

REPORT OF MEETING

2nd 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)¹, F Herrera¹, S Blanchoud², SM³ Leandro**

¹*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal*

²*Department of Biology, Université de Fribourg, Germany*

³*Escola Superior de Turismo e Tecnologia do Mar, Peniche, Portugal*

This is an open access article published under the CC BY license



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

L Gomes, R Lares, V Marques, F Brigham, P Lamosa, L Gafeira, AV Coelho

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

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

C Ferrario^{1,2}, Y Ben Khadra³, A Czarkwiani⁴, A Zakrzewski⁴, P Martinez^{5,6}, F Bonasoro¹, MD Candia Carnevali¹, P Oliveri⁴, M. Sugni^{1,2}

¹*University of Milan, Department of Environmental Science and Policy, Milan, Italy*

²*University of Milan, Center for Complexity and Biosystems, Department of Physics, Milan, Italy*

³*Université de Monastir, Institut Supérieur de Biotechnologie de Monastir, Laboratoire de Recherche, Génétique, Biodiversité et Valorisation des Bioressources, Monastir, Tunisia*

⁴*University College London, Department of Genetics, Evolution and Environment, London, United Kingdom*

⁵*Universitat de Barcelona, Departament de Genètica, Microbiologia i Estadística, Barcelona, Spain*

⁶*Institut Català de Recerca i Estudis Avançats, Barcelona, Spain*

Echinoderms are often subjected to traumatic amputations that damage or remove whole body parts i.e. arms. After such severe injuries, the repair phase must be effective with rapid emergency reaction and re-epithelialization as well finely regulated extracellular matrix (ECM) remodeling to ensure subsequent arm regeneration.

Here, we used the brittle star *Amphiura filliformis* (Ophiuroidea) and the starfish *Echinaster sepositus* (Asteroidea) as valid deuterostome marine invertebrate models to study similarities and differences in the repair phase phenomena of these two echinoderm species and discuss them in comparison with those of animals with limited regenerative abilities (i.e. mammals). To achieve this goal, we used an integrated approach based on both microscopy and molecular analyses.

We showed that in both echinoderm models, immediately after injury, emergency reaction and re-epithelialization are extremely rapid and more efficient than those displayed by mammals. The remodeling and the formation of the ECM, mainly collagen, is ensured by delayed activation of ECM genes and protein deposition and, together with absence of fibrosis (i.e. over-deposition of ECM), seem to be advantageous for regeneration-competent animals in comparison to mammals.

Overall, we found that the echinoderm species here studied show comparable repair events. The differences between regeneration-competent and non-competent animals suggest that rapid wound closure and delayed ECM deposition are necessary to ensure an effective regeneration of whole lost body parts. Further molecular and functional analyses must be performed to confirm this hypothesis.

Complement components as markers of hemocyte differentiation in the colonial ascidian *Botryllus schlosseri*

A Peronato, N Franchi, L Ballarin

Department of Biology, University of Padua

The complement system is one of the immune modulator mechanism of metazoans. The complement system of vertebrates is a complex array of soluble and membrane proteins able to orchestrate ancient immune responses such as inflammation and phagocytosis. Three complement-activation pathways are known in vertebrates: the classical, the alternative and the lectin pathways: all of them converge on the cleavage of C3.

Complement in invertebrates have been much less studied; however, C3 genes have been identified in representatives of all the major invertebrates phyla, starting from basal metazoans such as Porifera. As an invertebrate, the compound ascidian *Botryllus schlosseri* relies only on innate immunity for its defense and immunocytes (i.e., cells with defined roles in immunity) represent the great majority of the circulating hemocytes: they include cytotoxic morula cells and phagocytes. In the same species, we demonstrated the presence of the lectin

and the alternative pathways. All the complement components identified so far (C3, Bf, MBL, ficolin and MASP), are expressed by morula cells, the most abundant circulating hemocyte, the other immunocytes being represented by phagocytes.

My project aims to use C3 transcript as a signature of morula cells to study their differentiation from hematopoietic cells during ontogenesis and blastogenesis. In the first case, I will carry out in situ hybridization and PCR on larvae, whereas, in the case of blastogenesis, I will investigate the quantity of C3-positive cells in the pharyngeal niches recently identified in the various phases of the colonial blastogenetic cycle.

