REPORT OF MEETING 2nd general meeting and working group meetings of the COST Action 16203: STEM CELLS OF MARINE/AQUATIC INVERTEBRATES: FROM BASIC RESEARCH TO INNOVATIVE APPLICATIONS (MARISTEM), November 28-30, 2018, Marine Biology Station -Laboratoire Arago, Banyuls-sur-Mer, France Organizer: A-M Genevière

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Organizer: **A-M Genevière**

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Introduction to MARISTEM - stem cells of marine/aquatic invertebrates: from basic research to innovative applications

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Marine/aquatic invertebrates constitute the largest biodiversity and the widest phylogenetic radiation on Earth, from morphologically simple organisms (e.g., sponges, cnidarians), to the more complex mollusks, crustaceans, echinoderms, and protochordates. Today, adult marine/aquatic invertebrate stem cell (MISC) biology is of prime research and medical interest. However, studies on stem cells from organisms outside the classical vertebrate (e.g., human, mouse, and zebrafish) and invertebrate (e.g., Drosophila, Caenorhabditis) models have not been pursued vigorously. These organisms contain a variety of MISC-types that allow the production of a large number of novel bioactive-molecules, many of which are of significant potential interest for human health. MISCs further participate in aging and regeneration phenomena, including whole-body regeneration.

For years, the European MISC-community has been highly fragmented and has established scarce ties with biomedical industries in an attempt to harness MISCs for human welfare. Thus, it is important to (i) consolidate the European community of researchers working on MISCs; (ii) promote and coordinate European research on MISC biology; (iii) stimulate young researchers to embark on research in MISC-biology; (iv) develop, validate, and share novel MISC tools and methodologies; (v) establish the MISC discipline as a forefront interest of biomedical disciplines, including nanobiomedicine; and (vi) establish collaborations with industries to exploit MISCs as sources of bioactive molecules. In order to fill the recognized gaps, the EC-COST Action 16203 “MARISTEM” has recently been launched.

At its initial stage, the consortium unites scientists from 24 EC countries, Cooperating countries, and Near Neighbor Countries.

General meeting

(Speakers in alphabetical order)

Evo-devo of non-embryonic development in colonial ascidians

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Ascidians of the Styelidae family regroup more than 550 species and comprise both solitary and colonial forms. Whereas solitary species can reproduce only sexually, colonial ones have the ability to also propagate asexually by different modes of non-embryonic development and often can regenerate the body completely after injury[1]. We recently generated a robust phylogeny of Styelidae based on 20 new transcriptomes, showing that asexual reproduction has been acquired twice

in this family by convergence[2]. Notably, the species *Polyandrocarpa zorritensis* acquired asexual reproduction independently of all other species and displays a unique mode of bud formation that is a combination of epithelial morphogenesis and proliferation of putative blood stem cells[3]. In order to understand the molecular bases of convergent acquisition of budding across ascidian species, we are conducting a comparative transcriptomic study of budding tissues between distant colonial species (e.g. including *Polyandrocarpa zorritensis* and *Botryllus schlosseri*). In addition, we will generate transcriptomes of single blood cells in colonial and solitary species - with an emphasis on candidate totipotent stem cells - to provide molecular-level description of blood cells in ascidians, and pave the way to their functional characterization during budding. Taken together, this ongoing work will help to elucidate the plastic evolution of non-embryonic development in ascidians, in order to better understand why colonial species are able to propagate asexually, while solitary species are not.

[1]Tiozzo S. *et al*. (2008) Regeneration and stem cells in ascidians. In: Bosch TCG (ed) Stem Cells. Springer, Dordrecht, pp. 95-112.

[2]Alié A. *et al*. (2018) Convergent acquisition of nonembryonic development in styelid ascidians. Mol Biol Evol. 35: 1728.

[3]Scelzo *et al*. In prep.

Tissues regeneration and in hospite microalgae proliferation in the photosymbiotic marine flatworm *Symsagittifera roscoffensis* (Xenacoelomorpha, Acoela)

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Some marine animals evolved long-term functional partnership with photosynthetic micro-algae they reared inside the animal tissues. The biology and physiology of the green flatworm *Symsagittifera roscoffensis* show how a population of around 100.000 photosynthetically active green unicellular algae (*Tetraselmis convolutae*) is controlled beneath the epidermis of this animal. The non-photosymbiotic juvenile animal must ingest (not digest) micro-algae found in the surrounding environment or die (after 15 days in the lab) if no ingestion occurred. Once ingested, algae divide, confer the green color to the animal and supply energy, releasing in the tissues various photosynthates, the unique source of food for the animal.

