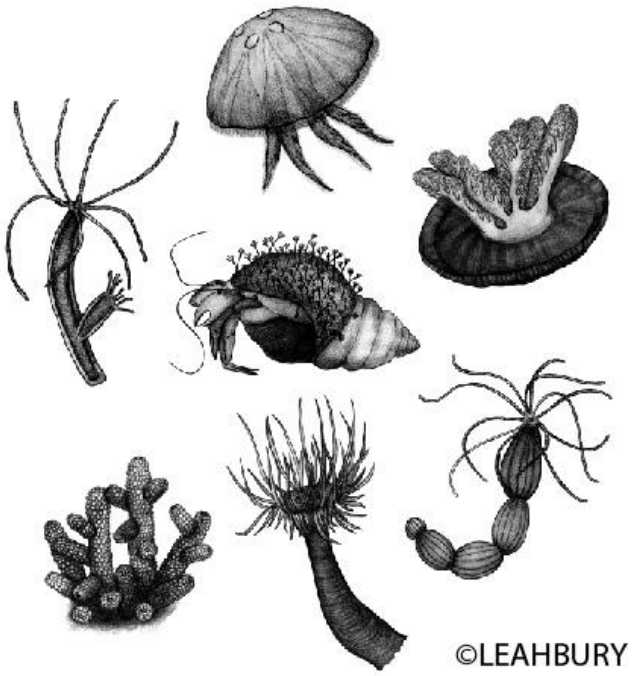
First Biennial Cnidofest:
The Cnidarian Model Systems Meeting

Whitney Laboratory for Marine Bioscience,
University of Florida, St. Augustine, FL
September 6-9, 2018



Co-Organizers:
Christy Schnitzler
Celina Juliano

THE WHITNEY LABORATORY
for MARINE BIOSCIENCE
UNIVERSITY OF FLORIDA

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About Cnidofest

In September 2016, we organized the first North American meeting of hydroid biologists ("Hydroidfest"), which was attended by approximately 60 people and, according to a post-meeting survey and informal feedback, was a huge success. We exceeded our own expectations for enthusiastic participation by the community and we accomplished our stated goal of bringing together the North American hydroid research community. Our long-term goal is to grow the cnidarian scientific community in North America and Hydroidfest was a big step in that direction. We began planning for the 2018 meeting in mid-to-late 2017. After surveying the broader cnidarian research community and speaking with the organizers of previous *Nematostella* meetings, it became clear that a broader meeting that encompassed all cnidarian model organisms was needed. Thus, the Cnidarian Model Systems Meeting (aka "Cnidofest") was born.

Thanks to rapidly advancing technologies, the cnidarian community is poised to make important breakthroughs; it is no longer necessary or even sensible to limit the number of model systems used to conduct detailed functional studies. Large collections of genomic and transcriptomic data have been generated and single-cell sequencing technologies are now beginning to refine these data sets further. These data, in combination with new gene editing capabilities, are opening novel experimental avenues and enabling us to use cnidarians to answer fundamental biological questions.

A number of North American laboratories, both well-established and newly established, have recognized the great potential of cnidarian models and are now using these animals to address fundamental biological questions. We are pleased that many of these laboratories are represented here at Cnidofest 2018. Our long-term goal is to hold our US-based meeting every two years, alternating between east and west coasts, thus balancing travel burdens for scientists in our growing community.

We welcome you to Cnidofest and look forward to an exciting and productive meeting. Thank you for joining us as we seize this opportunity to join together and celebrate our shared interest in and love of cnidarian model systems.

Follow Cnidofest on Twitter: @cnidofest

Use the hashtag #Cnidofest for all of your tweets!
Organizing Committee

Meeting Co-Organizers

Dr. Christine Schnitzler
Assistant Professor
University of Florida Whitney Laboratory for Marine Bioscience
Twitter: @christyschnitz

Christy is the on-site host and co-organizer. She uses *Hydractinia* to explore fundamental biological processes, such as stem cell-mediated regeneration, innate immunity, and cellular senescence. Read more about Christy at www.whitney.ufl.edu/christineschnitzler/

Dr. Celina Juliano
Assistant Professor
University of California, Davis
Twitter: @Juliano_Lab

Celina is one of our co-organizers. She is using *Hydra* as a model to study stem cells and regeneration. You can read more about Celina and her research at https://juliano.faculty.ucdavis.edu/

Organizing Committee
Committee Members

Dr. Matthew Nicotra
Assistant Professor
University of Pittsburgh
Twitter: @nicotralab

When he's not updating the Cnidofest 2018 website, Matt and his lab use *Hydractinia symbiolongicarpus* to study all aspects of allorecognition. Read about the lab at www.nicotralab.org.

Mr. Shuonan He
PhD Candidate
Stowers Institute for Medical Research

“Shuonan is the best thing since sliced bread.” - @MadScientist

Shuonan is a star graduate student in the laboratory of Matt Gibson. His research focuses on employing reverse genetic tools to dissect the function and regulation of Hox genes in the starlet sea anemone, *Nematostella vectensis*. Due to the unique phylogenetic position of Cnidarian animals, his studies provide valuable insights regarding the origin and evolution of Hox genes.
http://research.stowers.org/gibsonlab/shuonan-he.html

Dr. Juris Grasis
Assistant Professor
University of California, Merced
Twitter: @immunoviromics

Juris is our social media coordinator and tech guru. He uses *Hydra* as a model for host-holobiont interactions, with a focus on viral interactions. You can read more about Juris and his research at www.immunoviromics.org

Cnidofest 2018 Schedule At A Glance

Thursday, September 6

11:00 am – 1:00 pm	Registration Check-in
1:00 – 1:15 pm	Welcome
1:15 – 2:30 pm	Session 1: Genomics
2:30 – 3:00 pm	Coffee Break
3:00 – 4:15 pm	Session 2: Neurobiology I
4:15 – 4:45 pm	Coffee Break
4:45 – 5:25 pm	Lightning Talks I
5:25 – 7:30 pm	Poster Session I with drinks and appetizers
7:30 – 9:30 pm	Dinner
After 9:30 pm	Know about each other in the bar

Friday, September 7

8:00 – 9:00 am	Breakfast
9:00 – 10:15 am	Session 3: Development I
10:15 – 10:45 am	Coffee Break
10:45 am – 12:00 pm	Session 4: Development II
12:00 – 1:00 pm	Lunch
1:00 – 2:45 pm	Free Time
2:45 – 4:00 pm	Session 5: Development III
4:00 – 4:15 pm	Cnidofest 2018 Group Photo
4:15 – 4:45 pm	Coffee Break
4:45 – 5:25 pm	Lightning Talks II
5:25 – 7:30 pm	Poster Session II with drinks and appetizers
7:30 – 9:30 pm	Dinner
After 9:30 pm	Know about each other in the bar

Saturday, September 8

8:00 – 9:00 am	Breakfast
9:00 – 10:15 am	Session 6: Evolution
10:15 – 10:45 am	Coffee Break
10:45 am – 12:00 pm	Session 7: Neurobiology II
12:00 – 1:00 pm	Lunch
1:00 – 2:30 pm	Free Time
2:30 – 3:45 pm	Session 8: Immunology
3:45 – 4:15 pm	Coffee Break
4:15 – 5:30 pm	Session 9: Symbiosis
5:30 – 6:30 pm	Keynote Session
6:30 – 7:30 pm	Appetizers and Drinks
7:30 – 10:00 pm	Banquet Dinner
After 9:30 pm	Know about each other in the bar

Cnidofest 2018 Schedule At A Glance

Sunday, September 9

8:00 – 9:00 am	Breakfast
9:00 – 10:15 am	Session 10: Ecology
10:15 – 10:45 am	Coffee Break
10:45 am – 12:00 pm	Session 11: Cell Biology and Biophysics
12:00 – 12:45 pm	Lunch
12:45 – 1:00 pm	Awards Ceremony and Closing Remarks

Cnidofest 2018 Program

Thursday, September 6

11:00 am - 1:00 pm	Registration and Check-in
1:00 - 1:15 pm	Welcome
1:15 - 2:30 pm	**Session 1: Genomics** *Session Chair: Andreas Baxevanis* Christy Schnitzler (p. 13) - "New kid on the block: Placing the *Hydractinia* genome within the context of established cnidarian genomes" Joe Ryan (p. 14) - "Attaching thousands of leaves to the cnidarian tree of life" Wai Yee Wong (p. 15) - "Evolutionary Dynamics and Function of *Hydra* Transposons" Steve Sanders (p. 16) - "CRISPR/Cas9-mediated gene knockin in the hydroid *Hydractinia symbiolongicarpus*"
2:30 - 3:00 pm	Coffee Break
3:00 - 4:15 pm	**Session 2: Neurobiology I** *Session Chair: Michael Layden* Jacob Robinson, Invited Technology Speaker (p. 17) - "Micro- and nano-technologies to reveal neural and behavioral states in small invertebrates" Krishna Badhiwala (p. 18) - "Automated tracking of locomotion pattern in *Hydra vulgaris*" Yuki Noro (p. 19) - "Functional imaging of Hym-355 and Hym-121 peptidergic neurons in *Hydra*"
4:15 - 4:45 pm	Coffee Break
4:45 - 5:25 pm	Lightning Talks
5:25 - 7:30 pm	Poster Session 1 with drinks and appetizers 01 - Cheryl Ames (p. 20) 02 - Belinda Artes (p. 21) 03 - Sofia Barreira (p. 22) 04 - Sydney Birch (p. 23) 05 - Elisabeth Caoili (p. 24) 06 - Ruoxu Chen (p. 25) 07 - Michael Connelly (p. 26) 08 - Christophe Dupre (p. 27) 09 - Guillaume Duret (p. 28) 10 - Maddison Harman (p. 29) 11 - Shuonan He (p. 30) 12 - Alexandra Hernandez (p. 31) 13 - Grace Jean (p. 32)

14 - Kade Muffett (p. 33)
15 - Elizabeth Lanphear (p. 34)
16 - Dmitrij Ljaschenko (p. 35)
17 - Yui Matsumoto (p. 36)
18 - Susan McLaughlin (p. 37)
19 - Yu-ichiro Nakajima (p. 38)
20 - Aki Ohdera (p. 39)
21 - David Plachetzki (p. 40)

7:30 - 9:30 pm — Dinner

Friday, September 7

8:00 - 9:00 am — Breakfast

9:00 - 10:15 am — **Session 3: Development I**

Session Chair: Athula Wikramanayake

Angelika Böttger (p. 41) - "Investigating Notch-signalling in *Hydr*a by differential gene expression profiling combined with analysing single cell sequencing data"

Febrimarsa Febrimarsa (p. 42) - "Accumulation of N6-methyldeoxyadenosine precedes the zygotic genome activation during early embryogenesis in *Hydractinia*"

Ahmet Karabulut (p. 43) - "Electroporation of small hairpin RNAs for rapid and efficient gene knock-down in the sea anemone, *Nematostella vectensis*."

Cheng-Yi Chen (p. 44) - "Finding their way: Primordial germ cell specification and migration in *Nematostella vectensis*"

10:15 - 10:45 am — Coffee Break

10:45 am - 12:00 pm — **Session 4: Development II**

Session Chair: David Plachetzki

Bert Hobmayer (p. 45) - "Cnidarian sFRPs in primary body axis patterning - an update"

Hongyan Sun (p. 46) - "Evolution of Axin function in the Wnt/β-catenin pathway in metazoans: Insights from sea anemones and sea urchins"

Jack Cazet (p. 47) - "Chromatin remodeling during *Hydra* regeneration"

Naga Nakanishi (p. 48) - "CRISPR knockouts reveal an endogenous role for ancient neuropeptides in regulating developmental timing in a sea anemone"

12:00 - 1:00 pm — Lunch

1:00 - 2:45 pm — Free Time

2:45 - 4:00 pm — **Session 5: Development III**

Session Chair: Celina Juliano

Jeff Farrell, Invited Technology Speaker (p. 49) - "Spatial and temporal reconstruction of embryogenesis using single-cell RNAseq"

Stefan Siebert (p. 50) - "Stem cell differentiation trajectories in *Hydra* resolved at single cell resolution"

Flora Plessier (p. 51) - "Targeted cell labelling and single-cell transcriptomics to explore neural developmental trajectories in the anthozoan *Nematostella vectensis*"

4:00 - 4:15 pm — Group Photo

4:15 - 4:45 pm — Coffee Break

4:45 - 5:25 pm — Lightning Talks

5:25 - 7:30 pm — Poster Session 2 with drinks and appetizers

22 - Rebecca Helm (p. 52)
23 - Remi Ketchum (p. 53)
24 - Whitney Leach (p. 54)
25 - Jason Macrander (p. 55)
26 - Shelcie Menard (p. 56)
27 - Jason Presnell (p. 57)
28 - Abby Primack (p. 58)
29 - Gonzalo Quiroga-Artigas (p. 59)
30 - Kevin Rodriguez (p. 60)
31 - Miguel Salinas-Saavedra (p. 61)
32 - Hiroshi Shimizu (p. 62)
33 - Jennifer Spillane (p. 63)
34 - Joshua Swore (p. 64)
35 - Bryan Teefy (p. 65)
36 - Matthew Travert (p. 66)
37 - Rui Wang (p. 67)
38 - Wataru Yamamoto (p. 68)
39 - Kelsey Yetsko (p. 69)
40 - Benjamin Young (p. 70)
41 - Paige Zhang (p. 71)
42 - Bob Zimmerman (p. 72)
43 - Alexandrea Duscher (p. 73)