Stem cells roles in the aging marine colonial invertebrate model animal, *Botryllus schlosseri*

O Ben-Hamo^{1,2}, B Rinkevich¹, R Ben Shlomo³, L Ballarin⁴

¹*Israel Oceanographic and Limnological Research, National Institute of Oceanography, P.O. Box 8030, Tel Shikmona, Haifa 31080, Israel*

²*Department of Evolutionary and Environmental Biology, University of Haifa, Israel*

³*Department of Biology and Environment, Faculty of Natural Sciences, University of Haifa – Oranim*

⁴*Department of Biology, University of Padua, Padua, Italy*

The aging process of living beings is one of the most intriguing and less understood biological phenomena. Stem-cells (SCs) may participate as effectors in aging. *Botryllus schlosseri*, a marine colonial invertebrate is an interesting model for the studying of aging since it ages both at the level of the entire colony, a process that takes months/years, and at the level of the temporary modules that live in a colony for three weeks and constantly replaced by younger asexually budded modules (20 °C, temperature-dependent). In *Botryllus* Cell-island (CI) niches of SCs were discovered in proximity to the endostyle organ of the zooids (mature modules). In diverse SC niches in mice and human, their number rises throughout the course of aging, while their functionality declines, and they undergo inevitable exhaustion. Our research aims to elucidate changes in hematopoietic stem cell (HSCs) numbers/behaviours along two aging processes in *Botryllus*. The research focuses on SCs in the hemolymph and in the CI niches of *Botryllus*. Preliminary observations using electron microscopy (TEM) on labelled SCs in the CI niches in *Botryllus* show that old colonies (8 months old) have 6 times more SCs than young colonies (3 weeks old). Regarding the level of the zooid, old zooids have 3.6 times more SCs compared to young zooids. Further observations should be carried out soon to delineate the pattern of SCs in the hemolymph of old/young colonies and of zooids along the life cycle.

Transcriptomic profiling of the mussel *Mytilus trossulus* with a special emphasis on integrin-like genes during development

M Maiorova¹, N. Satoh², K. Khalturin², N. Odintsova¹

¹National Scientific Center of Marine Biology; The Far Eastern Branch of the Russian Academy of Sciences, Vladivostok; Russia

²Marine Genomics Unit, Okinawa Institute Science & Technology; Okinawa; Japan

A prerequisite for the emergence of long-lived multicellular organisms was the evolution of intercellular adhesion mechanisms. A set of positively selected genes related to integrin complex was identified in the transcription profiles of the bivalve mollusk *Mytilus trossulus*, and changes in expression of candidate genes involved in cell adhesion were determined. The study is based on the Illumina RNA-sequencing data obtained for a *de novo* assembly of the transcriptome from early developmental stages and some tissues and cells. Our transcriptome dataset supplements to the genetic databases of non-model animals such as Bivalves and represents the first characterization of expressed sequences during early development of *M. trossulus* from the Sea of Japan. A total of 200079 contigs were obtained, and based on the GO terms, the number of annotated contigs was estimated to reach 19.96 %. The main findings include evidence that the predicted mussel β integrin-like protein sequences are most closely related to the integrins sequenced for all classes of Mollusca, while the highest similarity is observed between mussel and oyster proteins. Additionally, an analysis of the transcriptome revealed four full-length transcripts that seemed to be isoforms of two genes encoding β integrin-like proteins. The present study provides a transcriptome that can serve as a reference for future studies of *Mytilus* in the marine ecosystems.

Whole genome sequencing and gene editing via silencing RNA in some botryllid ascidians

FN Oğul, A Karahan

Institute of Marine Science, Middle East Technical University, Mersin, Turkey

Botryllid ascidians are the closest relative of vertebrates, they have notochord in their free swimming larval stage and by metamorphosis they lose their notochord and live as sessile near shoreline. They can reproduce both sexually and asexually. By asexual reproduction they form colonies from an individual (zooid) following sexual reproduction. During asexual reproduction each zooid regresses in approximately one week, and next generation is formed by budding and this process is called blastogenesis. Furthermore, it is known that they undergo whole body regeneration although closest relative vertebrates have limited capacity of regeneration at tissue or organ level. During both blastogenesis and whole body regeneration, inhibitor apoptosis proteins have a great importance in order to control apoptosis. We also know that inhibitor apoptosis proteins are related a variety of human diseases such as cancer, thus it is important to understand the mechanism of them. Botryllid ascidians have an important evolutionary spot and they can be a great model organism for further understanding of vertebrate evolution. In

the present study we will determine a model organism from botryllid ascidian species and conduct a whole genome sequencing in order to determine apoptosis related genes. Once we determine the genes we will conduct a series of knockdown experiments by using siRNA and CRISPR method. At the end of the study we believe that the study provides extensive insight on the signal mechanism and roles of inhibitor apoptosis proteins.

