ASCIDIAN NEWS*

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Many many thanks to the large number of AN readers who sent in contributions and for letting me know how important AN continues to be! There are **89** new publications listed at the end of this newsletter. Please keep in touch and continue to send me contributions for the next issue! Keep safe, keep working, and good luck to everyone.

***Ascidian News is not part of the scientific literature and should not be cited as such.**

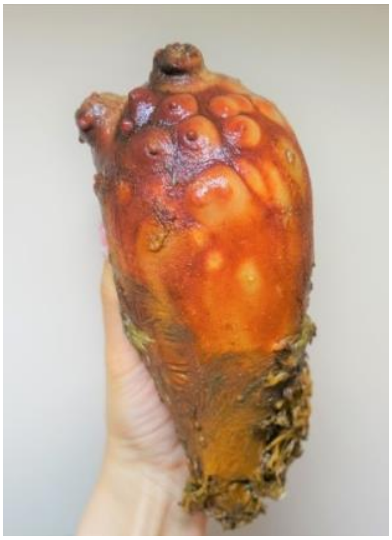
NEWS AND VIEWS

1. The 11th Intl. Conference on Marine Bioinvasions is scheduled for 15-19 May 2023 in Baltimore, Maryland. Deadline for early submission of Abstracts and travel award applications is Dec. 4, and early registration discount deadline is Jan. 15, 2023. To register, go to https://marinebioinvasions.info/register.

2. From Lion Novak and Noa Shenkar, Tel-Aviv University**:** A custom-made Toblerone chocolate bar just for tunicatologists! Lion was in Barcelona airport duty free on his way back from a conference a few months ago when he was offered this promotion to customize your own Toblerone, so of course he made for us a "Tunicata" chocolate bar! shenkarn@tauex.tau.ac.il, lionn@tauex.tau.ac.il .

3. Casey Bazeel, online: “Japan’s hoya [*Halocynthia roretzi*] is a straight-up edible video game monster, and here’s how to prepare/eat it.”

https://soranews24.com/2022/06/13/japans-hoya-is-a-straight-up-edible-video-game-monster-and-heres-how-to-prepare-eat-it%E3%80%90photos%E3%80%91/



“I’m not kidding on the “monster” part, as punishingly difficult video game *Elden Ring’s* world is stocked with giant hoya for you to fight.”

A very funny website, definitely worth visiting!

4. From longtime colleague **Don Cadien** (DCadien@lacsd.org): Asajiro Oka published many ascidian papers in the late 1920s-30s in the Proceedings of the Imperial Academy. The entire Proceedings papers are now online as pdfs, with free access. Go to https://www.jstage.jst.go.jp/browse/pjab1912/list/-char/en to download any you want.

5. From **Anna Di Gregorio**, New York Univ. (adg13@nyu.edu): I am organizing an online symposium dedicated to recognizing and celebrating the contributions of women to the field of ascidian biology. The symposium would be held via Zoom during the third or fourth week of March 2023, will be 1-2 days long, depending on interest and participation, and will include short talks and discussion on this subject. All ascidian researchers are invited, and all are welcome to provide names, ideas, stories, write-ups and other helpful information on women who have advanced the field of ascidian biology throughout the years with their research, teaching, mentoring and initiatives.

6. From **Lionel Christiaen**, New York Univ. (lc121@nyu.edu): Dear Colleagues of the Tunicate Community, It is with great sorrow that I am writing to announce the passing of my, and many others’, PhD advisor Jean-Stéphane Joly, [in September] in Paris, France. Jean-Sté had been suffering for many years from a devastating disease, but he remained a force of nature, always full of energy and enthusiasm for science, which he approached with an extraordinary spirit. He will be missed, but his memory will remain an inspiration.

From your AN editor: My database of ascidian papers includes many in which Jean-Stéphane was an author or co-author. He worked on ascidians for many years and greatly helped to advance our knowledge of this group.

6. From **Hitoshi Sawada** (sawada@kinjo-u.ac.jp): My mentor Dr. Hideyoshi Yokosawa, an Emeritus Professor of Hokkaido University, passed away on March 14, 2022, due to pancreatic cancer at the

age of 77. I learned from him a lot when I was an undergraduate student, and he was a supervisor of my Ph.D. thesis about the sperm proteases involved in ascidian fertilization. He used to invite me to go to Izakaya, a Japanese-style bar, for drinking and discussion about research and my private matter.

When he became a Full Professor in Hokkaido University, he recommended me to join his lab as an Associate Professor. Then, I moved to his lab of Hokkaido University from Hoshi's Lab of Tokyo Institute of Technology in 1991. In 2002, I got a position of Full Professor at Sugashima Marine Biological Laboratory, Nagoya University. He visited my lab in Sugashima MBL, which was also memorable for me. After his retirement from Hokkaido University, he moved to RIKEN for one year, and then to the School of Pharmacy, Aichi Gaukuin University, Nagoya, as a Professor. Since our apartments at Nagoya were very close, we used to drink as usual. He looked very young, like 50s, even at the age of 70s (see attached photos). A lot of his friends and former students gathered to Nagoya and attended his funeral. I would like to express my deepest condolences.