Controlling the life cycle in captivity of this flatworm – including the induction of photosymbiosis (i.e culture of the free living photosynthetic partner) - allows having access to any developmental stages in order to explore the intimate trophic relationship and other features. In the animal tissues algae are fully dedicated to photosynthesis as suggested by the absence of typical structures expressed in the algae free-living state for which lot of energy is allocated, such as the synthesis of body-wall (complex polysaccharides) and the movement flagella. The *in hospite* algae take advantage of the animal nitrogen waste and recycle them. At the very beginning of the establishment of the symbiosis algae also recycle the uric acid crystals that accumulate in the non-photosymbiotic juveniles flatworm. Intertidal natural *S. roscoffensis* colonies submitted to submarine groundwater discharge, enriched in nitrogen also show that the flatworms are nitrate interceptors.

In the natural environment, animals are exposed, several hours each day, to sun rays and are adapted to overcome the excess of sun (including UVs) and high oxygen concentration (from photosynthesis) in their tissues.

Beside coping with putative detrimental physiological conditions (oxidative stress) *S. roscoffensis* also exhibits strong capacities of tissue regeneration including brain.

After more than a decade of functional exploration related to *S. roscoffensis*,

- Tuning the techniques for completing life cycle in captivity
- Investing huge efforts in genomics/transcriptomics,
- Morphological (cell types) characterization advances,

many conditions are met for starting a formal exploration of the molecular and cellular mechanisms underlying brain and peripheral nervous system regeneration.

Corals as sources of bioactive molecules

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The corals, organisms relatively under-studied compared to many other species over the world, must represent a very diverse source of active substances with potential applications in the fields of human well-being and health. CORALIOTECH, young Startup of Marine Biotechnology located in Monaco, proposes an ecological technology of production of active products (proteins and peptides) originating from corals. Based on genetic engineering and biotechnologies, CORALIOTECH uses the coral genetic information provided by its scientific partners (such as the Centre Scientifique de Monaco) to clone genes of interest before implementation of an artificial process of production and purification of the corresponding proteins. Our technology does not require the use or exploitation of the coral itself. In addition to guaranteeing the preservation of natural ecosystems, our production activity also has the advantage of being clean (waste of essentially biological nature), safe (fully automated processes) and scalable (possibility for a rapid rise towards industrial productions). After R&D proof of concept, the process is performed at pilot scale before initiation of product evaluation assays. Most of our innovative effects are valorized through patenting. Our B2B activity targets more specifically the pharmaceutical, biotech and cosmetic companies. Finally, CORALIOTECH aims to progressively enrich its product pipeline and contribute, through its scientific partnerships, to a better knowledge of coral biology.

Marine/aquatic invertebrate stem cells as promising models in environmental toxicology: future prospects and research needs

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Most of ecotoxicity studies have been carried out in whole organisms at various developmental stages. Selected cell lines have been used less frequently to elucidate the mechanisms of toxicity. There are many reasons for that, but the lack of proper research models and biomarkers to identify physiological modes of toxic action of environmental pollutants is among the most important ones as pointed by G. Vogt (2011) in his paper entitled "Hidden Treasures in Stem Cells of Indeterminately Growing Bilaterian Invertebrates". However, it is also true that advance of a scientific discipline could generate new research models to better address scientific questions. This holds true for aquatic invertebrate stem cells and their potential applicability in environmental toxicology as proper research models. For example, a number of environmental toxicology studies have already been done using digestive gland cells, hepatopancreas of isopods (crustaceans) as a test model. In addition to existing endpoints, one could use also stem cells of hepatopancreas located at the blind ends of the digestive gland tubules (hepatopancreas) which are resembling the apical meristem of plants (Zimmer, 2002). Also, hematopoietic organs provide an environment where undifferentiated stem cells could be used to measure of response (endpoint) to variable conditions and agents. Another possible biomarker of cellular development and morphogenesis is the alteration of the cholinergic system, which is involved in embryonic development. Paraoanua et al (2007) report that locally produced acetylcholine might function as an intercellular signal, modulating the proliferation of stem cells. In our previous study, we have demonstrated altered activity of AChE to be related to altered sea urchin early development (Mesarič et al, 2015). Further, echinoderms represent a phylum with exceptional regenerative capabilities that can reconstruct both external appendages and internal organs (Reinardy et al., 2015). Understanding how regenerative processes respond to changing environmental conditions (contamination of ecosystem and variation in normal weather patterns) is paramount to predict the future vulnerability or success of these keystone marine animals. It is a privilege of MARISTEM project to transform the tremendous potential of research outcomes on marine invertebrate stem cells into guidelines for animal (humans included) health and environmental protection.