7:30 - 9:30 pm — Dinner

Saturday, September 8

8:00 - 9:00 am — Breakfast

9:00 - 10:15 am — **Session 6: Evolution**

Session Chair: Paulyn Cartwright

Natasha Picciani (p. 74) - “Prolific origination of eyes in Cnidaria with co-option of non-visual opsins”
E. Sally Chang (p. 75) - “Species delimitation and the evolution of freshwater tolerance in the invasive hydrozoan *Cordylophora* using phylogenetic, population genomic and environmental evidence”
Lara Adolfo (p. 76) - “Gap junction distribution in Cnidarians”
Meg Daly (p. 77) - “A quick fuse: melded trees highlight progress and opportunities in anemone biology”

10:15 - 10:45 am — Coffee Break

10:45 am - 12:00 pm — **Session 7: Neurobiology II**
Session Chair: Hiroshi Shimizu
Dylan Faltine-Gonzalez (p. 78) - “Investigating neuronal subtype development in *Nematostella vectensis*”
Jamie Havrilak (p. 79) - “Investigation of nervous system dynamics in *Nematostella vectensis*”
Jonathan Lovas (p. 80) - “A phase transition in activity of *Hydra’s* nervous system as it reassembles from individual cells”
Leslie Babonis (p. 81) - “A cnidocyte-specific gene regulatory network from *Nematostella vectensis*”

12:00 - 1:00 pm — Lunch

1:00 - 2:30 pm — Free Time

2:30 - 3:45 pm — **Session 8: Immunology**
Session Chair: Matthew Nicotra
Aidan Huene (p. 82) - “Third allodeterminent found in *Hydractinia* allorecognition complex.”
Nikki Traylor-Knowles (p. 83) - “Cellular and genomic immunity of *Pocillopora damicornis*”
Leah Williams (p. 84) - “Cnidarian toll-like receptor signaling”
Eviatar Weizman (p. 85) - “Chromatin dynamics enable transcriptional rhythms in the non-symbiotic cnidarian *Nematostella vectensis*”

3:45 - 4:15 pm — Coffee Break

4:15 - 5:30 pm — **Session 9: Symbiosis**
Session Chair: Jason Macrander
Juris Grasis (p. 86) - “Viruses as intimate partners in the *Hydra* holobiont”
Lorraine Ling (p. 87) - “The possible role of C-type lectins in establishing specificity in Cnidarian-*Symbiodinium* symbiosis”
Thomas Gilmore (p. 88) - “Transcription factor NF-κB is modulated by symbiotic status in the sea anemone *Aiptasia*”
Cassandra Newkirk (p. 89) - “Acquisition and proliferation of algal symbionts in polyps of the upside-down jellyfish”

5:30 - 6:30 pm	**Keynote Address** Virginia Weis (p. 90) - “Symbiotic sea anemones as model systems for the study of corals: Easier, faster, softer”
6:30 - 7:00 pm	Appetizers and Drinks
7:00 - 10:00 pm	Banquet Dinner

Sunday, September 9

8:00 - 9:00 am	Breakfast
9:00 - 10:15 am	**Session 10: Ecology** *Session Chair: Ann Tarrant* Adam Reitzel (p. 91) - “Variation in transcription and protein-protein interactions of Hsp70 in the anemone *Nematostella vectensis*” Hanny Rivera (p. 92) - “Plasticity in parental effects confers rapid thermal tolerance to *Nematostella vectensis* larvae“ Sergio Stampar (p. 93) - “Ceriantharia (Cnidaria) as an alternative model to study evolution and life cycles” Grace Snyder (p. 94) - “Transcriptional and microbial characterization of scleractinian coral cell populations separated by Fluorescence-Activated Cell Sorting (FACS)”
10:15 - 10:45 am	Coffee Break
10:45 am - 12:00 pm	**Session 11: Cell Biology and Biophysics** *Session Chair: Christophe Dupre* Helen McNeill (p. 95) - “Atypical cadherin Fat in *Hydra* development” Tapan Goel (p. 96) - “Mechanical coupling coordinates mouth opening in *Hydra*” Lauren Vandepas (p. 97) - “Chitin prevalence and diversity of chitin synthase genes across Cnidaria” Taylor Skokan (p. 98) - “Contractile actin rings suggest an engulfing role for ectodermal cells in *Hydra vulgaris*”
12:00 - 12:45 pm	Lunch
12:45 - 1:00 pm	Awards Ceremony and Closing Remarks

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New Kid on the Block: Placing the *Hydractinia* Genome within the Context of Established Cnidarian Genomes

Christine E. Schnitzler[1], Anh-Dao Nguyen[2], Sergey Koren[2], James M. Gahan[3], Sofia Barreira[2], Steven M. Sanders, Sebastian Gornik[4], Paul Gonzalez[2], Nicola Wong[5], Oleg Simakov[5], Adam Phillippy[2], Jim Mullikin[2], Paulyn Cartwright[6], Matthew Nicotra[7], Uri Frank[8], and Andreas D. Baxevanis[2]

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Cnidarian genomes provide a framework for exploring fundamental questions about the evolution of complex biological processes such as embryonic development, regeneration, self-recognition, and aging. We are focusing on the colonial cnidarian *Hydractinia*, a hydrozoan representative that has lost the medusa stage and produces gametes directly from sexual polyps. *Hydractinia* forms colonies of clonal polyps that are typically found on shells inhabited by hermit crabs. In the lab, we culture colonies on glass microscope slides and study gene function using several established functional genomics tools, including CRISPR/Cas9 genome editing. *Hydractainia* are highly regenerative, as they possess a lineage of migratory stem cells known as 'i-cells'. We have generated high-quality, high-coverage genome and transcriptome assemblies for *H. echinata* and its sister species, *H. symbiolongicarpus*, using PacBio long-read and Dovetail-based strategies for the genome and Illumina data for the transcriptome. The genome assemblies are 419 Mb for *H. echinata* (84x coverage, 1,582 scaffolds, N50 of 1.51 Mb) and 308 Mb for *H. symbiolongicarpus* (94x coverage, 392 scaffolds, N50 of 3.31 Mb), placing them among the most contiguous invertebrate genomes. Similar to *Hydra*, both genomes are AT-rich (65%) and highly repetitive (>50%). We are using a comparative genomics approach that includes ortholog clustering to determine how closely these genomes resemble other established cnidarian model genomes. Our results reveal both the conserved features and extensive evolutionary novelties contained within these *Hydractinia* genomes.

Attaching thousands of leaves to the cnidarian tree of life

Melissa B. DeBiasse[1], Ariane Buckenmeyer[1], Leslie Babonis[1], Bastian Bentlage[2], Allen G. Collins[3], Marymegan Daly[4], Jason Macrander[5], Adam M. Reitzel[6], Sérgio N. Stampar[7], and Joseph F. Ryan[1]

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[2]University of Guam, Mangilao, GU, USA
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[5]Florida Southern College, FL, USA
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Cnidarians are a stunning group of animals with diverse ecology, life history, and morphology. Resolving the relationships within Cnidaria is the essential first step to understanding the fascinating evolutionary processes that occurred within Cnidaria and for reconstructing the biology of the cnidarian-bilaterian ancestor, an animal that prospered in Precambrian oceans. Nevertheless, the position of cnidarians in the tree of life have been in flux over the last decade, and the position of several nodes, especially within the Anthozoa, are not satisfactorily established. Here we take an innovative, synergistic approach that combines a backbone phylogeny constructed from 110 cnidarian tips. We then run a phylogeny of thousands of cnidarian 18S sequences constrained by this backbone to produce the most comprehensive cnidarian phylogeny to date. Our phylogenomic dataset contains 32 new taxa including five new deeply sequenced cerianthid (tube anemone) transcriptomes, which allows us to shed light on this very interesting and controversial lineage. This ginormous tree of cnidarian life will serve as the foundation for future trait-based analyses and pave the way for understanding the evolutionary history of cnidarian innovations.

Evolutionary Dynamics and Function of *Hydra* Transposons

Wai Yee Wong[1], Daniel E. Martínez[2], Thomas Holstein[3], Charles N. David[4], Robert E. Steele[5], and Oleg Simakov[1]

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[4] Ludwig Maximilian University of Munich, Munich, Germany
[5] University of California at Irvine, California, United States

More than half of the *H. magnipapillata* genome is composed of transposable elements (TEs). While TEs are thought to be a major player in facilitating adaptation by providing new genetic material, regulating gene expression, and changing the genomic architecture, the role of the reported TE expansions in hydra remains unclear. In this study, we aimed to examine the evolutionary history of TEs and their function in hydra by a comparative genomic approach. To improve on the fragmented annotation during TEs classification, we developed a novel meta-pipeline, RepeatCraft, which defragments closely spaced repeat loci in the genomes. After applying the pipeline, the total number of repeat loci in *H. magnipapillata* was reduced by about 10%. We then performed a comparative analysis using the transcriptome and genome data from a green hydra (*Hydra viridissima*) and four brown hydras (*Hydra circumcincta*, *Hydra oligactis*, *Hydra vulgaris* strain AEP and Zurich), in reference to the *H. magnipapillata* genome. The result reveals that there was a significant expansion of a single LINE family specific to brown hydras, compared with the much less abundant LINE elements in the green hydra. We propose that the expansion of this LINE family led to the significant genome size increase in brown hydras. We are studying the role of this TE expansion in hydrozoan evolution, unraveling changes in genomic architecture associated with it, as well as its impact on genes active during development or regeneration.

Belyayev, A. (2014). Bursts of transposable elements as an evolutionary driving force. *Journal of Evolutionary Biology*, *27*(12), 2573–2584.
Chapman, J. A., et al. (2010). The dynamic genome of Hydra. *Nature*, *464*(7288), 592.
Petersen, H. O.,et al. (2015). A Comprehensive Transcriptomic and Proteomic Analysis of Hydra Head Regeneration. Molecular Biology and Evolution, 32(8), 1928–1947.
Smit, AFA, Hubley, R. (2008-2015). *RepeatModeler Open-1.0*. http://www.repeatmasker.org

CRISPR/Cas9-mediated gene knockin in the hydroid *Hydractinia symbiolongicarpus*

Steven M. Sanders[1,2], Zhiwei Ma[1,2], Julia M. Hughes[1,2], Brooke M. Riscoe[1,2], Gregory A. Gibson[3], Alan M. Watson[3], Hakima Flici[4], Uri Frank[4], Christine E. Schnitzler[5], Andreas D. Baxevanis[6], and Matthew L. Nicotra[1,2,7]

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[7] Department of Immunology, University of Pittsburgh, Pittsburgh, PA, USA

Hydractinia symbiolongicarpus, a colonial cnidarian, is a tractable model system for many cnidarian-specific and general biological questions. Until recently, tests of gene function in *Hydractinia* have relied on laborious forward genetic approaches, randomly integrated transgenes, or transient knockdown of mRNAs.Here, we report the use of CRISPR/Cas9 genome editing to generate targeted genomic insertions in *H. symbiolonigcarpus*. We used CRISPR/Cas9 to promote homologous recombination of two fluorescent reporters, eGFP and tdTomato, into the *Eukaryotic elongation factor 1 alpha* (*Eef1a*) locus. We demonstrate that the transgenes are expressed ubiquitously and are stable over two generations of breeding. We further demonstrate that CRISPR/Cas9 genome editing can be used to mark endogenous proteins with FLAG or StrepII-FLAG affinity tags to enable *in vivo* and *ex vivo* protein studies. This is the first account of CRISPR/Cas9 mediated knockins in *Hydractinia* and the first example of the germline transmission of a CRISPR/Cas9 inserted transgene in a cnidarian. The ability to precisely insert exogenous DNA into the *Hydractinia* genome will enable sophisticated genetic studies and further development of functional genomics tools in this understudied cnidarian model.

Research supported by NSF Grant IOS-1557339 and NIH Grant T32AI074490

Micro- and nano-technologies to reveal neural and behavioral states in small invertebrates

Krishna Badhiwala[1], Daniel Gonzalez[2], and Jacob T. Robinson[1-4]

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[3]Department of Electrical and Computer Engineering, Rice University, 6100 Main Street, Houston, Texas 77005
[4]Department of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, Texas, 77030

Millimeter-sized invertebrates like *C. elegans* and *Hydra* with only a few hundred to a few thousand neurons raise the exciting possibility of completely understanding the relationship between neural activity and behavior. To reach this goal we believe that it is critical to measure and manipulate neural activity while we simultaneously quantify the animal's behavior and control properties of the environment. In this talk I will discuss how microfluidics devices enable this type of quantitative neural and behavioral interrogation in small invertebrates. Specifically, we will compare microfluidic technologies for high-throughput electrophysiology and fluorescence imaging in *C. elegans* and *Hydra*, and describe how these devices can help answer fundamental questions about sensory motor transformations, neural control, and the relationship between neural and behavioral states.