Hideyoshi (left), Hitoshi (right)

Hitoshi (left), Hideyoshi (right)

Work in Progress

1. Susanna Lopez-Legentil, Patrick Erwin, Lauren Stefaniak, and **Marie Nydam** have begun work on an NSF funded project: Development and application of genomic resources for ascidian taxonomy and holobiont evolution. Our goals are developing new molecular markers for ascidian families Ascidiidae, Didemnidae, Pyuridae and Styelidae, to create the first catalog of ascidian diversity in artificial (harbor) and natural (reef) habitats in Belize, and to characterize ascidian gut microbiomes and map variation in structural (taxonomic groups) and functional (specific gene) features across the ascidian tree of life. We have sequenced whole genomes from 15 species at 31x coverage in order to develop markers. We will share these WGS data with any interested labs. We, along with two graduate students, sampled in Belize from July 20 - July 28, 2022. We visited 10 marinas and 3 mangrove sites. We tentatively identified 55 morphotypes to the species level, 32 to the genus level, 42 to the family level, and 19 to the order level. mnydam@soka.edu, lstefania@coastal.edu, erwinp@uncw.edu, lopezlegentils@uncw.edu .

2. From **Alexandre Alié**, Regeneration and Pluripotency team (Tiozzo lab), Institut de la mer de Villefranche, Villefranche sur Mer, France alexandre.alie@imev-mer.fr

Our group just got a grant from the French ANR for a 4 year project on thaliaceans. Thaliaceans form impressive planktonic swarms during the blooming season, thanks to asexual proliferation. We want to study the cellular and molecular mechanisms of asexual reproduction in salps to better understand the evolution of budding across tunicates. This project will be conducted in collaboration with the group of Bettina Meyer, from the Alfred Wegener Institute, to further study how environmental fluctuations may impact asexual reproduction and demography in salps. More on tiozzolab.org

3. https://www.prnewswire.com/news-releases/ascidian-therapeutics-launches-to-rewrite-rna-301646826.html
NEW YORK and BOSTON, Oct. 12, 2022 /PRNewswire/ -- ATP, a leader in life sciences venture capital, today announced the launch of Ascidian Therapeutics, a biotechnology company built and developed by ATP and funded with a $50 million Series A financing. With a focus on treating human diseases by replacing mutated exons at the RNA level, Ascidian's technology enables therapeutic targeting of large genes and genes with high mutational variance while maintaining native gene expression patterns and levels. This approach is designed to provide the durability of gene therapy while reducing risks associated with DNA editing and manipulation. Ascidian is advancing its lead program for *ABCA4* retinopathy in IND-enabling studies while it progresses its pipeline of programs in ophthalmology, and neurological, neuromuscular, and rare diseases.

https://www.biopharmadive.com/news/ascidian-atp-rna-exon-editing-startup/633875/
With $50 million in funding, Boston-based Ascidian Therapeutics claims its RNA “exon editing” approach could match the durability of gene therapy while avoiding some of the risks that come with editing DNA. Its platform is designed to correct for mutations in exons — the regions of DNA that contain information needed to make proteins. Ascidian aims to do this by replacing mutated exons with functional RNA copies as DNA is being converted into its chemical cousin. The company will first target a genetic eye condition called Stargardt disease, which is the most common form of inherited macular degeneration and results in vision loss.

4. From **Delphine Dauga** (contact@bioself-communication.com): ANISEED is short for Ascidian Network for In Situ Expression and Embryological Data. It is both a collection of tools and a database, which as of November 2022, are undergoing refurbishment and a server migration by Nassif, the latest addition to the team and the new main developer of ANISEED. Soon, the new official ANISEED platform will be readily available for you to visit, use, and report back via the feedback button.

As for the annotation of articles, it is now back on track for ANISEED. After a very quiet period, we want to catch up on the literature, with a one-year contract 100% dedicated to annotation that started early November 2022 (ANISEED's curation has no sustainable funding and can only be achieved thanks to the labs participation). If you want your article(s) to be annotated, please contact Marion Guéroult-Bellone (m.gueroultbellone@gmail.com) and be prepared to send HD embryo images, gene IDs, cis-regulatory sequences... or anything that cannot be easily found in the article.

ANISEED's curation has no dedicated funding and can only be achieved thanks to individual lab donations. Thank you for the generosity of those who have already contributed! Please contact Delphine if you can help and do not forget to ask for some money for the ANISEED curation in your grant applications.