Mesaric T, *et al*. Sperm exposure to carbon-based nanomaterials causes abnormalitiesin early development of purple sea urchin (*Paracentrotus lividus*). Aquatic Toxicology 163: 158-166, 2015.
Paraoanu LE, *et al*. Expression and possible functions of the cholinergic system in a murine embryonic stem cell line. Life Sciences 30: 80, 2375-2379, 2007.
Reinardy HC, *et al*. Tissue Regeneration and Biomineralization in Sea Urchins: Role of Notch Signaling and Presence of Stem Cell Markers. PLoS ONE 10: e0133860, 2015.
Zimmer M. Nutrition in terrestrial isopods (Isopoda: Oniscidea): an evolutionary-ecological approach. Biol Rev. 77: 455-493, 2002.
Vogt G. Hidden treasures in stem cells of indeterminately growing bilaterian invertebrates. Stem Cell Rev. 8(2): 305-17, 2012.

Stem cells cell in sponges (Porifera): an update

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Sponges (Porifera) are thought to be the sister group of all other animals and the earliest branching multicellular lineage of extant animals and as such a key group for understanding of the evolutionary history of animal stem cells and their regulation. Sponges are known to possess remarkable reconstitutive and regenerative abilities and high cell dynamic.

There is a widespread opinion that all sponges cells are capable of transdifferentiation and under certain conditions exhibit properties of pluripotency. However, the experiments on the regeneration and reaggregation of dissociated cells, have shown that not all cells exhibit the properties of stem cells.

Sponges do not have well-established stem cell lineages. Furthermore, presumable stem cells differ between four sponge classes. The most consistent model of the stem cell system is elaborated for fresh-water Demospongiae. According to this model demosponges have two stem cell lineages: archaeocytes and choanocytes. Both express the ortholog of the stem cell marker Piwi and show the proliferation activity in the intact sponges (Funayama, 2018). During regeneration in demosponges these cell types play an important role: they give rise to the new exopinacoderm and participate in the restoration of the choanosome structures. Additionally, the archaeocytes and choanocytes have ability for (trans)differentiation to various cell types during the restoration processes after sponge tissue dissociation (Borisenko et al. 2015; Lavrov, Kosevich, 2016). Despite the importance of archaeocytes as stem cells of demosponges, there is still no ultrastructural characterization of this type of cell; moreover, there are contradictory and unclear interpretations of the morphology of this cell type.

However, both Calcarea and many Homoscleromorpha do not have mesohyl cells similar to demosponges archaeocytes. In these sponge clades choanocytes and pinacocytes exhibit properties of polypotentiality, as follow from gametogenesis, experiments on regeneration, and

cell dissociation. These cells can directly transdifferentiate into other cell types without archaeocytes-like stage (Ereskovsky et al. 2015; Lavrov et al. 2018). Finally, it is necessary to emphasize the importance of models diversification: the comparison between different sponge taxa may help to shed light on the diversity of stem cells in Porifera and their properties.

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Coral skeletal proteins and their function in mineral formation

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Coral biomineralization is important at the organismal, ecosystem, and global scales, yet the biological component has not been well understood. In particular, identities, roles, and environmental susceptibility of the proteins retained in coral skeleton were previously unknown. Understanding the cellular and molecular responses of stony corals to ocean acidification is key to predicting their ability to calcify under projected high CO2 conditions. Of specific interest are the links between biomineralization proteins and the precipitation of new calcium carbonate (CaCO3), which potentially can provide a better understanding of the biomineralization process. To address this, we developed a novel coral tissue cultures to investigate the biophysical mechanism of calcification in corals. Our goals were to establish an experimental system in which calcification is facilitated at the cellular level, while simultaneously allowing in vitro manipulations of the calcifying fluid, and to study the mineral initiation mechanism in corals.