Dynamics of circulating coelomocytes populations during starfish regeneration

B Simões¹, B Oliveira¹, S Guatelli², M Sugni², R Zilhão³, AV Coelho¹

¹*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal*

²*Department of Biosciences, University of Milan, Milan, Italy*

³*Faculdade Ciências Universidade Lisboa, Lisboa, Portugal*

The potential for regeneration has its maximum expression in echinoderms. Preliminary studies of regeneration in echinoderms were based on the determination of growth rates and on the morphological, histological and cellular basis of this phenomenon. More recently, some advances have been made in the characterization of the molecular mechanisms involved in the regeneration process of tissues and organs. Studies that have been developed in our laboratory focus on a starfish species common in Portuguese coast with high regeneration capability, *M. glacialis*. Coelomic fluid, the main intra-tissue communicating medium in echinoderms, contains different types of cells, generally called coelomocytes, which are believed to participate in several functions such as, nutrient storage, gas exchange, production of connective tissue components, immune defense and tissue regeneration upon natural amputation. Although there is a generally accepted morphological identification of five types of coelomocytes there is no uniform criteria until now on their classification, as well as a correlation between morphology and the above specified functions. Despite, this lack of knowledge, coelomocytes are hypothesized as the most actively involved elements during the repair phase of asteroid arm regeneration.

Coelomocyte populations from *M. glacialis* were characterized by flow cytometry and microscopy in our group. These results were used to define sorting strategies by flow cytometry, in order to perform their individual transcriptome characterization. This characterization will be used to identify coelomocytes functional activities and to follow their circulating dynamics during regeneration. Additionally, identified specific biomarkers for each population will allow to generate probes for flow cytometry and *in situ* microscopy localization of tissue localized coelomocytes.

Weekly regeneration of central nervous system in the colonial tunicate *Botryllus schlosseri*

C Anselmi, F Gasparini, L Manni

University of Padua, Department of Biology

The study of neural stem cells and CNS development, regeneration, and organization, is an active field of research aiming to identify neural stem cells, diverse neurons, and CNS supporting cell types, to better understand the individual pathways and cell intrinsic factors leading to the formation of an adult brain. We used *Botryllus schlosseri*, an invertebrate chordate with a simple CNS and a robust regenerative capacity to investigate the possible presence and role of adult neural stem cells. Unlike most species, where the body is long lived and maintained by cellular replacement, *B. schlosseri* regenerates new colonial units (buds) on a biweekly basis from stem cells that remain for life, replacing the previous generation zooids, which then die through massive apoptosis. This cycle of development includes the formation of all body organs, including the neural complex. The latter is composed of a cerebral ganglion (brain), associated to a neural gland and a dorsal organ. During bud development, the neural gland and dorsal organ rudiments produce the brain cells. We studied the brain organization during the adult life using immunolabeling and confocal microscopy, finding that its cell number changes with a specific trend. Moreover, histological serial sections of adult neural complex at different stages of adult life indicated that the neural gland and the dorsal organ could represent a source of new neurons also during adult life. All these evidences suggest that *B. schlosseri* possesses adult stem cells involved in the neural complex maintenance.

Proteomic approaches for shell biominerals: insights into the biomineralization of Mediterranean spiny oyster *Spondylus gaederopus*

J Sakalauskaite^{1,2}, B Demarchi¹, Marin F²

¹*Department of Life Sciences and Systems Biology, University of Turin, Italy;*

²*UMR CNRS 5561 Biogéosciences, University of Burgundy-Franche-Comté, Dijon, France.*

Mollusca is one of the most diversified metazoan phyla and their ability to form an exoskeleton represents a true innovation from an evolutionary point of view. Shell biominerals possess exceptional material properties (e.g. nacre is outstandingly resistant!) and long have been an inspiration for biomimetic materials.

The current knowledge on shell biomineralization processes is mainly attributed to the development of 'omics-based techniques which have completely revolutionized the field enabling to study the processes at the molecular level. Yet, uncovering the ancient 'biomineralization toolkit' seems still too ambitious, owing to the great diversity of shell structural architectures, size of

phylum and the lack of studies for non-model systems.

Spondylus gaederopus, an iconic shell of Mediterranean, is such an example. *Spondylus* diverged quite recently, but had a peculiar evolutionary pathway. The shell is very hard and it has a composite microstructure with more than three different layers (including the complex cross-lamellar). The spine growth is very fast and shells are able to repair them rapidly, indicating the evolution towards energy-effective biomineralization.