5. From **Hitoshi Sawada** (sawada@kinjo-u.ac.jp) : I’m now editing a Special Issue "Gametogenesis and Gamete Interaction", to be published by Biomolecules, a peer-reviewed open access journal (IF 6.064, 5-Year IF 6.191). Please submit your original paper or review article to this special issue. The Article Processing Charges (APCs) is 2100 CHF per accepted paper. Papers may be submitted from now until 22 February 2023 as papers will be published on an ongoing basis. We are looking forward to receiving your article. Thanks in advance for your cooperation. For more information, please refer to the following website. https://www.mdpi.com/journal/biomolecules/special_issues/44W044K023

6. From **Megan Powers**, Ph.D. student in Billie Swalla’s lab, Univ. of Washington Dept. of Biology, Seattle WA (mpowers4@uw.edu): This year, the Swalla lab has been working to survey invasive ascidians throughout the Puget Sound, Washington, USA. In the late 1990s, nonnative ascidians were surveyed at marinas throughout the Pacific Coast of the United States by Gretchen and Charles

Lambert, and five nonnative species were reported within the Puget Sound by the year 1998. We hypothesize that human activities like shipping and aquaculture have continued to cause the spread of invasive ascidian species to new locations throughout the Salish Sea in the past 24 years. This summer, we began to survey marinas in Puget Sound to assess the current distribution of invasive ascidians. I deployed settling plates at five marinas both with and without cages for predator exclusion as well as surveying the ascidian communities on floating docks. We plan to continue and expand this survey in the coming years to determine how many new nonnative species have arrived in the Salish Sea and how far invasive populations have extended within the Salish Sea in the past two decades.

Meetings Abstracts

(References are not included in those abstracts that included them. Please see the online abstract books.)

1. North Carolina Branch of the American Association for Microbiology, Boone, North Carolina, Nov. 5, 2022.

Functional redundancy in the ascidian microbiome: A metagenomic comparison of *Eudistoma capsulatum* microbiomes from marina and reef habitats in North Carolina. Brenna T. Hutchings, Susanna López-Legentil, Patrick M. Erwin, Dept. of Biology & Marine Biology, and Center for Marine Science, Univ. of North Carolina Wilmington, Wilmington, NC. lopezlegentils@uncw.edu

Ascidians (Chordata: Tunicata) are filter-feeding marine invertebrates found in benthic communities growing on artificial and natural substrates across the globe. The success of ascidians in colonizing a variety of habitats may be partly due to the specificity and dynamics of their microbial symbionts. Previous work revealed host-specific microbial symbionts in native (*Eudistoma capsulatum*) and nonnative (*Distaplia bermudensis*) ascidian species in North Carolina using amplicon (16S rRNA) sequencing. When sampled across artificial (marina) and natural reef habitats, the nonnative host exhibited stable microbiomes while the microbial communities of the native host (*E. capsulatum*) shifted between habitats. Here, we employed shotgun metagenome sequencing of symbiont communities of the same marina and reef populations of *E. capsulatum* to determine if compositional shifts result in changes to functional gene content. Metagenomic libraries (Illumina HiSeq) were constructed from 4 replicates of each species and at each site. Preliminary analyses were conducted by annotating unassembled (MG-RAST v 4.0.3) and assembled (KBase) datasets. Differentially abundant genes between habitats were identified in MicrobiomeAnalyst (edgeR and DESeq2 algorithms) based on KEGG Orthology (KO) and SEED Subsystems annotation databases. Based on KO, seventeen genes (~1.06% of all genes) were significantly more abundant in the marina-associated microbiomes than reef-associated microbiomes, while SEED outputs detected two genes (~0.07%) that were significantly more prevalent in the marina population. Metagenome-assembled genomes (MAGs) of 4 dominant symbiont taxa were reconstructed from the reef dataset and 6 dominant taxa were reconstructed from the marina dataset. Together, these results revealed similar functional gene content in *E. capsulatum* across habitats, thus suggesting negligible impacts of compositional shifts on overall holobiont metabolism due to functional redundancy. Future studies t

2. Proceedings of the EuroEvoDevo 2022 Conference, Naples, Italy, May 31-June 03, 2022.

a) On the complement system of protochordates: evolutionary clues from colonial ascidians. Anna Peronato[1], Nicola Franchi[2], Loriano Ballarin[1]. 1 Dept. of Biology, Univ. of Padova; 2 Dept. of Life Science, Univ. of Modena and Reggio Emilia.

The vertebrates complement system is a complex array of soluble and membrane proteins able to

sense nonself. Three activation pathways (alternative, lectin and classic) lead to the ultimate cleavage of C3 to C3a and C3b. C3a is a chemokine able to recruit immunocytes at the infection site, whereas C3b acts as an opsonin favouring phagocytosis of foreign material. A lytic pathway can also be activated leading to the polymerization of C9 in the membrane of nonself cells causing their lysis. Much less is known on the presence and roles of complement in invertebrates. A decade ago, data on ascidian complement were limited and mainly referred to the solitary species Ciona robusta where a couple of orthologous transcripts for C3, expressed by a fraction of haemocytes, were known as well as the involvement of C3a in the recruitment of circulating cells. Our interest in invertebrate immune responses, with particular reference to invertebrate-vertebrate transition, led us to investigate the complement system of the colonial ascidian Botryllus schlosseri. We characterized the presence of both the alternative and the lectin pathways in Botryllus. All the complement components (C3, Bf, MBL, ficolin and MASP) are expressed by morula cells, directly involved in the immune response. In addition, we demonstrated the important role of C3 activation in modulating the colonial blastogenetic cycle and phagocytosis, and the presence of a G-protein-coupled receptor for C3a/C5a. New studies will aim to acquire information on the regulators of complement activation and their role in Botryllus biologyhat leverage metatranscriptomic and comparative approaches with nonnative ascidian counterparts will provide additional insight into the impact of ascidian microbiomes on host fitness and distribution.

b) **Non embryonic development in *Botryllus schlosseri*: the role of stress granules.** Drago L., Santovito G., Ballarin L., Dept. of Biology, Univ. of Padova, Italy.

