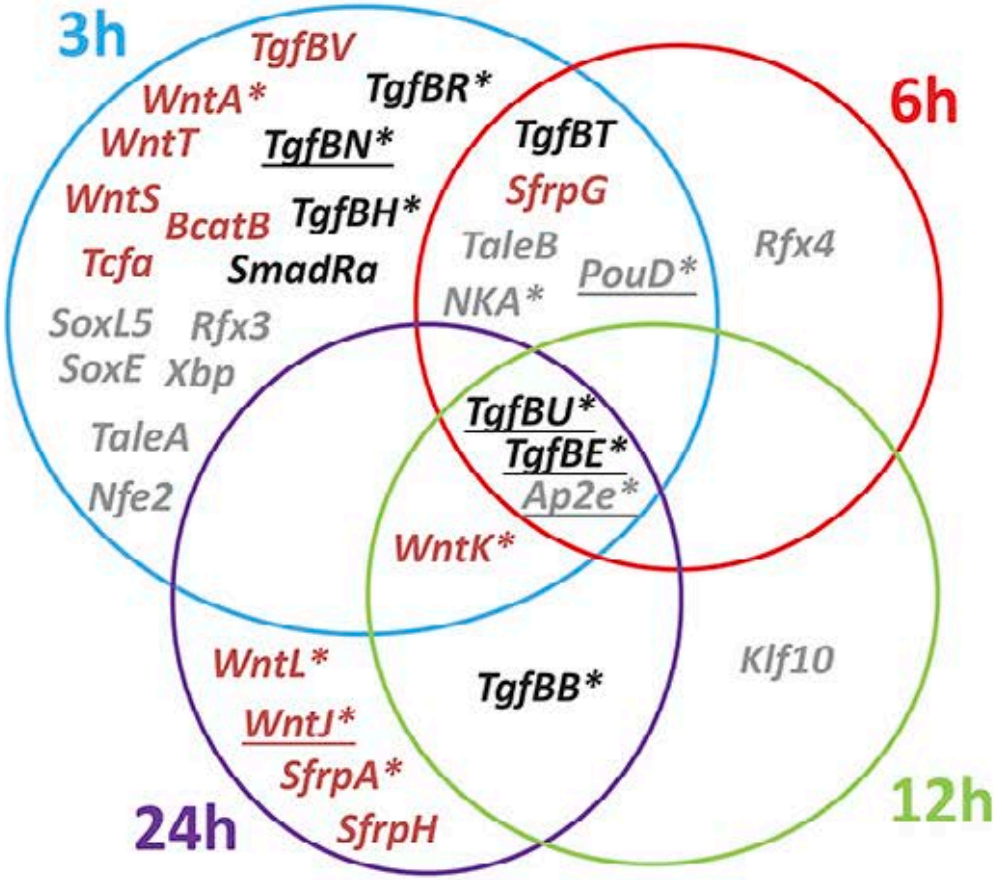


Regeneration in *Sycon*: detection of differentially expressed genes by RNA-Seq

Within hours of dissection, multiple Wnt and Tgf-beta pathway components, including their key transcrption factors are upregulated, as are also several other developmental transcription factors.

Many of these genes are highly expressed in the osculum of intact sponges.



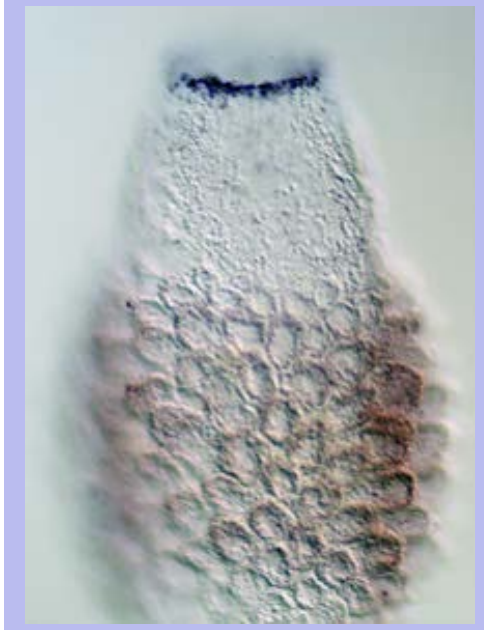


Genes* with statistically significant higher expression in top than bottom.
Genes* with statistically significant higher expression in top than middle.