Automated tracking of locomotion pattern in *Hydra vulgaris*

Krishna N. Badhiwala[1] and Jacob T. Robinson[1,2,3]

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[2]Department of Electrical and Computer Engineering, Rice University, 6100 Main Street, Houston, Texas 77005
[3]Department of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, Texas, 77030

Basic body movements like contraction, elongation and nodding are well characterized in *Hydra*, but questions remain regarding the purpose of these movements. Specifically, do *Hydra* link behavioral motifs together to achieve complex behaviors such as goal-directed locomotion? To answer this question, we have developed automated image processing algorithms to track *Hydra* movements in a variety of environments. From long-term, time-lapse images, we can extract positional information to identify body posture and translation. We then generate spatiotemporal maps of the detected behavioral motifs to analyze the combinations of movements that result in complex locomotion patterns. This approach complements recently demonstrated microfluidic behavioral chambers that enable time-lapse imaging of many animals in parallel. We show that together microfluidic technologies and automated image tracking enable studies of complex locomotive patterns like photo- and thermo-taxis. We also identify the basic body movements that could form the building blocks for complex locomotion patterns. Together these technologies support efforts to understand sensory motor transformations in *Hydra* by quantifying how the animals behave in different environments.

Functional Imaging of Hym-355 and Hym-121 Peptidergic Neurons in *Hydra*

Yukihiko Noro, Hiroshi Shimizu, Hanan Mahmood, Katsuhiko Mineta, and Takashi Gojobori

King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia

To understand how the brain works is one of the most challenging goals in modern biology. Although hydra is not believed to have the so-called brain, its simple nervous system and pattern of behaviors would be a good advantage to address this issue. More than ten years ago we successfully selected nine genes that are selectively expressed in neurons, out of which seven genes encoded neuropeptides and four of them had been already known (Hym-176, RFa, Hym-355, GLWa). This suggests that peptidergic neurons are major components of hydra nervous system and that these seven gene markers may cover all the peptidergic neurons in hydra.

In the previous hydroidfest 2016 meeting we showed the neuron subsets expressing two of the seven neuropeptide genes, Hym-176-coding gene (Hym-176A) and its paralogue (Hym-176B) respectively had motor functions, exerting longitudinal contractions. In this presentation we will show the functional imaging of another two peptidergic neurons expressing Hym-355 or Hym-121. Interestingly the excitation of these two neuron subsets was always synchronized within a subset and was not correlated to any movements of hydra. Therefore, we concluded these neurons subsets function as autonomic or sensory neurons.
Out of the seven selected neuropeptide genes, we have currently isolated functional promoters for five genes, for four of which we also raised GCaMP6s transgenic animals as we described above and for the rest of which we just raised GFP transgenic animals for now. We hope to isolate functional promoters for all the nine neuron-specific genes and to raise GCaMP6s transgenic animals for all of them in the end to see the activity of almost all neurons in hydra.

Hwang J. S. (2007). The evolutionary emergence of cell type-specific genes inferred from the gene expression analysis of *Hydra.* Proceedings of the National Academy of Sciences, 104 (37) 14735-14740

Field-forward DNA sequencing as a tool to establish the upside-down mangrove jellyfish *Cassiopea* as an indicator species

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The relative ease of operation, high throughput and cost of Next Generation Sequencing platforms have enabled the coupling of traditional field collection methods with laboratory-based metagenomic sequencing approaches to provide a molecular snapshot of species-diversity in many aquatic environments. Unfortunately, the time lag between field sampling, sequencing and endpoint bioinformatics analyses precludes the ability to provide a contemporaneous characterization of the target ecosystem, against the backdrop of shifting global climate. The growing decline of aquatic ecosystems due to chemical, physical and biological threats, along with issues related to invasive species, highlights the critical need for field-forward sequencing protocols that can provide rapid characterizations of environmental communities. The upside-down mangrove jellyfish *Cassiopea* has been gaining attention as an indicator species, with promising applications for coastal ecosystem management. We developed a field-forward DNA sequencing strategy to collect and analyze environmental DNA from *Cassiopea* populations inhabiting mangrove communities of Key Largo, FL in ecosystems affected by Hurricane Irma in July 2017. The prototype for this portable system provides: 1. A low-complexity protocol requiring minimal training for operation, 2. A relatively short “sample-to-answer” timeframe, 3. Field-forward DNA sequencing capabilities in austere environments, 4. Manual and/or battery-powered operation, 5. A small footprint and ease of portability, and 6. Multiplexing capabilities for the simultaneous assessment of multiple collection sites and/or genetic markers. We present here an assessment of the condition of three *Cassiopea* populations, based on the findings of our inaugural field-forward sequencing study.

Action of ß-Catenin in head- and foot-specific differentiation in regenerating *Hydra* polyps

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Maintaining the self renewal capacity of a stem cell or initiating the differentiation of a cell are fundamental mechanisms in regeneration processes and need to be orchestrated precisely. In the ancestral freshwater polyp *Hydra* the canonical Wnt pathway is known to initiate head organizer formation, to play a significant role in positional information along the single body axis, and to act in the pre-patterning phase in head regenerates. ß-Catenin was shown to act as early response gene within the first 30 minutes after decapitation. Here, we present data using the small molecule inhibitor iCRT14, which is able to inhibit ß-Catenin-Tcf interaction in the nucleus and thereby down-regulates canonical Wnt signaling. Treatment of multi-headed ß-Catenin transgenic animals with iCRT14 results in a rescue towards a normal wild type phenotype. Treatment of regenerates shows that both head and foot regeneration depend on nuclear ß-Catenin activity and that a block of ß-Catenin responsive transcription represses the terminal differentiation of head- and foot-specific cells. Furthermore, ß-Catenin and ß-Catenin induced target gene expression remains upregulated in head and foot regenerates when ß-Catenin responsive transcription is inhibited by iCRT14. We therefore propose, that a gene regulatory ß-Catenin activity is required for a transition from pre-patterning to position-specific differentiation. In addition, the spatiotemporal differences of transcriptional and translational profiles of the head-specific factor HyBra1 provide evidence that a translational control mechanism is involved at the onset of a position-dependent differentiation program.

Exploring the role of ribosomal gene repeats in regeneration

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Repetitive DNA has been implicated in chromatin organization, regulation of gene expression, genome replication, cell proliferation, and the maintenance of genome integrity, but large repeats are often not found in reference genomes. Establishing the organization and distribution of repetitive DNA within a genome is crucial to fully understanding cellular function and for identifying new targets for therapeutic applications through genome editing. In this study, we have focused on *Hydractinia*, a colonial marine cnidarian that is a valuable model organism for the study of regenerative medicine. Like human embryonic stem cells, *Hydractinia* stem cells are pluripotent and have homologs to human genes associated with the ability to self-renew and differentiate. Importantly, the regenerative process depends on proper cell growth throughout the numerous cycles of cell division which, in turn, depends on the timely and flawless assembly of ribosomes. We have already identified a complete ribosomal gene consensus sequence in *Hydractinia* and have determined the genomic architecture of its rDNA repeats. A comprehensive protein domain structural analysis indicates that, unlike human, mouse, and frog, *Hydractinia* does not possess the canonical UBF protein, a transcription factor that is known to bind to rDNA and is required for the recruitment of the Pol I transcription machinery during ribosome biogenesis. This opens the possibility that *Hydractinia* might employ a different mechanism for regulating transcription of rDNA genes and nucleolar formation than that used by higher eukaryotes, perhaps providing important insight as to the regenerative capacity of this organism. This overall approach and comparison of these repeats and transcription factors between regenerative and non-regenerative organisms might reveal mechanisms that are primitive and shared among animals (or evolutionarily derived ones); this will help address key questions in regeneration and prompt the development of new clinical approaches to improve human health.

The genomic characterization of larval settlement in the biofouling invertebrate *Ectopleura larynx*

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The hydroid *Ectopleura larynx* has an indirect lifecycle that produces a dispersive larval stage called actinula. Actinulae larvae select the substrate upon which they settle by integrating sensory cues from the environment. Previous research has investigated the settlement biology of actinulae larvae, however, to date, no study has combined sensory behavior experiments with molecular genetics. Here we examine the molecular genetics underlying the behavioral response to environmental cues during settlement of the actinula larva. We hypothesize that that light and biofilm-derived chemical cues are detected by cnidarian opsin and T1R taste receptors respectively and that these genes will be differentially expressed in sensory neurons as actinula development proceeds to settlement. We will test these hypotheses using RNAseq on various stages of actinula development through settlement and combine these data with behavioral experiments that examine the effects of light intensity, wavelength and biofilm-derived chemical cues on the propensity to settle. The goal of this research is to elucidate the interplay between behavior, genetics, and the sensory environment in the settlement of the actinula larva of *Ectopleura*.

We would like to acknowledge our funding from the USDA and the University of New Hampshire.

Determining the expression patterns of two *Brachyury* paralogs in *Hydractinia* head regeneration

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The gene *Brachyury* belongs to the T-box family of transcription factors and is widely known for its conserved role in mesoderm formation and body axis patterning across vertebrates. Despite its established function as a mesodermal marker, homologues of this gene have recently been discovered in diploblasts, including two paralogs (*HyBra1* and *HyBra2*) in the freshwater cnidarian *Hydra magnipapillata* (Bielen, 2007). RNAseq data from our lab and collaborators indicated that two paralogous *Brachyury* genes are also expressed in the colonial hydrozoan, *Hydractinia echinata*. Moreover, both of these genes are highly upregulated in blastema tissue during head regeneration at 24 hours post head removal. We now plan to visualize the spatial and temporal patterns of expression for both *Brachyury* paralogs through *in situ* hybridization at different time points during head regeneration in our lab's model organism, *Hydractinia symbiolongicarpus.* Defining the expression patterns of these *Brachyury* paralogs during regeneration and in intact adult polyps will serve as an informative first step in investigating the role of this conserved gene in *Hydractinia.* We will compare our results to recent expression studies of *Brachyury* genes in other model cnidarians.

Bielen, H., Oberleitner, S., Marcellini, S., Gee, L., Lemaire, P., Bode, H., Technau, U. (2007). Divergent functions of two ancient Hydra Brachyury paralogues suggest specific roles for their C-terminal domains in tissue fate induction. Development, 134(23), 4187. http://dx.doi.org/10.1242/dev.010173.

Mapping of sex determination loci in *Hydractinia symbiolongicarpus*

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Sexual reproduction is ubiquitous in eukaryotes, and sex determination strategies vary greatly among different species. Although many divergent sex determination strategies have been elucidated in bilaterians, we still know little about nonbilaterians. Notably, no sex determination mechanisms have been identified in any cnidarian. Here we report progress in identifying sex determination loci in the colonial cnidarian, *Hydractinia symbiolongicarpus*. Our hypothesis is that sex determination is regulated by genetic rather than environmental factors and that distribution of relevant genes may be either chromosomal or multiple loci. To test this hypothesis, we are constructing a linkage map of the *Hydractinia* genome which we will then use to identify quantitative trait loci (QTL) that co-segregate with sex. To do this, we have crossed two strains of *H. symbiolongicarpus* to create an F1 population. We then sequenced both parents and 96 offpspring on the Illumina platform to obtain 30X coverage per animal. We are currently identifying variants using the GATK pipeline. SNPs conforming to expectations of a pseudotestcross (heterozygous in one parent and homozygous in the other) will then be used to generate a linkage map, followed by the identification of sex-linked loci. This work has the potential to reveal the first cnidarian sex determination loci, and will provide chromosome-level linkage groups for the *Hydractinia* genome, which should be useful for future studies.

Differential immune gene expression of *Pocillopora damicornis* corals in response to antibiotics treatment, heat stress, and lipopolysaccharide exposure

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Corals establish diverse symbioses with dinoflagellate algae, bacteria, and micro-eukaryotes that benefit the health of the coral holobiont. Climate change is causing a global collapse of coral reefs, as rising temperatures disturb coral symbioses resulting in coral bleaching, disease and death. Activation of innate immunity has been observed during coral stress responses, and heat tolerance may be influenced by the coral bacteria community. To clarify patterns of immune gene expression in response to heat stress and bacterial community disruption, *Pocillopora damicornis* corals from Kenting National Park in Taiwan were subjected to separate and combined treatments with antibiotics (ampicillin and streptomycin), heat stress, and bacterial lipopolysaccharide (LPS), an immune stimulant. Differential gene expression analysis and weighted gene co-expression network analysis revealed that genes involved in microbial recognition, immune signaling and defense were upregulated in LPS and antibiotics treatments relative to control fragments, suggesting that antibiotics cause a dysbiotic bacterial community that drives immune inflammation. Additionally, immune genes were downregulated during heat stress, and activation of apoptotic pathways was observed in combined antibiotics and heat treatments. These results suggest that stability within the coral bacterial community is essential for maintaining coral immune function and holobiont health in a changing climate.