Stress granules (SGs) are membrane-less cellular foci, representing a conserved evolutionary strategy of stress response. SGs are formed through the over-expression of mRNA binding proteins, such as TIA-1 related nucleolysin (TIAR), able to sequester specific mRNAs and regulate their translation into anti-stress proteins. Tunicates are invertebrate chordates closely related to vertebrates. The colonial ascidian Botryllus schlosseri, under controlled laboratory conditions, undergoes weekly generation changes called take-overs (TOs), during which the old zooids will be replaced by their primary buds growing to the adult size. At TO, circulating phagocytes actively ingest effect cells of old zooids so that an increase in oxygen consumption (respiratory burst) takes place with the production of reactive oxygen species. In protection of the new zooid generation from oxidative damages, we hypothesize that SGs play a pivotal role. To verify this hypothesis and study the molecular evolution of SGs, we used the TIAR protein as marker to investigate the dynamics of SGs' formation during the blastogenetic cycle of B. schlosseri. We analyzed the modulation of mRNA transcription for TIAR by quantitative Real Time PCR, and the localization of its transcript in the hemocytes by in situ hybridization. We used an antibody specific for tunicate TIARs on hemolymph monolayers and on colony sections, to confirm the involvement of immunocytes in stress responses. Anti-TIAR antibody was microinjected in Botryllus circulation, and the morphologic effects on the colony were evaluated. Our results highlight the key role of stress granules in the regulation of the blastogenetic cycle, especially in detoxification.

3. 11th International Tunicate Meeting, Kobe, Japan, July 11-15, 2022.
https://sites.google.com/view/11th-itm/home
The complete abstract book is available on the website and also via the following link, and can be downloaded: https://www.konan-u.ac.jp/hp/devbiol/11itm/AbstractBook.pdf

a) The complement system of ascidians: clues from the colonial ascidian *Botryllus schlosseri*. Loriano Ballarin1*, Nicola Franchi2, and Anna Peronato1. 1 Dept. of Biology, Univ. of Padova, Italy, 2 Dept. of Life Sciences, Univ. of Modena and Reggio Emilia, Italy.

The complement system of vertebrates is a complex array of soluble and membrane proteins able to sense nonself and activate an immune response. Three (alternative, lectin and classic) activation pathways are known: they lead to the cleavage of C3 to C3a and C3b. C3a is a chemokine able to recruit immunocytes at the infection site, whereas C3b acts as an opsonin favoring phagocytosis of foreign material. The lytic pathway can also be activated leading to the ultimate polymerization of C9 in the membrane of nonself cells causing their lysis. In invertebrates, much less is known on the presence and roles of complement. As far as ascidians are concerned, a decade ago, data were limited and mainly referred to the solitary species Halocynthia roretzi and Ciona robusta, where transcripts for C3, expressed by a fraction of haemocytes, are known. Ascidian C3 is involved in the modulation of phagocytosis as well as, through its derivative C3a, in the recruitment of circulating cells. Our interest in the immune responses of colonial ascidians led us to investigate the complement system of Botryllus schlosseri. Our research started ten years ago and led to the demonstration of: i) the pivotal role of circulating cytotoxic morula cells in the synthesis of the major complement components of both the alternative and lectin pathways; ii) the importance of C3 activation in modulating phagocytosis; iii) the presence of a G-protein-coupled receptor for C3a/C5a expressed by morula cells and modulating the transcription of C3; iv) the presence of a soluble C1qDC protein involved in inflammation and able to modulate the degranulation of morula cells. New ongoing studies aim to acquire information on the regulators of complement activation and their role in Botryllus biology.

b) Stress granules in ascidians: an overview. Laura Drago1, Alessandro Pennati2, Ute Rothbächer2, Gianfranco Santovito1, and Loriano Ballarin1. 1 Univ. of Padova, Dept. of Biology, Padova, Italy; 2 Univ. of Innsbruck, Dept. of Zoology, Innsbruck, Austria.

