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## REPORT OF MEETING

**2<sup>nd</sup> workshop of the COST Action 16203 MARISTEM: OMIC APPROACHES TO IDENTIFY AND CHARACTERIZE MARINE/AQUATIC INVERTEBRATE STEM CELLS, Escola Superior de Turismo e Tecnologia do Mar, Peniche, Portugal**

Organizers: **A Varela Coelho (chair)<sup>1</sup>, F Herrera<sup>1</sup>, S Blanchoud<sup>2</sup>, SM<sup>3</sup> Leandro**

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**Identification and relative quantification of asterosaponins isolated from *Marthasterias glacialis* coelomic fluid during arm-tip/radial nerve cord regeneration**

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Regenerative potential is commonly observed in echinoderms. Starfishes are well-known echinoderms, which are capable of reconstructing external appendages and internal organs often subjected to amputation. The coelomic fluid bathes the internal organs, it transports the circulating cells and signaling compounds.

The objective of this work was to identify the molecular species present in the coelomic fluid that could play an important role in the regeneration process of the starfish *Marthasterias glacialis*.

Aiming that, a protocol to extract compounds present in the cell free coelomic fluid (CFF) from control and regenerating (2, 13 and 70 days post-amputation) groups was optimized. The regeneration process was induced by amputation of 2 arm tips or partial removal of 2 radial nerve cords per starfish. The solid phase extraction (SPE) chromatography with acetonitrile discontinuous gradient was used to elute CFF compounds. SPE eluted fractions were analyzed by ESI-MS/MS in positive and negative mode. More intense *m/z*

signals were detected for negative molecular ions with monoisotopic and mass losses characteristic of asterosaponins. These pentaglycoside or hexaglycoside sulfated steroids have several bioactive properties, namely at the antimicrobial, immunological, physiological and pharmacological levels.

Our results show reproducible asterosaponin profiles for each regeneration time point, suggesting their promising participation in the regeneration process.

**Echinoderms are valid deuterostome marine invertebrate models to study repair phase events after arm injury**

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investigating stem cell involvement in developmental pathways.

### **Components of TGF-beta signaling pathway in the sponge *Halisarca dujardini*.**

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TGF-beta pathway is one of the major signaling mechanisms that orchestrate development of multicellular organisms. Axial patterning in embryo development is the brightest example of its role. Thus, asymmetric expression of TGF-beta superfamily ligands determines of dorso-ventral axis in bilateral animals. Ligands from several families manage the different embryonic morphogenesis through regulation of proliferation, differentiation, apoptosis, cytoskeleton, adhesion and cell migration. Functions of TGF-beta pathway established early in evolution of multicellularity as ligands or receptors not found in Protista, whereas in Cnidaria this pathway determines the directive axis of body. At the same time, TGF-beta expression data from sponges' development tell us about involvement of TGF-beta pathway in axial patterning of Porifera embryo. Number of ligands may be as many as eight in demosponge *Amphimedon queenslandica* or twenty-two in calcisponge *Sycon ciliatum* so sponges are not simple in sense of ligand repertoire in comparison to Bilateria. In this study we search through transcriptome and analyze ligands, receptors and cytoplasmic messenger proteins of TGF-beta pathway in sponge *Halisarca dujardini* (Demospongiae). Eight ligand sequences were identified, and their primary structure and domain organization correspond to eumetazoan TGF-beta. Three of eight identified ligands can be classified as TGF-beta *sensu stricto* when next five are not fall into any of eumetazoan family of TGF-beta. With hybridization in situ and quantitative transcriptomics shown that expression of some ligands restricted to oscular tube similar to Wnt expression pattern. Similar situation described for Wnt pathway ligands in sponges, and it shows independent involvement end lineage-specific expansion of some signaling pathways members inside Porifera phyla.

### **Study of structural and functional proteins in the sea anemone *Actinia fragacea* (Cnidaria) and potential biomedical interest**

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Marine biological resources provide a diverse source of proteins with application in several

biotechnological fields due to their broad structural and biological properties.

Cnidarians are examples of marine animals with biotechnology interest. These soft body animals are distinct as they possess a unique organ specialized in the production of toxins. Research has been performed in several species due to their ecological importance (e.g. jellyfish blooms; coral reefs), regenerative capacity and for their bioactive compounds. More recently, other biotechnology interests emerged including collagen of jellyfish as an alternative to mammal collagen and adhesives proteins of hydrozoans, in view of the development of biomimetic adhesives and antifouling compounds.

Their basic features, ecology and high diversity make cnidarians interesting models in different biotechnological fields and many are potential sustainable resources making important the investigation in different fields (e.g. chemical and biochemical composition, physical-chemical features, screening of bioactive molecules and microbioma). In this study we propose to perform molecular biology, proteomic tools and other protein characterization techniques in the pedal disc and tentacles of *Actinia fragacea* to analyze the collagen and protein adhesive molecular features. This study could provide information of interest in the biomedical field, with focus on the development of biomaterials for tissue engineering, wound healing and drug delivery.