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Scalability in the nervous system of Hydra

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The nervous system can change in size dramatically while keeping its functions intact, which is illustrated by the fact that many species of vastly different sizes exhibit very similar behaviors. In rodents for instance, the brain size can span two orders of magnitude, with the mouse brain having about 70 million neurons whereas the capybara brain has about 1600 million neurons (Figure 1A) [1]. Uncovering the mechanisms underlying brain scalability can help understand fundamental principles of brain function, since brain size differences among closely related species are frequently observed throughout the evolutionary tree [2]. This problem is difficult to study in rodents because of the complexity of their brain, which makes a side-by-side comparison very challenging. Fortunately, many other animal species can change in size too, some of them placed very early on the evolutionary tree. Among them, the freshwater invertebrate Hydra is an ideal model to pursue such questions since its nervous system is very simple and the same animal can change in size repeatedly by up to an order of magnitude (Figure 1B). We aim at reconstructing the nervous system of Hydra using electron microscopy in order to describe how it is built and how it changes when the animal grows and shrinks.

References

1. Herculano-Houzel, S. (2009). The human brain in numbers: a linearly scaled-up primate brain. Front. Hum. Neurosci. 3, 31.
2. Neves, K., Ferreira, F.M., Tovar-Moll, F., Gravett, N., Bennett, N.C., Kaswera, C., Gilissen, E., Manger, P.R., and Herculano-Houzel, S. (2014). Cellular scaling rules for the brain of afrotherians. Front. Neuroanat. 8, 5.

Electrical activity of neuron in Hydra vulgaris

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Hydra vulgaris has emerged as a promising model organism to study the relationship between behaviors and neuronal networks. However, in order to understand the neural net, neurobiologists need a description of the individual neurons' electrical activity. Specifically, the way information is transported -through action potentials (digital) or graded signals (analog)- has major implications on understanding the sparsification and integration of information, and ultimately on modeling the *Hydra's* nervous system. Although the existence of neuronal action potential has been proposed in *Hydra*, they have never been recorded at the membrane level. The recent discovery of action potential in *C. elegans* neurons has highlighted the fact that stimuli can differ drastically in timing and intensity between organisms, and that action potentials can have various molecular basis. Therefore, detecting action potentials might require unconventional recording conditions (osmolarity, ions concentration) and stimulation protocols (intensity, duration). Keeping this in mind, we propose to adapt patch clamp electrophysiology to the recording of dissociated and *in situ Hydra* neurons. By varying the composition, concentration and osmolarity of the recording buffers, and by testing various stimulation protocols we hope to reliably describe the basis of neuronal signal propagation in *Hydra*. We are convinced that answering this question will shine light on the contribution of individual neurons to *Hydra*'s behaviors.

Investigating multiple strains of *Hydractinia* from three geographic locations in the United States

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Members of the colonial hydrozoan genus *Hydractinia* display an array of desired model cnidarian characteristics including their ability to grow rapidly via asexual reproduction, their ability to spawn daily in response to light, their small size, and their translucent tissues. They are also ubiquitously found in nature occurring on hermit crab shells inhabited by crabs from the genus *Pagurus*. *Hydractinia* spp. from North America express a variety of morphologies and behaviors. Currently, the Schnitzler Lab is culturing three strains of *Hydractinia* collected from the following areas: New Haven, Connecticut; Wachapreague, Virginia; and Cedar Key, Florida. The strains from Connecticut and Virginia are subpopulations of *Hydractinia symbiolongicarpus*. The strain from Florida is an undescribed species, *Hydractinia* sp. [GM]. We are studying differences in morphology, behavior, and development between these strains. We are amplifying and sequencing the mitochondrial 16S gene from these strains and placing them within an existing 16S species tree (Miglietta et al. 2009). We are also attempting to cross the strains with one another to determine whether they can produce viable offspring. Results from this study will increase our understanding of the diversity of characteristics among subpopulations of geographically isolated *Hydractinia*.

Miglietta, MP, Schuchert, P, Cunningham, CW. 2009. Reconciling genealogical and morphological species in a worldwide study of the Family Hydractiniidae (Cnidaria, Hydrozoa). Zoologica Scripta 38: 403-430.

An ancestral Hox code controls tissue segmentation and body patterning in the sea anemone *Nematostella vectensis*

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Hox genes encode conserved developmental transcription factors that govern Anterior-Posterior (A-P) pattering in diverse bilaterian animals. Although Hox genes are also present within Cnidaria, these simple animals lack a true A-P axis, leaving it unclear how and when a functionally-integrated Hox code arose during evolution. Here we utilized recently established shRNA-mediated knockdown as well as CRISPR/Cas9 mutagenesis to demonstrate that a Hox/Gbx network (consisting of *Anthox1a*, *Anthox8*, *Anthox6a* and *Gbx*) controls radial segmentation of the larval endoderm during development of the sea anemone *Nematostella vectensis.* Intriguingly, loss of Hox/Gbx activity also elicits striking defects in tentacle patterning along the directive axis of primary polyps. Detailed analysis of *Anthox8* upstream regulatory elements further revealed the existence of a Pbx-dependent auto-regulation mechanism. Based on these observations, we propose that an ancestral Hox code controlled body patterning and tissue segmentation prior to evolution of the bilaterian A-P axis.

Has horizontal gene transfer occurred between cnidarian endoparasites and their hosts?

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Myxozoa is a diverse group of cnidarian endoparasites that have a complex life cycle with alternating hosts. Their body sizes are highly reduced and they use nematocyst homologues (polar capsules) to assist them in attachment to hosts including invertebrates (annelids and freshwater bryozoans) and vertebrates (fish, amphibians, waterfowl, and shrews) as primary and secondary hosts, respectively. Together with the monotypic *Polypodium hydriforme*, myxozoans form a clade of endoparasitic cnidarians known as Endocnidozoa. *Polypodium* includes a free-living adult stage and larval stages that infect eggs of acipenseriform fishes (sturgeon and paddlefish). To our knowledge, horizontal gene transfer (HGT) has never been reported between animals. Nevertheless, due to the close association between host and endocnidozoan parasites, we hypothesize that HGT may have occurred in these systems. To explore this hypothesis, we apply a phylogenetic framework to try to identify horizontally transferred genes in our recently obtained genomes of *Buddenbrockia plumatellae* and *Polypodium hydriforme*.

Oral-Aboral Axis Specification in "Upside Down Jellyfish" *Cassiopea xamachana*

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Many decisions about the generation of the animal body plan, including axial patterning, are made in early development. However, many animals, such as the diverse group cnidarians, are capable of post-embryonic patterning (e.g. regeneration and asexual reproduction). Genetic networks coordinating embryonic axis specification are well-characterized in the sea anemone, *Nematostella vectensis*. In another cnidarian, *Cassiopea xamachana*, buds develop the oral-aboral axis at an angle to that of the parent polyp, presenting an interesting question of how this axis is established during asexual reproduction. Here we show a preliminary morphological and molecular analysis of *Cassiopea* embryonic development with the goal of characterizing the molecular basis of axis specification in sexual and asexual reproduction. Our results indicate that expression of certain genes with known involvement in *Nematostella* axial patterning are spatially restricted during the embryonic development, consistent with involvement of these genes in *Cassiopea* axial patterning. Differences in expression patterns between *Cassiopea* and *Nematostella* also suggest diverging roles for some of these genes. This study has progressed our understanding of embryonic axis specification and helps lay the groundwork for functional studies comparing embryonic development and asexual budding in *Cassiopea*.

DuBuc et al. Hox/Wnt interact to pattern the primary body axis of an anthozoan cnidarian prior to gastrulation. *Nat. Com.* **9**, 2007 (2018).

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Description of novel nematosome-like structures in the mucus of the upside-down jellyfish *Cassiopea*

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The upside-down jellyfish *Cassiopea* (Cnidaria: Scyphozoa: Rhizostomeae) is often the subject of studies on symbiosis, and was recently proposed as a model system for studying coevolution. Yet, we know little about one of *Cassiopea*'s most specialized behaviors: the production and release of mucus that results in a phenomenon known as "stinging water." Though a familiar irritation for mangrove snorkelers, the components of this exuded mucus and its ecological role are not well understood. We report that *Cassiopea* mucus is composed of innumerable, microscopic motile cellular masses, of irregular shape, consisting primarily of nematocytes (specialized stinging cells). Though analogous to nematosomes found in the mesenteries of the model anemone *Nematostella*, *Cassiopea* nematosomes are unique in many ways, in particular the presence of *Symbiodinium* endosymbionts within these structures. Our study is the first to report on jellyfish nematosomes since the detection of these unidentified "innumerable minute spherical bodies" within *Cassiopea* mucus by perplexed luminary Alfred G. Mayer more than a century ago. Using epifluorescence and confocal microscopy, as well as specialized microfluidic devices for extended photo and video documentation, we characterized the ultrastructure of *Cassiopea* nematosomes. Additionally, we investigated their potential utility in defense and predation. We provide the first detailed description of this innovative character, along with a preliminary evaluation of the potential ecological role played by this stinging mucus in the rising model organism *Cassiopea*.

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Linalool as a Potent and Reversible Anesthetic for *Hydra vulgaris*

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The ability to make transgenic *Hydra* lines has opened the door for quantitative in vivo studies of *Hydra* regeneration and physiology. An anesthetic with the ability to reliably and innocuously relax *Hydra* tissue or whole animals would provide researchers with improved control over their experiments and enable in vivo imaging with sub-cellular resolution. Urethane is frequently used to relax and immobilize *Hydra*. However, live animals in urethane are not sufficiently immobilized for live imaging at high magnifications. Linalool, a monoterpene alcohol derived from different plant extracts, is a reversible anesthetic previously studied in rats, mice, and humans. It operates in these systems cytostatically through competitive inhibition of glutamate. Behaviorally, *Hydra* react to linalool with a full extension of the body column, tentacles splayed outwards, a posture that is particularly useful for fine manipulations. As the animals are sufficiently immobilized in linalool, it is possible to take fluorescent multi-channel z-stacks at high magnification. Using the pinch test as a readout, we found that *Hydra* incubated in 1mM linalool are fully anesthetized in 5 minutes and become responsive again 10 minutes after being returned to *Hydra* medium. While long-term incubation in 1mM impairs head regeneration, at lower concentrations of linalool (≤0.25 mM), head regeneration is not impacted. The linalool concentrations that were deemed effective in the absence of overt toxicity for short and long-term applications, respectively, are now being evaluated for possible side-effects on the cell cycle, cell death, and build-up of tolerance. Our studies suggest that linalool is a promising agent for use in *Hydra* as it acts quickly, reversibly, and innocuously, allowing for unprecedented precision in manipulation and live imaging.

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The substrate of memory: Synaptic plasticity in a wiggly nervous system. A project idea

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Synaptic plasticity (SP), i.e. activity imposed changes on synaptic transmission, is widely accepted as the mechanism for learning and memory. Synaptic proteins, which are thought to be responsible to maintain those changes are, however, not stable enough to account for long term memory since they turn over within hours to days. Therefore, it is in question that not synaptic proteins but a different more stable biochemical substrate in cell bodies accounts for long-term memory (Arshavsky, Prog Neurobiol. 2006; 80(3):99-113). *Hydra*, with its regenerative capabilities, in conjunction with molecular-biological and other techniques, might be suitable to resolve this controversy. As a first step the existence of SP in *Hydra* needs to be evaluated. To this end, light-responsive neurons, located in the tentacles will be illuminated and postsynaptic cells could be identified by using calcium imaging. Then, channelrhodopsin2 (ChR2), a light gated ion channel, will be expressed in postsynaptic cells. To induce SP, the presynaptic cells, i.e. the light sensitive Neurons will be stimulated by light. In another set of experiments the postsynaptic cells will be stimulated by activating ChR2. The most interesting changes are expected after simultaneous stimulation of both sides of the synapse, a phenomenon called Hebbian plasticity (Ljaschenko et. al., Cell Rep. 2013; 3(5):1407-13). The functional changes of the synapses upon stimulation will be assessed by means of calcium imaging, voltage imaging, electrophysiology; structural changes by immuno-histochemical fluorescence microscopy. To test whether plasticity is stored as a stable biochemical substrate in somata of cells which showed functional and/or structural changes upon stimulation, body parts including those somata, but not the synapses, will be grafted onto non-stimulated individuals. If plastic changes are stored as a stable biochemical substrate in cell bodies, the functional plasticity should be present in the recipient organism.