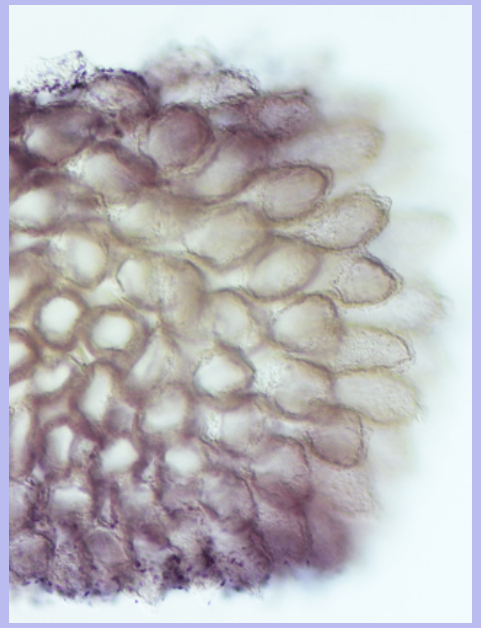
Adamska et al. In prep.

Expression of *SciTgfBU* during regeneration

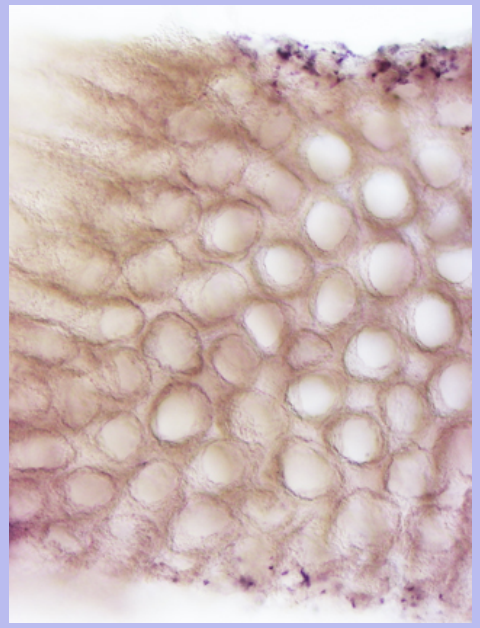
Intact specimen



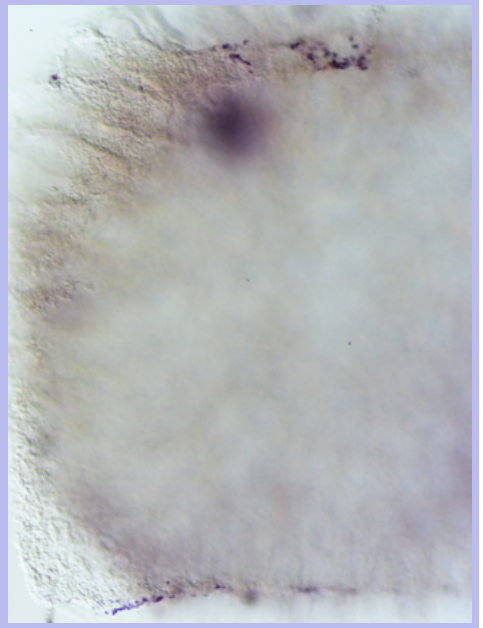
3 hrs



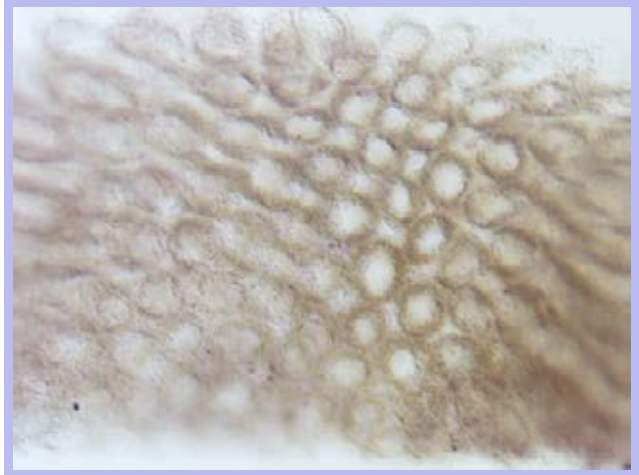
12 hrs



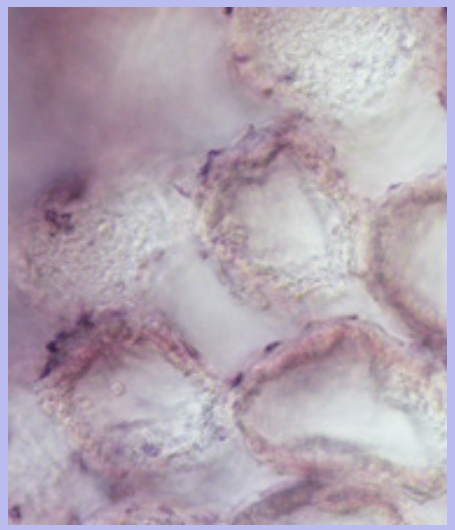
48 hrs



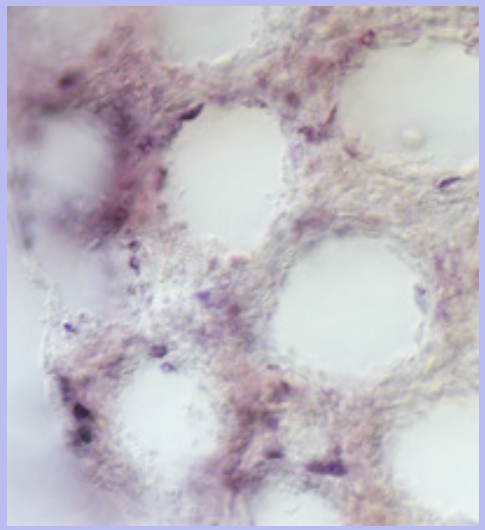
3/6 hrs control



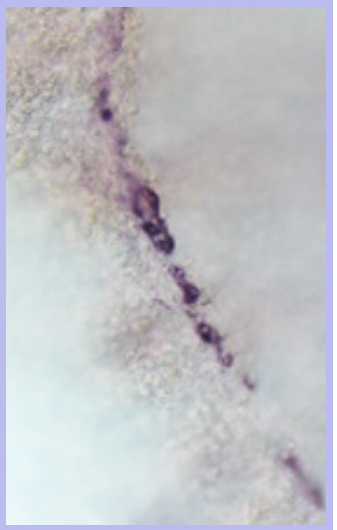
3 hrs



6 hrs



24 hrs

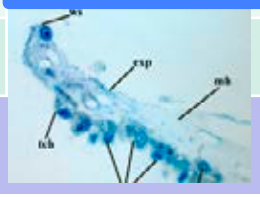


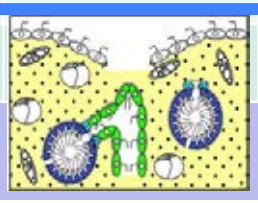
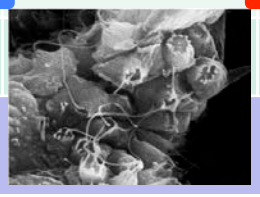
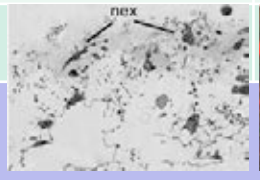
Main stages of regeneration in model sponges

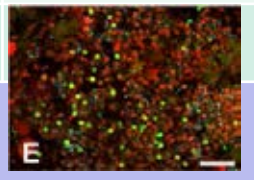
	Retraction of the wound surface	Regenerating "plug" formation	Alignment of the wound edges	Regeneration membrane (epithelization)	Formation of blastema	Restoration of ectosome & choanosome
Oscarella lobularis	+	+	+	+	-	+
Sycon ciliatum	-	+	+	+	-	+
Leucosolenia variabilis	+	-	+	+	-	+
Halisarca dujardini	+	+	+	+	+	+

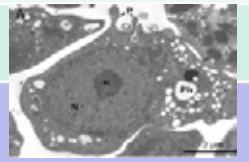
Basic morphogenetic and cellular processes during models regeneration

	Spreading (flattening) of epithelial sheets	Fusion of epithelial sheets	Cell transdifferentiation	Epithelial-mesenchymal transitions	Active local cell proliferation	Participation of stem-cells
Oscarella lobularis	+	+	+	-	-	+
Sycon ciliatum	+	+	+	-	-	+
Leucosolenia variabilis	+	+	+	-	-	+
Halisarca dujardini	-	-	+	+	+	+