Stress granules (SGs) are stalled translational initiation complexes preserving mRNAs for anti-stress proteins and so regulating stress responses. This is possible thanks to the presence of mRNA-binding proteins such as TIA-1 related nucleolysin (TIAR), considered an important core component of SGs [1]. They disassemble in the presence of an acute stress so to unlock the translation of mRNAs into anti-stress proteins [2]. Until now, very few works have been devoted to study SGs in invertebrates, especially in marine species. By using TIAR as SG marker we explored the possible roles of these foci in the solitary ascidian Ciona robusta and in the colonial ascidian Botryllus schlosseri, both from the Lagoon of Venice. We started with an evaluation of their involvement in the responses to oxidative stress induced by metals, such as Cu, Zn, Fe and Cd, the impact of which on marine ecosystems is well documented. We carried out gene expression studies by qRT-PCR and in-situ hybridization. To validate the hypothesis of SG post-transcriptional control, we used specific anti-TIAR antibody in immunocytochemistry and immunohistochemistry and visualized their subcellular localization in immunocytes through transmission electron microscopy. In addition, the importance of SGs in the regulation of stress responses during embryonic development was investigated in C. robusta, through electroporation experiments with construct for reporter gene (LacZ) expression, containing the promoter region for TIAR. Botryllus, due to its peculiar capability to reproduce sexually and asexually [3], was considered to investigate the SG role during non-embryonic development, with microinjection experiments of the anti-TIAR antibody. The latter experiments suggest that SGs is involved not only in the regulation of stress, for example the one related to the diffuse apoptosis in adult zooid tissues characterizing the weekly renewal of colony [4], but also in cell proliferation required for the full development of new adult individuals.

c**) Ascidians as model organisms for assessing the extent and impact of anthropogenic pollutants in marine environments.** Noa Shenkar[1,2]*, Dror Avisar[3], Aviv Kaplan[3], Gal Navon[1],Lion

Novak[1], Adi Torfstein[4], Gal Vered[1]. [1]Sch. of Zool., George S. Wise Faculty of Life Sci., Tel Aviv Univ., Tel Aviv, Israel. [2]Steinhardt Mus. of Natural History & Natl. Res. Center, Tel Aviv Univ., Israel. [3] The Water Research Center, Porter Sch. of the Environment & Earth Sci., Tel Aviv Univ., Israel. [4]Inst. of Earth Sci., Hebrew Univ., Jerusalem, & InterUniv. Inst. for Marine Sci., Eilat, Israel.

Coastal environments have been undergoing dramatic changes in the past few decades. Although environmental and governmental agencies invest much effort in general monitoring and protection of this environment, there is a gap in our knowledge of the physiological impacts of current environmental stressors on the marine fauna, and of the possible potential of marine organisms as biological indicators of environmental health. Our on-going study focus on promoting the use of solitary ascidians, in particular invasive species, as biological indicators of marine environments. As highly efficient filter-feeders inhabiting both pristine and polluted environments ascidians present fundamental opportunities for ecotoxicological studies. By combining a wide variety of methods, we are currently developing tools for ascertaining the extent and impact of anthropogenic pollutants such as micro-plastics, plastic additives,pharmaceuticals, and heavy metals along the Mediterranean and Red Sea coasts of Israel. Our ability to produce lab-grown culturesand to identify a wide suite ofcontaminants in seawater, sediment and ascidian tissues enable us to accurately quantify a variety of contaminants of emerging concern, andconduct controlled exposure experiments. Results of our project demonstrate theapplicability of invasive ascidians as biological indicators, and emphasize the immediate need for the improvement of current monitoring protocols and management plans of coastal environments.

4. 4th general meeting of the COST Action 16203: Stem cells of marine/aquatic invertebrates: from basic research to innovative applications (MARISTEM), Padova, Italy, October 20, 2021. ISJ Invert. Surv. J., 19: 37-41.

Preliminary data on senescence in haemocytes of the colonial ascidian *Botryllus schlosseri*. F Cima1, L Drago1, A Peronato1, N Franchi2, O Ben Hamo3, L Ballarin1. 1Dept. of Biology, Univ.y of Padova, Padova, Italy, 2Dept. of Life Sciences, Univ. of Modena and Reggio Emilia, Modena, Italy, 3National Institute of Oceanography, Haifa, Israel.

Senescence is a cellular response to damage that limits the proliferation of aged or effete cells and plays physiological roles as it is required for tissue homeostasis. Colonies of the protochordate Botryllus schlosseri, undergo cyclical generation changes or takeovers (TOs) during which adult zooids are replaced by their buds reaching adulthood. The period of time between two TOs is referred to as blastogenetic cycle. During the TO, cells of adult zooid tissues die by apoptosis and are cleared by circulating phagocytes that, in turn, undergo phagocytosis-induced apoptosis and are cleared by new phagocytes in a recurrent, apparently endless, process. In the present work, we demonstrate that phagocytes, after the engulfment of effete phagocytes enter a senescence status and home, in the following mid-cycle, in the ventral islands, on both sides of the endostyle, where undifferentiated (stem) cells are also found. From these sites, senescent cells cross the peribranchial epithelium and are released in the peribranchial cavity where they will be expelled with the exhalant water.

5. 5th Scientific Retreat of the Dept of Biology, Padova, May, 26-27, 2022.

a) Stem cell niches in the highly regenerative chordate *Botryllus schlosseri* and stem cell contribution in bud development. Vanni V., Caicci F., Peronato A., Asnicar D., La Torre F., Gasparini F., Martello G., Ballarin L., Manni L., Univ. of Padova, Padova, Italy.