Transcriptome assembly and comparative functional gene enrichment analyses of the reverse development and transdifferentiation in *Turritopsis dohrnii*

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The medusae (jellyfish) of *Turritopsis dohrnii* (Cnidaria, Hydrozoa) undergoes reverse development to avoid death caused by physical damage, adverse environmental conditions, or aging. Weakened or damaged jellyfish will undergo a whole-body transformation into a cluster of uncharacterized tissue, referred to as the cyst stage, which then will metamorphose back into an earlier lifecycle stage, the juvenile polyp. This unique ability has granted the species the name, the "Immortal Jellyfish". The underlying cellular mechanism that permits its reverse development is called transdifferentiation or cell reprogramming. Cell transdifferentiation allows fully mature and differentiated cells to reprogram themselves into a new cell type of any lineage. Thus, transdifferentiation is highly regarded in the biomedical sector as a potential mechanism to transform mature cells into any needed cell type after tissue damage. The polyp, jellyfish and cyst stage of *T. dohrnii* were sequenced through RNA-sequencing and the transcriptomes were assembled *de novo.* The transcriptomes were then annotated to create the gene expression profile of each stage. Comparative functional gene enrichment analyses with the cyst as the central stage of comparison reported significant Gene Ontology categories that were over-expressed, such as telomere maintenance and DNA repair, in the cyst as compared to other stages. The enrichment analyses also reported significantly under-expressed categories, such as mitotic cell division, cellular differentiation and development, in the cyst as compared to the other stages. Ultimately, our work produced a foundation to develop an alternative model system to further investigate and understand regeneration, cellular plasticity and aging in metazoans.

The Effect of Calcium Sensing Receptor Agonists and Antagonists on Hydra Feeding Behaviors and Cnidocyte Discharge

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Calcium sensing receptors (CaSRs) belong to the class C G-protein coupled receptor family, which includes glutamate, olfactory, vomeronasal and taste receptors. In vertebrates CaSRs respond to extracellular Ca^{2+} to mediate processes such as Ca^{2+} homeostasis, chemotaxis and nutrient sensing. Their response to Ca^{2+} is enhanced by amino acids and glutathione (GSH), and thus they appear to be good candidates for a chemoreceptor affecting hydra cnidocyte discharge and the feeding response. Blastp searches of the hydra genome identified at least 20 genes with homology to vertebrate CaSRs. Expression analysis indicates that one of these genes is expressed in hydra tentacles. Chemical modulators of the CaSR were tested to determine if they would affect hydra feeding behavior and cnidocyte discharge. The calcimimetic R568 activates the feeding response and also increases mechanically-stimulated cnidocyte discharge. The calcilytic NPS2143 inhibits GSH-stimulated cnidocyte discharge, but not cnidocyte discharge activated by mechanical stimulation alone. TNCA is tryptophan derivative that acts as a high-affinity CaSR co-agonist. Unlike R568 and NPS2143, which bind in the 7-transmembrane region, TNCA binds to the CaSR extracellular domain. In hydra, TNCA does not increase cnidocyte discharge or activate the feeding response, but it does decrease GSH-stimulated cnidocyte discharge provided it is introduced to hydra prior to the application of GSH. Gd^{3+} (an orthosteric CaSR agonist) increases GSH-stimulated mouth opening at low concentrations, but inhibits both mouth opening and cnidocyte discharge at higher concentrations, perhaps acting as a TRP channel blocker. It is interesting to note that CaSR receptors are known to interact with TRP channels, and that PKD2 TRP channel genes are expressed both in hydra tentacles and at the tip of the hypostome.

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Environmental control of life cycle in jellyfish *Cladonema pacifica*

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Hydrozoan jellyfish have various life stages including vegetatively propagating polyps, which are connected with stolons, and sexually reproducing free-swimming medusae. In the normal life cycle, medusae are produced by polyps, and this transition appears to depend on environment factors such as nutrition and temperature. However, the mechanism underlying the environmental control of life cycle is poorly understood. Here we use *Cladonema pacifica,* a hydrozoan jellyfish living in Japanese coastal waters, as a model to understand temperature-dependent control of life cycle. *Cladonema* polyps give rise to medusa in spring while staying as stolons during the cold season, suggesting that *Cladonema* takes distinct morphologies in different seasons. This seasonal change of life cycle can be recapitulated in the laboratory condition. We are currently addressing a question how *Cladonema* polyps alter morphologies by responding to temperature shifts, and trying to investigate molecular and cellular mechanisms that controls life-cycle transitions.

Box, stalked and upside-down? Draft genomes from diverse jellyfish (Cnidaria, Acraspeda) lineages: Alatina alata (Cubozoa), Calvadosia cruxmelitensis (Staurozoa), and Cassiopea xamachana (Scyphozoa)

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Anthozoa, Endocnidozoa, and Medusozoa comprise the three major clades of Cnidaria. Medusozoa is further divided into four clades, Hydrozoa, Staurozoa, Cubozoa, and Scyphozoa—the latter three lineages make up the clade Acraspeda. Acraspeda includes some of the most venomous organisms on the planet, numerous nuisance species, some of the most highly developed eyes in the animal kingdom, and it encompasses extraordinary diversity in terms of life history. Currently, no genomes are publicly available for any of these animals. Here we present three new draft genomes of Calvadosia cruxmelitensis (Staurozoa), Alatina alata (Cubozoa), and Cassiopea xamachana (Scyphozoa) for which we provide preliminary orthology analyses that includes an inventory of their known venom-related genes. To further demonstrate the utility of these datasets, we identify synteny between Pou and Hox genes that had previously been reported in a hydrozoan, suggesting that this linkage is highly conserved, dates back to at least the last common ancestor of Medusozoa, and is likely independent from the Hox-Pou linkages seen in vertebrates. These draft genomes provide a valuable resource for studying the evolutionary history and biology of these extraordinary animals, and for identifying genomic features underlying venom, vision, and life history traits.

Focusing in on Cnidarian Opsin Phylogeny

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All modes of animal vision depend on opsin proteins of the G protein coupled receptor (GPCR) class. Opsins are present across animals and cnidarian opsins were first described more than a decade ago. After much progress, fundamental questions stemming from the paucity of opsin data representing the major lineages of Cnidaria persist. Recent phylogenomic analyses have clarified cnidarian relationships and provide a comprehensive set of genome-scale datasets that could ameliorate these issues. Here we describe a new bioinformatic approach called Phylogenetic Focusing that progressively circumscribes complete orthologous clades of interest within their larger gene families. We applied phylogenetic focusing to a selection of 60 cnidarian and 25 outgroup genome-scale datasets and find that the GPCR neighborhood within which opsins reside is populated by several, previously undescribed clades of non-bilaterian GPCRs including major radiations in sponges, ctenophores and cnidarians. This finding challenges the view that melatonin receptors are the close evolutionary sister to opsins and highlights a hidden diversity of GPCRs in the close vicinity of opsins. In addition, cnidarians are inferred to have inherited the full complement of opsin types but have lost several of them in a lineage specific manner, leaving anthozoans as the cnidarian clade that best represents the ancestral cnidarian opsin palate. Finally, the rate of opsin gene duplication and loss is significantly higher for many cnidarian taxa as compared to other animals, indicating a tumultuous evolutionary history for cnidarian opsins. Our analysis also clarifies several features of the global metazoan opsin phylogeny.

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Investigating Notch-signalling in *Hydr*a by differential gene expression profiling combined with analysing single cell sequencing data

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Here we have investigated the function of Notch-signalling in *Hydra* by using the presenilin inhibitor DAPT, which blocks propagation of Notch-signals very efficiently. DAPT treatment prevents differentiation of proliferating nematocyte progenitor cells into mature nematocytes (Käsbauer et al., 2007). Moreover, it impedes the structure of the *Hydra* head, probably due to the impairment of the *Hydra* head organiser (Münder et al., 2013). In order to understand the role of the Notch pathway for these processes we have analysed differential gene expression in DAPT treated animals. This revealed downregulation of 806 genes and upregulation of 333 genes. By inspecting single cell sequencing data we found that almost half of the downregulated genes were expressed in nematocytes and nematocyte progenitors. This result is in perfect accordance with our experimental findings about the effect of DAPT on nematocye differentiation. Moreover, *gooscoid*, a gene associated with the Spemann organiser, was also downregulated by DAPT. Single cell sequencing data revealed 78 of the downregulated genes to be specifically expressed in the head or tentacles of *Hydra*. Their roles for the *Hydra* head organiser will be analysed in the future. In conclusion, this work shows the power of the *Hydra* cell type transcriptome atlas for interpreting data from functional differential gene expression experiments.

Käsbauer, T., Towb, P., Alexandrova, O., David, C.N., Dall'armi, E., Staudigl, A., Stiening, B., Böttger, A., 2007, Dev Biol 303, 376-390.
Münder, S., Tischer, S., Grundhuber, M., Buchels, N., Bruckmeier, N., Eckert, S., Seefeldt, C.A., Prexl, A., Käsbauer, T., Böttger, A., 2013, Dev Biol 383, 146-157.

Accumulation of N6-methyldeoxyadenosine precedes the zygotic genome activation during early embryogenesis in *Hydractinia*

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After the fusion of the two pro-nuclei, the newly formed zygotic genome is initially transcriptionally inactive. Hence, early development is driven by maternal mRNAs. The zygotic genome becomes transcriptionally activated several cell cycles later, depending on species, but the mechanism that initiates zygotic genome activation (ZGA) remains largely unknown in most animals. Here, we propose a role for N6-methyldeoxyadenosine in regulating ZGA during early development of the cnidarian *Hydractinia*.
Using ethyl uridine incorporation, we determined the stage of ZGA in *Hydractinia* to be at the 64-cell stage. We also find that *Hydractinia* accumulates N6-methyldeoxyadenosine (6m-dA), at up to 5-folds above background level, at the 16-cell stage, two cell cycles before the major wave of ZGA. This peak of DNA methylation is removed two cell cycles later, at the 64-cell stage, coinciding with ZGA. We hypothesize that 6m-dA acts to transiently halt zygotic transcription, while keeping relevant genes poised. Once removed after the 32-cell stage, transcription is free to commence in poised genes. This constitutes a simple way for temporal control of ZGA using a single step of DNA demetylation. We are studying the mechanisms of 6m-dA mediated transcription block. Our proposal is consistent with previous studies that provided evidence for a pausing effect of 6m-dA on RNApolymerase II (Wang *et al*).

Wang, Wei, *et al.* (2017) *J. Am. Chem. Soc.*, *139* (41): 14436

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Electroporation of small hairpin RNAs for rapid and efficient gene knock-down in the sea anemone, *Nematostella vectensis*.

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The *N. vectensis* research community is continuing to develop a wide repertoire of techniques and tools for experimental manipulation of gene function. Recently, gene-specific knockdown through microinjection of small hairpin RNA's was developed in our lab. Here, we present a protocol for shRNA-mediated gene knockdown by electroporation of eggs and embryos. The protocol enhances the power of shRNA-based knockdown with the speed and simplicity of electroporation, enabling processing of large numbers of eggs or embryos within minutes. In addition, we provide a detailed description of the electroporation procedure, including preparation of reagents, electroporation conditions, preparation of *N. vectensis* eggs for electroporation and follow up care of the electroporated embryos. Finally, we demonstrate the knockdown of several endogenous and exogenous genes with known phenotypes, and discuss the potential applications of this method.

Finding their way: Primordial germ cell specification and migration in *Nematostella vectensis*

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Multicellular organisms primarily rely on germ cells to transmit genomic information between generations. Understanding the mechanisms by which diverse species generate gametes is thus one of key questions in EvoDevo. While bilaterian animals harbor distinctive gonad tissues, many pre-bilaterian species have scattered multipotent stem cells that give rise to both somatic cells and germ cells (e.g. i-cells in Hydra). In contrast, the sea anemone *Nematostella vectensis*, like most bilaterians, harbors distinctive gonad tissue. In this sense, *Nematostella* may represent a transition point between the ancestral reproductive modality of multipotent germline stem cells and the more derived unipotent germline stem cells found in Bilateria. To study the developmental origins of *Nematostella* germ cells, we first confirmed the primordial germ cells (PGCs) are specified as two pharyngeal cell clusters during early juvenile stage. Next, we used shRNA to knockdown key germline factors, such as Vasa and Piwi, and demonstrate the necessity of these genes for PGC formation. Since PGC clusters initially form between the expression domains of the Hh ligand and its receptor, Ptc, we hypothesized that the Hh pathway is involved in PGC development. Surprisingly, by knocking down Hh1 and Gli3, we found the Hh pathway is required for PGC formation. Lastly, to determine how the two PGC clusters give rise to the eight gonads in adults, we traced PGC in juveniles. We found the initial clusters are epithelial cells which then delaminate and move into the underlying mesoglea to disperse along the gonad primordia. This observation suggests *Nematostella* PGCs mature by epithelial-to-mesenchymal transition and then become migratory. To more clearly resolve PGC specification mechanisms and the migration of PGCs, we are generating CRISPR KO and fluorescent transgenic lines. With these new tools, we expect our data will shed new light on the evolution of germline stem cells.