Homoscleromorpha and **Calcarea** regeneration conclusions

- 1 - The basic morphogenetic processes during **Homoscleromorpha** and **Calcarea** regeneration are spreading (flattening) and fusion of epithelial sheets.
- 2 - This regeneration accompanied by **transdifferentiation** of differentiated cells in the wound area.
- 3 - The regeneration in **Calcarea and Homoscleromorpha** is **morphallactic**, when lost body parts are replaced by the remodeling of the remaining tissue accompanying with cells transdifferentiation.
- 4 - The **main sources** of new exopinacoderm are: intact **pinacoderm**, surrounding the wound surface, intact **choanoderm**.

Demosponges regeneration conclusions

1 - The main mechanism during ***Halisarca*** regeneration **is a mesenchymal morphogenesis by mesenchymal-epithelial transformations**.

2 - This regeneration involves intervention of **polypotent cells** - ***archaeocytes*** and ***choanocytes*** - that migrate to the injured area where form a **blastema** with dedifferentiated cells.

3 - The **regeneration in *Halisarca*** has **epimorphosis** features that require blastema formation, active cellular dedifferentiation and proliferation prior to the replacement of the lost body part.

4 - There are **three main sources of the new exopinacoderm** during regeneration: choanocytes, archaeocytes and (rarely) endopinacocytes.

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