We focus the study on biomineralizing shell proteins, which result trapped inside the skeleton. By applying proteomic analysis on shells - ShellOmics, we aim to characterize the shell protein repertoire controlling the biomineralization. Here we present the preliminary data on *Spondylus*, discussing possible methods for "big data" analysis and interpretation, given the paucity of comparative 'omics datasets for non-model system. The work sheds light on the complexity and rapid evolution of molluscan skeletal matrices.

Botryllus schlosseri, a model ascidian for the study of stem cells

V Vanni, F Gasparini, L Manni

Department of Biology, University of Padua, Padua, Italy

The colonial ascidian *Botryllus schlosseri* emerged in 1950s as an important model organism for the study of developmental biology and comparative immunology. Here, we highlight *B. schlosseri* potential for the study of stem cells in asexual reproduction and regeneration. *B. schlosseri* is a colonial tunicate that can reproduce both sexually and asexually. The asexual cycle is characterized by the initial formation of a thickened disc of somatic stem cells in the lateral body wall of the parent zooid. This disk gradually becomes a vesicle where organogenesis takes place, developing an adult blastozooid. Stem cells are, therefore, essential for asexual development. When all the blastozooids and buds are surgically removed from a colony, leaving only the tunic with its vasculature, the remaining circulating cells are capable to regenerate new individuals through a process called vascular budding. Here, some stem cells aggregate to form a vesicle, which undergoes morphogenesis regenerating the whole colony. Colonies of *B. schlosseri* possess also the ability to fuse each other and create chimeras. Chimeras share the circulatory system that allows blood cells to circulate between the fused partners. This capability was used to show that, within the chimera, somatic and germline stem cells compete to populate niches and regenerate tissue or germline organs. In the last decade, dedifferentiation capabilities were also questioned: when buds are *in vitro* cultured, they form epithelial monolayers and show de novo emerged stemness signature. Altogether, these data show that *B. schlosseri* has great potential for

investigating stem cell involvement in developmental pathways.

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

I Borisenko¹, AV Ereskovsky^{1,2}

¹*Saint-Petersburg State University; Saint-Petersburg; Russia*

²*Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale; Marseille; France*

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

M Almeida^{1,2}, M Rocha^{1,2}, TH Silva^{1,2}, RL Reis^{1,2,3}

¹*3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Barco, Guimarães, Portugal*

²*ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal;* ³*The Discoveries Centre for Regenerative and Precision Medicine, University of Minho, Barco, Guimarães, Portugal*

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^{1,2,3}, FV Bolshakov^{1,2}, IE Borisenko², VS Frolova⁴, DB Tokina⁵, AV Ereskovsky^{2,5}

¹*Pertsov White Sea Biological Station, Biological faculty, Lomonosov Moscow State University, Moscow, Russia;*

²*Department of Embryology, Biological faculty, Saint-Petersburg State University, Saint-Petersburg, Russia*

³*Koltzov Institute of Developmental Biology RAS, Moscow, Russia*

⁴*Department of Embryology, Biological faculty, Lomonosov Moscow State University, Moscow, Russia*

⁵*Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Aix Marseille University, CNRS, IRD, Avignon University, Station Marine d'Endoume, Marseille, France*

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

B Temiz, E Ozturk, A Karahan

Middle East Technical University, Institute of Marine Sciences; Department of Marine Biology and Fisheries; Mersin; Turkey

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

L Iglesias Ollé, MC Vogg, B Galliot

Department of Genetics and Evolution, Institute of Genetics and Genomics in Geneva (iGE3), Faculty of Sciences, University of Geneva, Switzerland

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/ β -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/ β -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*

J Levanoni^{1,2}, B Rinkevich¹

¹Israel Oceanographic and Limnological Research, National Institute of Oceanography; Haifa; Israel

²Department of Marine Biology, Leon H. Charney School of Marine Sciences, University of Haifa; Haifa; Israel

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

are able of transdifferentiation between the various cell lineages, thus allowing prompt renewal of damaged tissues.

A previous research has shown a peculiar cluster of cells at the edge of a growing coral nubbin featuring a morphology unlike any ectodermal or endodermal tissues. We suggest that these cells might be pluripotent stem cells. Using histological methods as well as utilizing stemness markers, such as Piwi, Vasa and Nanos expression, we try to distinguish between this cluster of cells and typical differentiated somatic cells and evaluate their properties.