Cnidarian sFRPs in primary body axis patterning - an update

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sFRPs are secreted proteins acting as modulators of Wnt signaling by binding extracellular Wnt ligands and thereby prohibiting their binding to Frizzled receptors. Canonical Wnt signaling is regarded as a key factor in patterning the eumetazoan primary body axis. It is activated in the cnidarian blastoporal organizers and defines the oral end of cnidarian polyps such as the *Hydra* head organizer. Here, we demonstrate that sFRP1/2/5 is involved to specify the aboral end of the primary cnidarian body axis. Previously, we have shown that in *Nematostella* embryos *sFRP1/2/5* is transcriptionally activated complementary to *wnt* gene activation in the aboral domain starting at the late blastula stage. Morpholino-mediated knock-down of sRFP1/2/5 in *Nematostella* embryos terminates aboral development at the gastrula stage, but also affects positional values along the entire oral-aboral body axis. Throughout *Hydra* bud formation, sFRP1/2/5 expression defines the future aboral end of the newly forming oral-aboral body axis. In stage 1 buds, it starts with a ring-shaped expression domain around the newly forming head organizer, which is defined by *wnt3* activation. Thus, we propose that in a 2D circular bud anlage, oral and aboral positional values are defined early, and that the 3D bud protrusion emerges from this field by using morphogenetic processes including cell polarity changes, cell movement, and directed cell proliferation. Furthermore, *sFRP1/2/5* is activated in the tip of foot regenerating *Hydra* as an early response gene. Attempts to block the sFRP1/2/5 protein by using the small molecule inhibitor WAY-316606 resulted in the differentiation of reduced numbers of basal disc-specific epithelial cells and loss of ability to adhere to the substrate. Notably, in the treated regenerates we found tentacle-specific battery cells scattered throughout the newly formed foot, indicating that sFRP1/2/5 is not required for terminal differentiation per se but may specify a fate decision between head and foot in *Hydra*.

Evolution of Axin function in the Wnt/β-catenin pathway in metazoans: Insights from sea anemones and sea urchins

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Axin is a critical scaffold protein in a destruction complex that negatively regulates β-catenin stability in the Wnt/β-catenin (cWnt) pathway. The role of Axin in DV axis formation in vertebrates is fairly well understood. But, the role of Axin in primary axis patterning is still unclear in bilaterians and non-bilaterians. In bilaterians, Axin interacts with β-catenin through a conserved binding domain (β-catBD) and targets it for degradation. Intriguingly, non-bilaterian Axins lack the β-catBD, questioning a conserved role for this critical protein in regulating β-catenin stability across taxa. To address this question, I tested if Axin is functionally conserved between bilaterians and non-bilaterians using sea urchins and *Nematostella*. Overexpression of SUAxin in sea urchin embryos led to downregulation of cWnt and an anteriorized phenotype, but overexpression of *Nv*Axin in sea urchin embryos had no effect. Deletion of the β-catBD of sea urchin Axin to mimic *Nv*Axin still anteriorized embryos indicating that the lack of effect by NvAxin is not simply due to a missing β-catBD. To determine if Axin is required for normal endomesoderm formation Axin was knocked down using anti-Axin morpholinos. This led to strongly posteriorized sea urchin embryos and using animal half explants I showed that this phenotype was due to de-repression of cWnt signaling in animal half blastomeres. This indicated that a global potential to form endomesoderm is suppressed throughout the early embryo by Axin. I am now testing the role of Axin in *Nematostella* to determine if it plays a role in endomesoderm specification. This will allow me to determine if the biological role of Axin in endomesoderm specification is conserved between sea urchins and *Nematostella* even in the absence of critical protein-protein interaction domains on NvAxin. Elucidating how Axin regulates cWnt signaling in bilaterians and non-bilaterians will provide critical insight into the evolution of cWnt signaling.

Chromatin Remodeling During Hydra Regeneration

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Regeneration is a highly complex and coordinated response to injury that depends on extensive changes in gene expression. Understanding the nature of the transcriptional regulatory network underlying regeneration—in particular identifying key transcription factors and their targets—would provide insight into how a regenerative wound response is regulated and may highlight ways in which regeneration differs from non-regenerative wound healing. To better understand transcriptional regulation during regeneration, we performed an Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) on *Hydra* polyps during both head and foot regeneration three hours after midgastric bisection. By comparing these samples to uninjured tissue, we were able to identify thousands of loci genome-wide with significant changes in chromatin accessibility following injury. Injury-responsive peaks appeared largely identical in both head and foot regeneration, suggesting that the early injury response may be generic at the chromatin level. In addition, the vast majority of injury-specific peaks were located near transcription termination sites—indicative of increased transcription—with relatively few injury-specific peaks located near putative promoter regions. Thus, much of the initial wound response may result from increased transcription of poised genes with constitutively accessible promoter regions. Additionally, the promoters of injury-responsive genes showed a significant enrichment for the cAMP response element (CRE), which is bound by the basic leucine zipper (bZIP) family of transcription factors. Numerous bZIP genes are upregulated during early regeneration, suggesting these transcription factors may be key regulators of an initial generic injury response in *Hydra*. Future studies will seek to functionally test the importance of bZIP transcription factors during regeneration. Additionally, we will perform ATAC-seq on later regeneration timepoints to identify the regulatory regions associated with the specialization of head and foot regeneration.

CRISPR knockouts reveal an endogenous role for ancient neuropeptides in regulating developmental timing in a sea anemone.

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Neuropeptides are evolutionarily ancient peptide hormones of the nervous and neuroendocrine systems, and are thought to have regulated metamorphosis in early animal ancestors. In particular, the deeply conserved Wamide family of neuropeptides—shared across Bilateria (e.g. insects and worms) and its sister group Cnidaria (e.g. jellyfishes and corals)—has been implicated in mediating life-cycle transitions, yet their endogenous roles remain poorly understood. By using CRISPR-Cas9-mediated reverse genetics, we show that cnidarian Wamide—referred to as GLWamide—regulates the timing of life cycle transition in the sea anemone cnidarian *Nematostella vectensis*. We find that mutant planula larvae lacking GLWamides transform into morphologically normal polyps at a rate slower than that of the wildtype control larvae. Treatment of GLWamide null mutant larvae with synthetic GLWamide peptides is sufficient to restore a normal rate of metamorphosis. These results demonstrate that GLWamide plays a dispensable, modulatory role in accelerating metamorphosis in a sea anemone.

Spatial and temporal reconstruction of embryogenesis using single-cell RNAseq

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Cell type specification during embryogenesis is a complex process often initiated by developmental signals and executed by a cascade of transcriptional events that unfold across time and space. Single-cell RNAseq (scRNA-seq) provides a unique opportunity to query these transcriptional events, and generating scRNA-seq data is increasingly accessible as methods mature, cost decreases, and commercialized systems reduce the learning curve significantly. However, analysis and interpretation of the data remains challenging and under constant innovation. I will present two approaches for analyzing scRNA-seq data and their application to investigating cell type specification during early zebrafish development.
First, Seurat is an approach that infers the spatial origin of single-cell transcriptomes based on a small set of landmark gene expression patterns. We applied Seurat to spatially map 851 transcriptomes from zebrafish embryos just prior to gastrulation, inferring a transcriptome-wide atlas of gene expression patterns. Additionally, clustering uncovered a previously uncharacterized cell state that we believe represents a blastula-specific transcriptional response to DNA damage.
Second, URD is an approach that infers developmental trajectories in the form of a branching tree from scRNA-seq data. We sequenced 38,731 cells from zebrafish embryos at 12 developmental stages and identified the trajectories of 25 cell types from the onset of zygotic transcription through early somitogenesis. Gene expression analyses associated developmental trajectories with known and candidate regulators, classic and novel marker genes, and combining URD with Seurat identified the trajectories' spatial origin in the blastula. Additionally, we found that some developmental branchpoints contained intermediate cells expressing genes characteristic of multiple cell fates, which seem to switch specification from one fate to another.

Stem cell differentiation trajectories in *Hydra* resolved at single cell resolution

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Abstract

Differentiation from stem cells is restricted to certain developmental stages or confined to particular adult tissues in the majority of animals. In contrast, the adult freshwater polyp *Hydra* continually renews all of its cells from either stem cells or from somatic cells that acquire new function and therefore presents a valuable system to understand cellular decision making and differentiation pathways. In the homeostatic *Hydra*, continuous cell turnover and replenishment is supported by three lineage-restricted stem cell populations. The multipotent interstitial stem cells continuously give rise to neurons, gland cells, nematocytes and, under certain conditions, to the germline. The two epithelial lineages, ectoderm and endoderm, are supported by mitotically active epithelial stem cells in the body column that are continually displaced toward the oral and aboral ends where they give rise to the head and foot epithelia. Here we elucidate and construct the complete stem cell differentiation trajectories for all three lineages using a droplet based single cell sequencing approach (Drop-seq) on whole animals. We identify molecular signatures of stem cells, terminally differentiated cells, cells in the process of differentiation from stem cells, and cells undergoing changes in response to positional cues. These data allow us to identify key transcription factors with likely function in specific differentiation trajectories. The data sheds new light on the molecular diversity of certain populations of cells, such as neurons, and suggest that neurons and gland cells share a common progenitor in *Hydra*. All together, these data offer an entirely new window into understanding the molecular mechanisms underlying stem cell biology and regeneration in an animal that has a long history in developmental biology.

Targeted cell labelling and single-cell transcriptomics to explore neural developmental trajectories in the Anthozoan *Nematostella vectensis*

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Neurons are a metazoan innovation and represent a diverse class of cells across eumetazoan phyla. Neurogenesis has been well studied in bilateralian model organisms, underlining the conservation of neurogenic transcription factor families, but the patterning of Cnidarian nervous systems is not well characterized. Our lab has recently generated a single-cell atlas of the adult and larval stages of the Anthozoan *Nematostella vectensis*[1], which has highlighted a broad diversity of cell types, especially neurons. This static map of the adult nervous system has uncovered the molecular fingerprint of specific subsets of the nervous system, and their combinatorial transcription factor expression. An ongoing challenge is the reconstruction of developmental trajectories leading to these differentiated cell states. Thus, we present here a work-in-progress to explore specific transcriptional trajectories underlying neurogenic processes in *Nematostella vectensis*. Our approach combines targeted genetic labeling with single-cell transcriptomics to investigate the molecular outcome of tagged cells. As a proof-of-concept, we use microinjected embryos with a fluorescent reporter for the FoxL2 transcription factor, -whose expression can be seen in neurons and cnidocytes in the adult-, to assess the transcriptional state of cells that expressed this reporter at the gastrula stage. This targeted approach can enable the specific reconstruction of key neurogenic trajectories in *Nematostella vectensis*. Comparisons with bilateralian neurogenic mechanisms would help us better understand the evolution and development of nervous systems.

[1] Sebé-Pedrós A, *et al*. Cnidarian Cell Type Diversity and Regulation Revealed by Whole-Organism Single-Cell RNA-Seq. Cell. 2018 May 31;173(6):1520–1534.e20.

Life Cycle Evolution within Scyphozoa

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Complex life cycles are a textbook feature of Medusozoa, yet many lineages have lost or modified one or more life cycle stage. Scyphozoa is composed of roughly 200 species, yet few studies have examined life cycle evolution within this monophyletic lineage. In this work I examine scyphozoan life cycle variations in an evolutionary context. Within scyphozoan, monodisc strobilation has evolved at least twice independently, and is correlated with a small calyx size. The evolutionary loss of intermediate life cycle stages has occurred at least four times, while the reduction/loss of medusae may have occurred only once. In addition, there is a high degree of developmental and life cycle plasticity within species. This intra-specific plasticity may facilitate life cycle evolution, and I propose possible developmental mechanisms for the evolution of modified life cycles in Scyphozoa.

Microbial community dynamics of a keystone urchin species in the Arabian Peninsula

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Microbial communities of marine organisms have been shown to play a crucial role in the development, physiology, and thermal tolerance of their hosts. The rock-burrowing urchin, *Echinometra mathaei,* is found across the Indo-Pacific and plays a significant role in the health and dynamics of reef ecosystems as a major bioeroder. The range of *E. mathaei* extends into the thermally extreme Persian/Arabian Gulf (PAG) where present-day summer maxima (~35-37°C) exceed climate change predictions for the Indo-Pacific reefs in the next century. To date, there have been very few studies on microbial composition of marine organisms in this region and no studies that focus on the microbiome of *E. mathaei*, despite the potential role that it may play in this species ability to survive extreme conditions. To characterize the microbiome in the PAG, we collected fifteen *E. mathaei* from one site within the PAG and contrasted their microbial assemblage with fifteen individuals from one site in the neighboring Gulf of Oman (summer maxima of ~30-32°C). Further, we compared three common DNA extraction procedures and inferred bacterial diversity from each method through 16S ribosomal RNA (rRNA) gene amplicon sequencing. Our results indicate that 63.7% of the variation in the samples was explained by the location of where the samples were collected and the addition of a bead-beating and lysozyme step more effectively capture difficult to lyse taxa, such as gram-positive bacteria. Finally, DNA extraction method plays an important role in estimates of Shannon diversity, where diversity indices were significantly higher in both sites when a lysozyme and bead beating step was used.