### **Recovery process in sponges: morphogenesis and cell sources**

**AI Lavrov<sup>1,2,3</sup>, FV Bolshakov<sup>1,2</sup>, IE Borisenko<sup>2</sup>, VS Frolova<sup>4</sup>, DB Tokina<sup>5</sup>, AV Ereskovsky<sup>2,5</sup>**

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Sponges (Porifera) represent one of the most ancient metazoan lineages. They possess unique anatomical and tissue structure, making them promising models for evolutionary studies. The high plasticity of sponge tissue and cells provide them with outstanding recovery abilities, ranging from wound healing to re-building of a functional body from dissociated cells.

We have combined several microscopy techniques to elucidate morphogenesis, cellular mechanisms and cell sources during reparative regeneration and cell reaggregation in five species from different clades: *Halisarca dujardini* (Demospongiae), *Aplysina cavernicola* (Demospongiae), *Sycon* sp. (Calcarea),

*Leucosolenia variabilis* (Calcarea) and *Clathrina arnesenae* (Calcarea).

The main mechanism of reparative regeneration in studied Demospongiae is cell migrations and epithelio-mesenchymal transformations, involving archaeocytes and choanocytes, which are a cell source for the recovery of lost structures. In contrast, the reparative regeneration in Calcarea occurs due to extensive remodeling of intact tissues near the wound through epithelial morphogenesis, accompanied by cell transdifferentiations.

The cell re-aggregation in both Demospongiae and Calcarea involves mass cell dedifferentiation on the early stages of the process. During progressive development of multicellular aggregates, individual cell migrations and transdifferentiations ensure restoration of required cell types and intact anatomical structures. However, epithelial morphogenesis contributes to the development of calcareous sponge aggregates.

Thus, during recovery processes, sponges utilize diverse and complex morphogenetic mechanisms, with a particular importance of cell transdifferentiation. While all sponges demonstrate high recovery abilities, the morphogenesis and cell sources for the recovery of lost structures varies in different clades.

#### **Phylogenetic and phylogeographic resolution of *Botrylloides leachii* (Savigny, 1816) in North-eastern Mediterranean**

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Globally distributed sea squirts, tunicates, are one of the most diverse taxons among chordates. Two genera of colonial tunicates, *Botryllus* thought to originated in Mediterranean Sea including 32 and *Botrylloides* with 19 species, are botryllid ascidians spread among all of the world seas. The presence of 6 botryllid species in the Eastern Mediterranean Sea particularly in Israel, Egypt and Gulf of Suez shore are confirmed which are *Botrylloides leachii*, *Botrylloides niger*, *Botrylloides anceps*, *Botrylloides israeliense*, *Botryllus schlosseri* and *Botryllus rosaceus*.

Tunicates being the closest invertebrate relatives to the vertebrates are one of the widely used model organisms especially in developmental biology and immunology studies. Considering their unique characteristics as including the only chordate group having the ability of whole-body regeneration, *B. leachii* has a pivotal role in these studies with another special feature by undergoing into hibernation when the ambient conditions are not favorable. In this study, we target to determine the evolutionary phylogenetic status of *B. leachii* by estimating its resolution and biodiversity in the North-eastern Mediterranean Sea at 8 spatial stations from Antalya to Hatay regions employing mitochondrial and nuclear DNA markers (COI and H3) on over hundreds of samples. Also the morphological investigation of the species is

performed and blastogeny (an asexual reproduction type) is analyzed. Besides, Kizkalesi station selected as transient sampling residing is monitored through a year to understand the biodiversity characteristics combined with several ecological parameters such as salinity, temperature and tidal rhythms.

#### **Deciphering target genes of the *Hydra* head inhibitor Sp5**

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In 1744, Abraham Trembley discovered one of the most fascinating characteristics of the *Hydra* model organism: its amazing ability to regenerate from any missing body part. But how does this animal trigger regeneration? The head organizer, located in the most apical part of the *Hydra*, produces two signals, the head activator and the head inhibitor, the latter preventing ectopic head formation in intact and regenerating conditions.

Even though *Hydra* is used as a model system since more than 250 years, the *Hydra* head inhibitor, a protein named Sp5, was just discovered recently. The expression of Sp5 is under the control of Wnt/ $\beta$ -catenin signaling, is mainly expressed in the head and re-expressed during regeneration. Sp5 RNAi triggers multiple head formation in intact and regenerating animals and Sp5 inhibits Wnt/ $\beta$ -catenin signaling by repressing the expression of Wnt3. In addition, Sp5 has an activating effect on its own promoter, suggesting that Sp5 acts as a transcriptional repressor and activator (Vogg *et al.*, 2019 Nature Commun). Here we present an approach for the identification of Sp5 target genes. Finding Sp5 target genes and deciphering their role will help to understand if Sp5 works alone or in cooperation with other genes to restrict *Hydra* head formation.

#### **Stem cells Identification in regenerating tissues of the scleractinian coral *Stylophora pistillata***

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Stem cells in some cnidarian taxa were identified as far back as the 19th century. The first cells to be identified were the i-cells in *Hydra*. Since then, along with the constant increase in the number of tools in the histological and molecular toolbox, more data has been accumulated regarding stem cells and cell dynamics in the rest of the Cnidarian classes. One class that has been solely neglected is the Anthozoa, and specifically the scleractinian corals. Coral stem cells weren't documented so far, leading to the assumption that corals are lacking in somatic stem cells. Therefore, the common theory is that their ectodermal and endodermal epithelial cells