Viable cell cultures of the hermatypic, zooxanthellate coral, *Stylophora pistillata*, have been maintained for 6 to 8 weeks. Using an enriched seawater medium with aragonite saturation state which mimiks open ocean surface waters (Ω arag ~4). We have shown that within 72 hr after isolation, cultures of separated coral cells aggregate into proto-polyps and form an extracellular organic matrix (ECM) and precipitate aragonite crystals at a rate comparable to the intact organism and with geochemical properties similar to parent skeleton.

Responses of molluscan cells to ultra-low temperature exposure

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The study is focused on the alterations that occur in mussel larval cells both in standard culture conditions and in response to ultra-low temperature exposure. Development of this direction is important for understanding the mechanisms of cold susceptibility of marine organisms. The main pathways of molluscan cell death have been found to be associated with mechanical cellular disruption caused by the freezing-thawing processes themselves, as well as with apoptosis and necrosis. Apoptosis was not the main death pathway after a freeze-thaw cycle, but it was induced in a significant part of mussel cells immediately after thawing and depended mostly on the cryoprotectant used. Nothing is known about the causes of apoptosis in mollusks now, although this phenomenon is described in different classes of mollusks. We suggested that the use of the apoptotic inhibitors, known to mammalian cells, could provide a higher yield of viable cells after thawing. For comparison, we used primary mouse embryonic fibroblasts and human colon tumor cells. Additionally, the analysis of nuclear aberrations (such as few multipolar mitoses or the absence of a division spindle in mitotic cells) has been shown to be a useful tool for assessing cell disruption of molluscan cells regardless of the used cryoprotectant. The best cryoprotectant for bivalve cells was 5% dimethyl sulfoxide without any additives. Only staurosporine resulted in evident apoptosis in molluscan larval cells. Unfortunately, we did not reveal apoptotic inhibitors that could significantly reduce apoptosis in molluscan cells after freezing-thawing.

This study was partially supported by the COST.

Reverse development and stem cells in the cnidarian *Turritopsis dohrnii*

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The medusa of the Mediterranean jellyfish *Turritopsis dohrnii* can revert into the preceding polyp stage, completing a full morph rejuvenation, by a process known as reverse development (RD). RD is achieved through different stages ending with the formation of a ball-like cyst from which eventually a new stolon will form. Stem cell proliferation and differentiation are supposed to play key roles during RD. Hydrozoans possess a population of stem cells, called interstitial cells, characterized by a large nuclear to cytoplasmic ratios and prominent nucleoli. Cells with these characteristics are mainly present in the manubrium and in the canal system of *T. dohrnii* medusa, as revealed by TEM analysis. In cnidarians, orthologs of c-Myc and Sox2 have been found expressed in stem cell lines of *Hydra* polyps and in *Clytia hemisphaerica* medusae and planula larvae. We identified homologues of these genes in *T. dohrnii* and studied their expression pattern. We found that

they are mainly expressed where i-cells are localized in the medusa. Their expression is turned off during the early stages of RD, whereas transcripts can be detected again during late RD stages. Moreover, by EdU incorporation assays, we demonstrated that proliferation is an active process in the medusa and during the late stage of RD. Our results suggest that stem cells proliferation and differentiation may play a role primarily during the cyst stage and the formation of the stolon.