Effects of chronic and acute salinity stress on the behavior of a burrowing sea anemone, *Nematostella vectensis*

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Marine environments, specifically within shallow estuaries and tidal pools, experience tremendous shifts in abiotic factors seasonally and daily (i.e., salinity, oxygen, UV). The effects of this wide range of natural variability exerts physiological stress for animals in these habitats. *Nematostella vectensis*, a burrowing sea anemone found in brackish coastal pools, has been observed to modify their burrowing habits in response to oxygen and temperature stress; however, their behavioral response to salinity stress is unclear. In order to investigate how *Nematostella* responds to variable salinities, we measured burrowing behavior of animals exposed to acute and chronic salinity. We observed significant differences in burrowing time and overall movement for sea anemones in high and low salinity. Of particular interest, *Nematostella* chronically exposed to high salinity burrowed similarly to control conditions, suggesting acclimation, but when chronically exposed to low salinity they did not burrow at all. Our data suggest an asymmetric response to salinity where acclimation occurs only under high salinity conditions in *Nemastostella*. Susceptibility to low salinity may result in a predisposition for higher extinction rates in populations that are closer to freshwater inputs.

Toxin assemblage and prey community in the model sea anemone *Nematostella vectensis* throughout their natural range

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The resulting diversity of toxic proteins found in most venomous animals are thought to be an end product of strong environmental selective pressures influenced by interactions with predators or prey, which has been deduced from venomous animals that are prey specific consumers or predator specific defenders. The sessile starlet sea anemone (*Nematostella vectensis*), however, encounters a variety of potential prey and predators throughout their natural distribution in the shallow estuarine ponds in which they are found. In order to better understand co-evolutionary mechanisms in venomous animals that are less specific with their potential targets we use a next-generation sequencing approach to characterize the toxin diversity in *N. vectensis* throughout their distribution, in addition to COI DNA barcoding to identify potential prey items consumed by *N. vectensis* polyps. We find that despite their population spanning over 2000 kilometers, sequence variation among key toxin proteins found in adult *N. vectensis* remain relatively conserved throughout their distribution. We also find that prey diversity varies tremendously across their distribution; including lineages that were previously not thought to be prey for *N. vectensis*. Although the toxin workhorses (Nv1, a sodium channel toxin) did not seem to vary tremendously, we did find subtle variation across what were presumed to be ancillary proteins (NvPTx1 and sodium channel toxins), which may carry more functional importance during development. Overall, our results provide significant insight into toxin diversity found in *N. vectensis* and highlights the importance of toxic workhorses in non-specific sessile venomous animals, a sharp contrast to the evolutionary arms race often referred to when studying toxin diversity.

Toxic effects of streptomycin on hair cells of the sea anemone, *Nematostella vectensis*

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Aminoglycoside antibiotics, such as streptomycin, produce ototoxic effects in vertebrate animals, but it is unknown if long-term exposure to aminoglycosides causes damage to the hair cells of invertebrate animals. This study investigated the effects of streptomycin on the epithelium of tentacles of the sea anemone, *Nematostella vectensis*. Streptomycin significantly reduced the number of hair bundles at the bases of tentacles of sea anemones. Streptomycin also significantly reduced the number of MitoTracker-labeled mitochondria at both the tips and bases of the tentacles. Labeling of nuclei with EdU, a thymidine analog, significantly increased at the bases of tentacles of sea anemones within the first hour after exposure to streptomycin. Furthermore, labeling of nuclei with Hoechst significantly increased in the tentacle epithelium within 4 hours after exposure to streptomycin, indicating an increase in cell number. These results indicate that long-term exposure to streptomycin is harmful to and induces cell proliferation within the epithelium of tentacles of sea anemones. It is likely that the induction of cell proliferation is at least partially intended to replace hair cells lost (reduction in both hair bundles and active mitochondria) due to exposure to streptomycin.

Development of genome editing techniques in the sea anemone *Exaiptasia pallida*, a model for cnidarian-dinoflagellate symbiosis

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Onset and establishment of cnidarian-dinoflagellate symbiosis is mediated by the host innate immune system through interpartner signaling between host receptors and symbiont surface proteins. For example, it is known that symbiont glycan and host lectin binding is crucial for symbiont specificity in Aiptasia (*Exaiptasia pallida*), a sea anemone that is widely used as a model for cnidarian-dinoflagellate symbiosis. Although transcriptomic data have identified many candidate genes and pathways that are correlated with symbiosis, the causal genetic mechanisms underlying onset and establishment of symbiosis are still largely unknown. This lack of knowledge is mostly due to the paucity of tools and techniques for assessing gene function in the context of symbiosis. Here we discuss our initial efforts in developing molecular genetic tools including CRISPR/Cas9 mediated gene knockout and gene tagging in Aiptasia to investigate the mechanisms underlying the onset and establishment of symbiosis.

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Nervous system plasticity and regeneration in *Hydra*

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In the small freshwater cnidarian *Hydra,* all differentiated cells in the homeostatic adult animal are replaced every 12-20 days, including the entire nervous system. *Hydra* is also able to regenerate its nervous system following catastrophic injury. Our ultimate goal is to understand the general principles of *Hydra* neural plasticity in both homeostatic and regenerative conditions. As a first step towards this goal, we are using single cell RNA-sequencing (scRNA-seq) to build a complete molecular map of the *Hydra* nervous system. We have thus far identified eight neuron subtypes with unique molecular signatures and are mapping the location of these subtypes in the *Hydra* nervous system using in situ hybridization. Based on our preliminary scRNA-seq data and published literature, we hypothesize that continual renewal of the *Hydra* nervous system under homeostatic conditions is accomplished by a combination of two mechanisms: 1) specification of new neurons from stem cells (neurogenesis) and 2) transdifferentiation between neuron subtypes. In our future work, we aim to use our scRNA-seq data to identify and test transcription factors unique to neurogenesis and transdifferentiation, thus gaining insight into the regulatory control of nervous system plasticity. Additionally, we plan to build transgenic reporter lines to quantify the number of neurogenesis and transdifferentiation events that occur during both homeostatic maintenance and regeneration of the nervous system. Through these exploratory studies, we hope to elucidate the molecular mechanisms that underlie neuronal plasticity and regeneration.

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A detailed head regeneration timeline in the cnidarian *Hydractinia symbiolongicarpus*

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Regeneration of body parts, a complex biological process almost entirely lost in live mammals, is widespread throughout the animal kingdom. In our lab, we are studying the colonial cnidarian *Hydractinia symbiolongicarpus* to better understand the process of tissue regeneration in this emerging regenerative model. *Hydractinia* can fully regenerate its head upon dissection in about 72 hours (Bradshaw et al., 2015). The process of head regeneration depends on a pool of migratory stem cells, known as interstitial cells or 'i-cells' based on their location in between epithelial cell spaces (Gahan et al., 2016; Plickert et al., 2012). Here, by using a series of imaging, immunohistochemistry, and cell proliferation assays, we provide a better understanding of the regeneration process in this species. Most polyps completely regenerated their head within ten days, with first tentacle buds occurring between 48 and 72 hours post dissection. Immunofluorescence staining showed wound closure within four hours, while nervous system regeneration and the appearance of stinging cells around the newly formed mouth and in the budding tentacles occurred between 48 and 72 hours after dissection. EdU staining and pulse-chase experiments revealed the essential contributions made by the pool of proliferating i-cells to all regenerated head structures. Our results confirm initial observations made in a sister species of *Hydractinia* and provide a more detailed understanding of the head regeneration process in *Hydractinia* that will be used as a basis for future studies of this highly regenerative species.

Bradshaw, B., Thompson, K., Frank, U., 2015. Distinct mechanisms underlie oral vs aboral regeneration in the cnidarian hydractinia echinata. Elife 2015, 1–19.

Gahan, J.M., Bradshaw, B., Flici, H., Frank, U., 2016. The interstitial stem cells in Hydractinia and their role in regeneration. Curr. Opin. Genet. Dev. 40, 65–73.

Plickert, G., Frank, U., Müller, W.A., 2012. Hydractinia, a pioneering model for stem cell biology and reprogramming somatic cells to pluripotency. Int. J. Dev. Biol. 56, 519–534.

Early Transcriptional Response of *Pocillopora damicornis* in Reaction to Physical Wounds

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Healthy coral reefs are some of the most valuable ecosystems on earth. They provide mankind vast nutritional, economic, medical, and recreational value, and are also considered to be the most biodiverse ecosystems on the planet. However, despite this importance, corals are threatened by physical injury from environmental and anthropogenic sources on a daily basis (e.g. storms, anchor damage, careless recreational divers, coastal construction, and other human disturbances). Little is understood about the molecular mechanisms activated when a coral is wounded and more specifically when these mechanisms are initiated within the wounded tissues. In this study, high-throughput RNAseq was utilized to determine the differential gene expression profiles in fragments of *Pocillopora damicornis* every hour over a five-hour time period to identify the early transcriptional response of wound healing in corals. We predict that essential wound healing genes such as Grainyhead (GRH), which are responsible for the formation and repair of epidermal barriers in wounded tissue, will be differentially expressed early in wound healing. Deciphering the differential expression of early expressed wound healing genes will lead to a better understanding of the mechanisms activated in corals after physical injury.

Trans-epithelial regulation of cell-cell adhesion in Nematostella vectensis embryos

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The maintenance of an epithelial integrity in animal tissues is a critical process in development. In triploblastic animals, Par-proteins regulate cell-polarity and adherens junctions, maintaining the integrity of both ectodermal and endodermal epithelia. But, in embryos of the diploblastic cnidarian Nematostella vectensis, Par-proteins are degraded in all cells in the endomesodermal epithelium. Using immunohistochemistry, CRISPR/Cas9 mutagenesis, and mRNA overexpression, we describe the functional association between Par-proteins and differential cell-cell adhesion in *N. vectensis* embryos. We demonstrate that endomesodermal epithelial cells are organized by a different cell-adhesion system than overlying ectoderm. Furthermore, our results demonstrate that the proper cell-polarity and cell-cell adhesion of the ectodermal layer somehow regulate trans-epithelially the integrity of the endomesodermal layer because these Par genes are not expressed in the endomesoderm. This regulation may maintain the tension between cells during invagination at gastrula stages, or, in conjunction with the extracellular matrix (ECM) and basal cues, it may influence signaling patterns necessary to organize epithelial layers during N. vectensis embryogenesis. These data provide molecular insight into the evolution of epithelial structure and distinct cell behaviors, opening questions on the role of ECM and 'intermediate' tissues in metazoan embryos.

Search for Gravity Sensing Structure and Mechanism in Hydra

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It has been reported that Hydra shows tactic movements e.g. positive phototaxis (Singer et al., 1963) and negative geotaxis (Ewer, 1947). Unfortunately, in both phenomena, experimental data presented in the reports are statistically not satisfactory or convincing while the mechanism of sensing light and gravity remains unclear. Even in modern era, expression analysis of opsin genes has been unsuccessful whereas statocyst that senses gravity in hydrozoan jellyfish has not been found in Hydra polyps. We have been engaged in investigation of geotaxis and its underlying mechanisms. Here we show recent progress of the project by providing results that Hydra indeed shows negative geotaxis and that a possible gravity sensing structure is in the cnidocil of nematocytes termed Nematocilin (Hwang et al., 2008). In order to examine negative geotaxis, pattern of migration of Hydra polyps that are attached to a vertical sidewall of culture tank was analyzed in the dark condition and atmosphere free environment to avoid phototaxis and aerotaxis. We obtained a strong tendency of upward locomotion of polyps demonstrating that negative geotaxis is significant. As a search for the position of gravity sensing tissue, deletion of parts of the animal was performed and its effect was examined. The whole elimination of tentacles or the head also eliminated the tendency of upward locomotion suggesting the presence of “gravity sensor” in the tentacles. For further analysis, we employed “3View System” by GATAN, inc. This system enables “automate sectioning and image capture of 3D ultrastructure using serial block-face scanning electron microscopy”. Statocyst senses gravity by sensing the movement of solid spherical material termed statolith. The aim of the search by 3View System is to search for alternative solid structure in the tentacles. The search is still in progress and so far a candidate structure is Nematocilin, lamin-like protein of 47kD that have repeat of seven amino acids forming coiled-coil structure. Nematocilin is localized in the core of cnidocil of Nemtocytes largely distributed in the tentacles. Since the displacement of Nematocilin in rod shape is sensed by stereocilia that surround the Nematocilin, and since there are no other solid structures in the tentacles, we currently hypothesize that the Nematocilin and stereocilia that surround it are responsible for gravity sensing in Hydra.

Applying long-read sequencing to the genome of *Cerianthus borealis*

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Members of the anthozoan clade Ceriantharia are tube anemones with several distinguishing features, including a novel cnidocyte type (ptychocytes), a novel larval type (cerinula), a secreted composite tube into which they retract when disturbed, and two whorls of tentacles that surround the oral opening. Recent phylogenomic analyses demonstrate that Ceriantharia derive from the earliest split in Hexacorallia and, as the sister lineage to the remaining hexacorals, offer a critical window into the origins and evolution of this important clade. Whole genome assembly is an invaluable first step in answering questions about an organism's evolutionary history and genomic complexity in relation to other taxa. Short read sequencing technologies are inexpensive and provide a low error-rate, but reads consist of only a few hundred base pairs and are unable to resolve repetitive regions or complex genomic architecture. Oxford Nanopore sequencing technology leverages a fundamentally different approach to sequencing that promises to provide long sequencing reads that can help alleviate some of the problems encountered with short-read platforms. However, many questions related to best practices and library preparation protocols remain for this young technology. Here, we test the utility of Nanopore reads alongside shorter Illumina reads in the genome assembly of the Cerianthid *Cerianthus borealis*. By using both short reads for accuracy and longer reads for contiguity of the assembly, we create a mixed strategy assembly and evaluate it compared to assemblies from individual sequencing technologies.