Uncovering the mechanisms driving regeneration and stem cell maintenance in highly regenerative organisms is of central importance in stem cell biology and regenerative medicine. The only chordates able to regenerate complete individuals from a few cells are colonial tunicates, representatives of the sister group of vertebrates. Therefore, we studied the stem cell properties in

the colonial tunicate Botryllus schlosseri, together with the microenvironments (niches) allowing their maintenance and differentiation, ultimately to better understand the reasons underlying their success in regeneration. We characterized for the first time the anatomy and development of known stem cell niches (that are transient), using histology and 3D reconstructions. We verified the candidate stem cell contribution to bud development, by using cell sorting, in vivo labelling and transplantation. Using live imaging and confocal microscopy, we found that candidate stem cells, which express stemness factors, do infiltrate and differentiate into several tissues, such as muscle, gonad components, several epithelia, and nervous system. These experiments allowed us to define a new permanent niche whose presence is essential to isolated bud development.

b) On the complement system of protochordates: evolutionary clues from colonial ascidians. Peronato A., Drago L., Franchi N., La Torre F., Vanni V., Manni L., Ballarin L.

The vertebrates complement system is a complex array of soluble and membrane proteins able to sense non-self. Three activation pathways (alternative, lectin and classic) lead to the ultimate cleavage of C3 to C3a and C3b. A lytic pathway can also be activated leading to the polymerization of C9 in the membrane of non-self cells causing their lysis. Our interest in invertebrate immune responses, with particular reference to invertebrate-vertebrate transition, led us to investigate the complement system of the colonial ascidian Botryllus schlosseri. We characterized the presence of both the alternative and the lectin pathways in Botryllus. All the complement components (C3, Bf, MBL, ficolin and MASP), are expressed by cytotoxic morula cells, that are directly involved in the immune response. In addition, we demonstrated the important role of C3 activation in modulating the colonial blastogenetic cycle and phagocytosis, the presence of a G-protein-coupled receptor for C3a/C5a. New studies aim to acquire information on the regulators of complement activation and their role in Botryllus biology.

c) Non-embryonic development in *Botryllus schlosseri*: the involvement of stress granules. Drago L., Santovito G., Ballarin L. Dept. of Biology, Univ. of Padova, Padova, Italy.

Stress granules (SGs) are cellular foci acting in the regulation of gene expression in response to various kind of stress. Their formation occurs with the over-expression of mRNA binding proteins, such as TIA-1 related nucleolysin (TIAR), considered a solid marker of SGs. The model organism chosen for this study is Botryllus schlosseri, a colonial invertebrate chordate from the Lagoon of Venice, belonging to the Tunicate subphylum. A curious aspect of this animal is its ability to undergo weekly generation changes called take-overs (TOs), during which the adult animals of the colony, called zooids, are replaced by their primary buds growing to adult size. At TO, circulating phagocytes actively ingest effect cells of old zooids causing an increase in oxidative stress. In protection of the new zooid generation from oxidative damages, we hypothesize that SGs play a pivotal role. To verify this hypothesis, we used anti-TIAR antibody, in immunocytochemistry and microinjection experiments, to investigate the dynamics of SGs' formation during the non-embryonic development of B. schlosseri.

6. XXII Meeting of the Italian Association of Developmental and Comparative Immunobiology, Padova February, 16-18, 2022.

a) Characterization and functional role of a novel C1qDC from a colonial ascidian. I. Surv. J. 19: 69-70. Peronato1, N Franchi2, M Tabarelli3, L Ballarin1 1Department of Biology, University of Padua, Padua, Italy; 2Dept. of Biotechnology and Life sciences, Univ. of Modena and Reggio Emilia, Modena, Italy; 3PhD school in Agricultural Science and Biotechnology, Univ. of Udine, Udine, Italy.

The complement system is present in all the metazoans as a complex array of soluble and membrane proteins able to orchestrate innate immune responses such as inflammation and phagocytosis. Although the complement system of invertebrates has been much less studied than that of vertebrates, however, it is equipped with at least the alternative and the lectin activation