First record of non-indigenous *Botrylloides anceps* (Herdman, 1891) species along the Turkish Levantine Coasts, Confirmed by DNA Barcoding

E Öztürk, B Temiz, A Karahan

Middle East Technical University, Institute of Marine Sciences, Erdemli, Mersin, Turkey

There are nearly 3000 Ascidian species identified and they are all found in seas. However, the studies on tunicates on Turkish coasts are limited; thus, not much known about the ascidian fauna of Turkish Seas. In this study, presence of the *Botrylloides anceps* (Herdman, 1891) species along the Mediterranean coast of Turkey has been proven by application of DNA barcoding tool for the first time. Sampling was performed in coastal area of the Hatay-Konacık region on 26 September 2018. Mitochondrial Cytochrome Oxidase I (COI) and nuclear H3 genes were used for identification.

Although *B. anceps* were observed in Israel, Australia, India, Brazil and Japan, only Israel and Australia provided gene sequences to the database. In the present study, one *B. anceps* (Israel) species haplotype data was obtained from the Genbank to compare with the present study sample. Phylogeographic resolution of these 2 haplotypes was investigated by using NETWORK software. Five mutation steps differences were observed between present study and previously studied (in Israel) *B. anceps* sample for COI gene and four for H3 gene.

This study is a very first record in terms of using DNA barcoding tool on the *B. anceps* identification for the sample that was collected in Turkish Seas. It is being planned to do whole genome sequencing of the species as a next project.

Contributing of “omics” for the understanding of *Chondrosia reniformis* collagen aggregation phenomenon

MS Rocha^{1,2}, D Fassini^{1,2}, E Martins^{1,2}, AL Alves^{1,2}, RL Reis^{1,2,3}, TH Silva^{1,2}

¹*3B's Research Group, I3Bs Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Barco, Guimarães, Portugal*

²*ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Portugal*

³*The Discoveries Centre for Regenerative and Precision Medicine, Barco, Guimarães, Portugal*

Collagen is the most abundant structural protein, being extremely similar in both vertebrates

and invertebrates. Currently, the main sources of collagen are bovine and porcine by-products, but risks of zoonosis and religious constraints have encouraged research for alternative collagen sources. In this sense, marine organisms have gained wide acceptability.

Although being promising biomaterials for biomedical applications, marine origin collagens have inferior mechanical properties than their mammalian counterparts. This can be mitigated by collagen crosslinking reactions, though these often involve cytotoxic chemicals; thus, a challenge for the biomedical community is the development of effective enzymatic crosslinking systems.

Marine sponges (phylum Porifera) are a sustainable source of collagen which possess dynamic collagenous tissues (DCT), a singular physiological adaptation that allows them to regulate the mechanical properties of the connective tissue matrix. This calcium-dependent phenomenon can reversibly stiffen or soften the tissues in a short time-span.

In our model, *Chondrosia reniformis*, this phenomenon is mediated by at least one stiffening factor which we recently acknowledged and partially purified, with the extract fraction interacting with collagen. However, neither the identity of the enrolled compound(s) nor the mechanisms behind the DCT's adaptability are fully comprehended. Considering this, proteomic approaches are envisaged for identification and characterization of stiffening factor(s), fundamental for comprehending the DCT phenomenon, aiming at the development of a novel collagen crosslinking method fitting for biotechnological applications.

Raveling cell type diversity and cell type regulation of the coral *Stylophora pistillata* by whole-organism single-cell transcriptome

S Levy¹, A Sebe-Pedros², T Mass¹, A Tanay²

¹*Marine Biology Department, The Leon H. Charney School of Marine Sciences; University of Haifa; Haifa; Israel*

²*Department of Computer Science and Applied Mathematics and Department of Biological Regulation; Weizmann Institute of Science; Rehovot; Israel*

Corals that are part of the phylum Cnidaria, are among the earliest metazoans that possess an organized body structure. The Indo-Pacific scleractinian coral *Stylophora pistillata* is a colonial zooxanthellate coral that is one of the main builders of the Gulf of Aqaba reefs. The coral body structure consists of two cell layers; an ectoderm and an endoderm, separated by a non-cellular gelatinous matrix called mesoglea. In this study we aim to reveal cell type diversity and the genes define each cell type. To do so we performed whole-organism single-cell transcriptome using massively parallel single-cell RNA-sequencing (MARS-Seq) analysis. Our main interest is to identify and characterize the calciblastic cells, which are involved in calcification and skeleton formation. We are also interested in the cells that host the algal symbionts (*Symbiodinium*), to better understand the role of the symbionts in coral

calcification. In addition, corals as most cnidarians have high regenerative capacity, however their stem cells population was not yet defined, therefore we would like to identify and characterize

Stylophora stem cells. Our preliminary results show that *Stylophora* cells are grouped into 24 cell clusters. Further analysis needed in order to identify and characterize each cluster.