Stem cells in marine invertebrates - an overview

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Stem cells are unspecialized cells in multicellular organisms that have the capabilities to differentiate into other types of cells and can also renew themselves to produce the same type of stem cells. Most interesting are the adult stem cells (ASCs; somatic stem cells) that are found throughout the body after development, and in the vertebrates are the cellular tools used particularly to replenish dying cells and to regenerate damaged tissues (as multipotent cell types). In these organisms they form all/many cell types of the organ from which they originate. In contrast to what is known from the vertebrates, many marine invertebrates reveal significant different characteristics for ASCs. These include high abundancy of ASCs in marine invertebrates (up to 30% of total cells), pluri- and even totipotency, very limited dependency on niches (also the unique appearance or transitory niches), the frequent dedifferentiation and transdifferentiation associated with ASCs, the expression of germ cells markers in somatic stem cells (e.g., specific markers for the germ line are equally expressed in somatic stem cells, such as Piwi; further highlighting that there are no boundaries between somatic and germ cells lineage in many marine invertebrates) and the consideration of ASCs as units of selections and units of regeneration. In a wide range of marine invertebrates, ASCs may emerge *de novo* and are contributors to dramatic changes in biological features, such as whole body regeneration, re-juvenilization, torpor phenomena (hibernation and aestivation) and more. ASCs in marine invertebrates may also reveal unique phenomena such as germ cell transformation to somatic stem cells and vice versa. ASCs of marine invertebrates differ structurally from the typical ASCs in the vertebrates and even between different marine phyla (such as the interstitial cells in hydrozoans, the neoblasts in flat worms, the archeocytes in sponges and the lymphocyte-like cells in tunicates), and there are unknown tumors of ASCs, rarely any neoplastic or age-related diseases. The above and other characteristics may point to new perspectives for the evolutionary forces that dictate the development of ASCs.

Tissue crosstalk is required to induce a potential stem cell based regenerative response in the anthozoan cnidarian *Nematostella vectensis*

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Whole body regeneration in anthozoan cnidarians is poorly understood but has recently been investigated using the sea anemone *Nematostella vectensis*. In this organism, while cell proliferation is required for regeneration, stem cells have yet to be identified.

In order to highlight the location of the potential stem cells involved in regeneration in *N. vectensis*, we focused our efforts on a very detailed characterization of the regenerative capacities of various body parts. In addition, to gain a better understanding of the cellular mechanisms involved in the regenerative response, we analyzed the cellular behavior during regeneration using pulse and chase as well as irradiations experiments.

Our analysis revealed that body parts depleted of a specific structure are unable to regenerate. Further, we have shown that this particular structure is essential for inducing proliferation at the amputation site. The analysis of cell behavior during regeneration suggests that two populations of potential stem cells, located in different structures of the animal body, are reactivated in response to the injury.

Taken together, our results strongly suggest that a tissue crosstalk is required to induce a potential stem cell based regenerative response in the anthozoan cnidarian *N. vectensis*.

Insights in the evolution of mechanisms controlling commitment of neural stem cells

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Commitment of stem or progenitor cells to undergo terminal divisions and differentiation is a critical step during development, tissue maintenance or regeneration. While the molecular processes that are involved in cell cycle control and cell growth are conserved among eukaryotes the processes that mediate the progressive commitment of stem cells and progenitors remains largely elusive. We have recently identified the zinc finger transcription factor Glass to play a critical role in neural progenitors to mediate commitment towards a defined photoreceptor cell fate in the fruit fly *Drosophila melanogaster*. In order to explore if this function of Glass is evolutionarily conserved, we have analyzed the expression of Glass in the marine

annelid *Platynereis dumerillii*. Interestingly in *Platynereis* we could not detect expression of glass in photoreceptors. Similarly, analyzing published single cell RNAseq data of the flatworm *Schmidtea mediterranea* indicates that opsin genes are not co-expressed with glass. These findings suggest that in distinct animal clades different developmental mechanisms act to specify a similar neural fate. To further explore the diversity of neural stem cells and progenitors we have initiated a single-cell transcriptomic in cnidarians and acoels.

Application of nano titanium dioxide treated surfaces for marine organisms growth inhibition

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As producer of nanomaterial titanium dioxide we have been asked to search for the solution of slippery access to the sea on the concrete surfaces at the beaches. The challenge is to find a solution that could be applied to existing surfaces and would sufficiently slow the growth of marine organisms on the treated objects. We have prepared several mixtures of the fast setting cement based material and applied them to the demonstration object. To be able to understand the mode of action of the photocatalytic surfaces on the growth of marine organisms, we approached Biotechnical Faculty of University of Ljubljana to help us in characterization and understanding of the desired and eventual unintentional effects of our materials. We plan to observe the dynamics of the growth of organisms in one year period.