pathways. The C1q-domain-containing (C1qDC) proteins are a large family of proteins, present in both vertebrates and invertebrates, characterized by one or more globular C1q (gC1q) domain(s) at the C- terminus. C1qDC proteins are distinguished in C1q- like proteins, with a gC1q domain and a collagen-like region at the N-terminus, and globular head C1q proteins (ghC1q) with one or more gC1q domains and a short N-terminus with no defined domains. The latter can be further divided into proteins without a signal peptide (cellular ghC1qs or cghC1qs) and proteins endowed with a signal peptide (secreted ghC1qs or sghC1qs). The gC1q domain has a typical jelly roll topology of five pairs of anti-parallel â-strands creating two â-sheets, with eight conserved hydrophobic amino acids and can interact with a large variety of ligands, both self and non-self. The same topology is present in the tumor necrosis factor (TNF) domain of protein of the TNF family so that a C1q-TNF superfamily of proteins (C1q/TNF-related proteins or CTRPs) has been defined. The mammalian complement component C1q, a subunit of the C1 complex of the classical complement activation pathway, has been the most thoroughly studied vertebrate C1qDC protein. In addition to activating C1r and C1s (and, as a consequence, in C3), C1q can also act as pattern recognition receptor (PRR) as, through its qC1q domain, it can recognize and bind pathogen-associated molecular patterns (PAMPs) on the surface of microbes and modulate their phagocytosis. Most of the C1qDC proteins have only a gC1q domain but the presence of molecules with multiple tandem C1q domains have been reported in both invertebrates and vertebrates; among the latter, CTRP4 is the only protein with two C1q domains described in mammals, birds, reptiles, amphibians and teleosts. The compound ascidian Botryllus schlosseri is a chordate invertebrate that relies only on innate immunity for its defense. 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 this same species, we identified the key components of the lectin and the alternative pathways. All these complement components (C3, Bf, MBL, ficolin and MASP), are expressed by morula cells, the most abundant circulating hemocyte. In this study, we mined the available transcriptomes and identified, in B. schlosseri, a novel multidomain C1qDC protein (BsC1qDC). It belongs to the sghC1q proteins and contains two gC1q domains, a signal peptide and present high similarity with human CTRP4. We followed the expression of BsC1qDC during the colonial blastogenetic cycle and in colonies injected with Gram (+) bacteria and identified its mRNA location by in situ hybridization (ISH). The expression trends during the colonial blastogenetic cycle suggest the presence of checkpoints modulating the transcription of bsc1qdc. The protein is synthesized and released by morula cells and a minority of phagocytes. When we knocked down the gene, we observed a decrease in phagocytosis of target particles, probably related to the involvement of BsC1qDC in the opsonization of non-self, as well as a decrease in degranulation and is involved in. Ongoing studies are trying to better clarify the role of bsc1qdc in Botryllus immune modulation and its interplay with the other complement components such as complement control proteins.

b) Tissue distribution of a rhamnose binding lectin in the colonial ascidian *Botryllus schlosseri* and effects of microinjections of the specific antibody into the circulatory system.
Bovo G., Ballarin L. I. Surv. J. 19: 72. Dept. of Biology, Univ. of Padua, Padua, Italy.

Lectins are non-enzymatic and non-immunoglobulin proteins, or glycoproteins, that bind carbohydrates with their Carbohydrate Recognition Domains (CRDs). They are involved in various biological processes, including host-pathogen interaction and intercellular communication. They play pivotal roles in the immune system of invertebrates by binding pathogens directly and opsonizing them. Botryllus schlosseri in a cosmopolitan ascidian, considered a reliable model organism for studies on the evolution of the immune system. B. schlosseri Rhamnose-Binding Lectin (BsRBL) acts as a chemokine and opsonin by interacting with different cell types. Although described in previous works, many aspects and roles of this lectin remain unknown. Here we studied the changes in tissue distribution of BsR BL during immune responses using light and electron microscopy. In addition, following the hints from extant data, suggesting a possible role of BsRBL in the process of takeover,

we investigated the effects of the removal of this protein, using a specific antibody, during the generation change, opening new queries on the roles of this lectin in Botryllus biology.

c) Dynamics of formation of stress granules during the colonial blastogenetic cycle of *Botryllus schlosseri*. Drago L., Santovito G., Ballarin L. Invertebr. Surv. J. 19: 75. Dept. of Biology, Univ. of Padua, Padua, Italy.

Stress granules (SGs) are cellular ribonucleoprotein foci preserving mRNAs for anti-stress proteins and so regulating stress responses.This is possible thanks to the presence of mRNA-binding proteins such as TIA-1 related nucleolysin (TIAR), considered an important core component of SGs. Botryllus schlosseri is a colonial ascidian easily found in the Lagoon of Venice, which undergoes weekly generation changes called take-overs (TOs). A blastogenetic cycle is defined as the period between two successive TOs. During the TO, lasting 24-36 h, a diffuse apoptosis occurs in tissues of old zooids, which will be replaced by their primary buds representing the new generation. At TO, an increase in oxygen consumption (respiratory burst) takes place with the consequent production of reactive oxygen species representing a stressful condition for the new zooid generation. We suppose that SGs can play a pivotal role in the protection from oxidative damages. To verify this hypothesis, in this work we used the TIAR protein as marker to study the dynamics of formation of SGs during the colonial blastogenetic cycle of B. schlosseri. At first, we analyzed the modulation of mRNA transcription levels for TIAR by quantitative Real Time PCR (qRT-PCR) and the location of its transcript in the hemocytes through in situ hybridization (ISH). Then, we used an antibody specific for TIAR on hemolymph monolayers, and on colony paraffin sections, to confirm the involvement of immunocytes in detoxification. Our results agree with the idea that immunocytes represent the major detoxification system in ascidians, active in the control of TIAR protein synthesis and, therefore, in SGs formation.

d) Insight on the signal transduction pathways involved in morula cell degranulation in the colonial ascidian *Botryllus schlosseri.* Peronato A., Ballarin L. I. Surv. J. 19: 84. Dept. of Biology, Univ. of Padua, Padua, Italy.

Morula cells are granular cells constituting the majority of hemocytes of the hemolymph of botryllid ascidians. They are the first cells sensing nonself and, as a consequence of the recognition, they synthesize and release cytokines and trigger and inflammatory process by release their granular content through exocytosis (degranulation). They are directly involved in the formation of the necrotic points of rejection along the contact border between incompatible colonies. During this process, they are selectively recruited and gather in the ampullae (the blind endings of the colonial circulation) close to the contact region before crossing the vascular epithelium and entering the tunic where they degranulate releasing their granular content, in primis the cytotoxic enzyme phenoloxidase and its polyphenol substrata. The degranulation reaction can be mimicked in vitro by exposing hemocytes to cell-free hemolymph from genetically incompatible colonies of to microbial cells such as Bacillus clausii cells. In the present research we induced in vitro degranulation to study the signal transduction pathways involved in the process using specific inhibitors of a series of kinases. Preliminary results indicate the involvement, in morula cell degranulation, of the pathways mediated by PKA, PKC and Jnk.

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MAP kinases involvement in stress signaling during the colonial blastogenetic cycle of *Botryllus schlosseri*. Drago L., Santovito G., Ballarin L. J. Biol. Res. 95 (s1): 13. Dept. of Biology, Univ. of Padova, Padova, Italy.

Botryllus schlosseri is a colonial ascidian easily found in the Lagoon of Venice, which undergoes

weekly generation changes called take-overs (TOs) [1]. A blastogenetic cycle is defined as the period between two successive TOs. During this phase, lasting 24-36 h, a diffuse apoptosis occurs in tissues of old zooids, which will be replaced by their primary buds representing the new generation [2]. An increase in oxygen consumption (respiratory burst) is observed, due to phagocytes removing apoptotic cells, which causes the production of reactive oxygen species (ROS) [3]. In order to protect the new zooid generation from ROS damages, these animals have evolved stress defense mechanisms, which imply the activation of anti-stress proteins through stress signaling transduction pathways, possible driven by mitogen-activated protein kinases (MAPKs). MAPKs are a family of highly conserved serine-threonine protein kinases important for the regulation of cell growth and differentiation and apoptosis [4]. With this study, through the use of specific inhibitors for Erk, JNK and p38, the three main MAPK subfamilies, directly micro-injected in Botryllus circulation, we want to evaluate the importance of MAPKs in the regulation of the blastogenetic cycle both from a morphological and a molecular point of view. Differences in transcription levels of stress-related genes, i.e. superoxide dismutase (sod), glutathione synthase (gs), glutathione peroxidases (gpxs), tia-1 related nucleolysin (tiar) and tristetraprolin (ttp), have been evaluated by quantitative real time PCR (qRT-PCR). The last two genes are involved in the formation of stress granules, important cell foci involved in post-transcriptional control of stress-related genes [5].

Thesis Abstracts

Decoding the Cis-regulatory control of stage-specific notochord gene expression by Brachyury. Lenny J. Negrón-Piñeiro, New York Univ. Ph.D. thesis, defended on August 15th, 2022; advisor Anna Di Gregorio. He is currently a postdoctoral fellow in Anna's lab. adg13@nyu.edu

The expression and function of Brachyury (Bra) – a member of the T-box family of transcription factors (TFs) – have been under investigation since this gene was discovered to be indispensable for mesoderm development. In particular, Bra is a key regulator of the formation of the notochord, an axial mesodermal structure considered a defining characteristic of chordates. The notochord acts as a signaling center and structural support for the surrounding embryonic tissues. Our lab uses an invertebrate chordate, the ascidian *Ciona robusta*, to elucidate the structure of the notochord gene regulatory network (GRN), in which *CionaBrachyury*(Ci-Bra) and *Foxa*.a (ortholog of *Foxa2*) sit upstream of several other TFs. Hundreds of Ci-Bra-downstream genes with different onsets of expression have been identified through multiple screens, and they encode for proteins that are involved in different cellular functions, such as TFs, extracellular matrix proteins and growth factors, among others. An outstanding question regarding the ascidian notochord GRN is how Ci-Bra, which is expressed in the notochord throughout all stages of its development, can control the expression of notochord genes that display a temporally staggered expression during notochord development. Our lab has uncovered that within notochord regulatory sequences, known as *cis*-regulatory modules (CRMs), the number of functional Ci-Bra binding sites correlates with the onset of expression of the genes that they control. Based on these results, we have proposed that the information controlling the stage-specific notochord gene expression by Ci-Bra is encoded in the number of binding sites for this TF.

In addition to testing this mechanistic hypothesis, this study provides a time frame of Ci-Bra expression – from transcript to mature and active protein within the nuclear compartment – and offers insights into the molecular events that lead to lineage specification in notochord cells.

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