Elucidating the role of Gap Junctions in Contractile Behavior of Hydra

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Within every nervous system there are neural circuits dedicated to the physiology and behavior of the organism. These circuits perform different functions that have been finely tuned through evolution. In humans, there are circuits that produce a reflex response, regulate body homeostasis and sense our environment. The complexity involved in the vertebrate nervous system makes it challenging to identify independent circuits and the function they perform. The freshwater cnidarian, *Hydra vulgaris*, is an ideal model to understand neural circuits and the phenotype they produce as they have a simple nerve net controlling their complex behavior. One of these behaviors exhibits itself as spontaneous contractile bursts (CB). The animal begins in an elongated state and contracts into a smaller "ball". Studies have identified a network of neurons located near the peduncle (foot region) that fire in conjunction with the contractile behavior[1]. Previous data also suggests that *innexin2* may coordinate this activity. In an effort to further identify the role gap junctions play in synchronizing this behavior we have used pharmacological inhibitors of gap junctions (Heptanol, Mefloquine, Carbenoxolone) while recording frequency and velocity of contractile events. While this data suggests that gap junctions are involved in these events it does not give us insight into the genes, innexins, coding for these gap junctions. To identify specifically which innexins are involved in coordinating this behavior we propose to knockdown and ectopically express innexin genes while observing contraction frequency, orientation and velocity. We also propose to perform the same experiments in the GCaMP line of hydra to understand how connectivity changes in the nervous system.

Dupre, C. & Yuste, R. Non-overlapping Neural Networks in Hydra vulgaris. *Curr Biol* 27, 1085–1097 (2017).

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PIWI-piRNA Pathway Targets Transposons in *Hydra* Somatic Stem Cells

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Transposable elements (TEs) are hypothesized to be a driver of aging in animals; increased transposition in aging somatic cells increases genome destabilization (Wood *et al*., 2016). The PIWI-piRNA pathway, a small RNA pathway that represses TE mobilization, is expressed in non-aging tissues including the germline, some cancers, and the somatic cells of long-lived animals (Kyriazis M., 2014). Our previous work demonstrates that the PIWI-piRNA pathway is present in all somatic stem cells of *Hydra*, which has an estimated lifespan of at least a few hundred years (Juliano et al., 2014; Schaible et al., 2015) Thus, we hypothesize that PIWI-mediated TE repression in the somatic stem cells of *Hydra* is necessary for maintaining genomic stability thereby promoting longevity. However, TE repression by the PIWI-piRNA pathway in somatic cells outside of the gonad has not been convincingly demonstrated. Our previous study found that somatic expression of the *Hydra* PIWI protein Hywi is necessary for animal survival (Juliano *et al*., 2014). Here we provide evidence that TEs are targets of the PIWI-piRNA pathway in the somatic stem cells of *Hydra*, supporting our hypothesis that TE repression mediated by the PIWI-piRNA pathway is required for somatic longevity in *Hydra*.

1.Wood, J. G. et al. Chromatin-modifying genetic interventions suppress age-associated transposable element activation and extend life span in Drosophila. Proc. Natl. Acad. Sci. U. S. A. 113, 11277–11282 (2016).
2.Kyriazis M Reversal of informational entropy and the acquisition of germ-like immortality by somatic cells. Curr. Aging Sci. 7, 9–16 (2014).
3.Juliano CE, Reich A, Liu N, Gotzfried J, Zhong M, Uman S, Reenan RA, Wessel GM, Steele RE, Lin H. 2014. PIWI proteins and PIWI-interacting RNAs function in Hydra somatic stem cells. Proc Natl Acad Sci U S A 111: 337-342.
4.Schaible, R. *et al.* Constant mortality and fertility over age in Hydra. Proc Natl Acad Sci U S A 112:15701–15706.

A medusa-specific homeobox gene in Cnidaria

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Medusozoans are characterized by the presence of a medusa stage as part of the life cycle, whereas all anthozoans lack this life cycle stage. Within the medusozoan class Hydrozoa, the medusa has been lost or reduced several times independently. Using publicly available genome and transcriptome data from species distributed across the phylum Cnidaria, we found that the presence of the homeobox gene *Tlx* is correlated with those species that have a medusa stage as part of the life cycle. Interestingly, the homeobox gene *Tlx* is highly conserved across metazoans but is lacking in the genomes of anthozoans and in those hydrozoans that have lost the medusa stage. Furthermore, available RNA-seq data for three medusozoan classes (Hydrozoa, Scyphozoa and Cubozoa) highlighted an upregulation of the expression of *Tlx* in medusa-related stages. Here we present the phylogenetic distribution of the *Tlx* gene throughout Cnidaria and the characterization of *Tlx* gene expression during medusa development in the hydrozoan *Podocoryna carnea.* Our findings support the unexpected scenario of an ancestral medusa for Cnidaria and the concomitant loss of the *Tlx* homeobox gene with medusa loss during cnidarian evolution. The characterization of *Tlx* and the functionally associated genes throughout cnidarians could offer a framework to investigate both the establishment of the developmental regulation context and potential syntenic relationships that could have led to the acquisition of the medusa stage.

Oscillation pattern shift during *Hydra* sphere regeneration is determined by mouth function

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Osmotically driven mechanical oscillations are not causally linked to symmetry breaking in regenerating Hydra spheres. Previous work on Hydra regeneration (1) assumed that cut tissue pieces do not retain polarity if they are sufficiently small, because they round up into a hollow sphere. Furthermore, it was claimed that regeneration of tissue piece spheres and of cell aggregate spheres follows the same process of osmotically driven oscillations and symmetry breaking. The latter was proposed to be correlated with a shift in oscillation cycles from large amplitude, low frequency to low amplitude, high frequency. More recent work, however, has shown that tissue pieces of any size are not equivalent to cell aggregates, as they retain polarity due to actin organization (2). As those authors did not examine oscillations, this begs the question of whether and how the oscillations and the cycle transition are related to symmetry breaking. By quantitatively analyzing oscillation dynamics and regeneration outcomes of tissue pieces, we show that the cycle transition occurs only in roughly half of all cases and is thus not required for regeneration. Oscillation amplitude and frequency in spheres that do not show a cycle transition are indistinguishable from pre-transition cycles of spheres that do. Our results suggest that tissue pieces may be able to follow two different trajectories to regeneration. Thus, we show that a cycle transition is not linked to biochemical symmetry breaking and propose a new explanation for the transition.

References

1. Soriano, Jordi, et al. "Mechanogenetic coupling of Hydra symmetry breaking and driven turing instability model." Biophysical journal 96.4 (2009): 1649-1660.
2. Livshits, Anton, et al. "Structural inheritance of the actin cytoskeletal organization determines the body axis in regenerating Hydra." Cell reports 18.6 (2017): 1410-1421.

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Neural and muscular dynamics of behaving *Hydra* under different environmental conditions

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To understand the neural code, i.e., the relation between the activity of a nervous system and the behavior it generates, we study *Hydra vulgaris*, which, as a cnidarian, represents some of the earliest nervous systems in evolution. Indeed, *Hydra* has a simple nervous system of 600-2,000 neurons, organized in three independent nerve nets in the ectoderm and endoderm, and which are distributed through the body of the animal but without any cephalization or ganglia. In recent work, we have genetically engineered the calcium sensor GCaMP6s in every neuron (Dupre and Yuste, 2017) or every muscle cell (Szymanski and Yuste, in prep.) of transgenic Hydra, and developed machine learning method to systematically analyze its behavior (Han et al, 2018). This makes it possible to reconstruct the entire neuronal and muscle activity of *Hydra* during behavior and analyze these databases to decipher the neural code.

As a step in this direction, we have explored how freely-behaving *Hydra* responds to environmental stimuli and conditions that affect its survival, while measuring its entire neural and muscle activity dynamics. Experimental conditions include high osmolarity (50mM sucrose), high temperature (30 degree Celsius), scarce of food (1 week starvation), and smaller body size. We acquired movies using calcium imaging and then processed and analyzed the data to extract neural and muscle activity during spontaneous contraction and elongation using custom ImageJ and MATLAB code.

To study changes in neuronal and muscle activity during different conditions, we focused on two previously identified non-overlapping circuits (Dupre and Yuste, 2017): contraction burst (CB) neurons that trigger body wall contractions and rhythmic potential 1 (RP1) neurons whose firing correlates with elongation. We found that activation of the muscle correlates to the activity of CB neurons during contraction and there was no difference in number of contractions or frequency of RP1 firing under different conditions. However, the frequency of CB firing decreased in high temperature or high osmolarity, (n = 3-7 replicates). These results indicate that *Hydra's* nerve net possess intrinsic control mechanisms to respond and adapt to the environment.

Dupre, C., and Yuste, R. (2017) Non-overlapping Neural Networks in Hydra vulgaris. Curr Biol 27, 1085-1097
Han, S., Taralova, E., Dupre, C., and Yuste, R. (2018) Comprehensive machine learning analysis of Hydra behavior reveals a stable basal behavioral repertoire. eLife 7, e32605

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Estimating heritability in thermal tolerance and identifying stress markers that correlate to survival at higher temperatures in *Acropora cervicornis*

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Climate change has resulted in warmer ocean temperatures that are having a negative impact on corals, which are highly susceptible to changes in temperature. Understanding the degree to which species vary in their thermal tolerance and whether this variation is heritable is important in determining their ability to adapt to climate change. To address this, *Acropora cervicornis* fragments from 20 genetically distinct colonies were kept at either ambient or elevated temperatures, and mortality was monitored for 26 days. Heritability of thermal tolerance was estimated using both a clonal method using a one-way ANOVA, as well as a marker based method using the program MARK (Ritland, 1996). To understand the physiological basis of thermal tolerance, tissue samples from both treatments were taken after 12 hours to investigate gene expression associated with sub-lethal temperature stress at both the mRNA and the protein level. It was found that this population has a relatively high amount of total genetic variation in thermal tolerance (H^2 = 0.528), but low additive genetic variation for this trait (h^2 = 0.032). In addition, both gene expression and protein expression among colonies were highly variable and did not show consistent patterns related to differences in thermal tolerance among colonies. While this population may have a limited capacity to respond to projected increases in ocean temperatures, the molecular basis of thermal tolerance in this species appears to be complex, and there may be many potential genotypic combinations that can result in heat-tolerant phenotypes.

Ritland, K. (1996). A marker-based method for inferences about quantitative inheritance in natural populations. Evolution 50(3): 1062-1073.

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Signatures of disease resistance for the threatened Caribbean branching coral, *Acropora palmata*

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Coral reefs are important ocean ecosystems that provide biodiversity and economic stability. Despite this value, they are under threat from anthropogenic stressors that have caused drastic decreases in global coral cover. In the Caribbean, White Band Disease has caused tremendous declines in the critical ecosystem building coral, *Acropora palmata*. With disease incidence and virulence rising, an in-depth knowledge of disease resistance dynamics is needed for all coral species. To maintain the critical ecosystem functions *Acropora palmata* provides, disease resistance dynamics for the already decimated wild populations are invaluable in ensuring its survival.

Previous observational work with *Acropora palmata* genotypes has shown large differences in disease tolerance, with percent disease transmission ranging from 0% to 100%. In this study RNA-seq was used to look at the differential gene expression of 12 *Acropora palmata* genotypes, with different disease tolerances, in 2016 and 2017. Fragments were sampled before disease exposure, and after a 7-day disease exposure. We hypothesise that differential gene expression will elucidate the expression of important immune genes and identify signatures of disease resistance. Preliminary results indicate significant gene expression differences among genotypes of varied disease susceptibility within each year. Large differences between genotype gene expression in year is also apparent, indicating that virulence of disease or environmental conditions are strong drivers of disease resistance. This work will contribute to scientifically driven restoration work by informing outplanting efforts of disease resistant individuals.

Aurelia (Scyphozoa) Gene GC content, DNA Methylation, and Conservation Across Metazoa

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The GC content of genes is emerging to be an important factor that influences the epigenetic regulations of transcription, through such mechanisms as DNA methylation. Methylated cytosines undergo deamination and are replaced by thymines over evolutionary time. In the moon jelly, Aurelia sp.1, genes can be grouped into two categories based on their relative density of cytosine-phospho-guanine (CpG) dinucleotides, which is a proxy for historic level of DNA methylation. Our result is consistent with other invertebrates, and contrasts with vertebrates and plants, where genes show a unimodal distribution in terms of CpG density. DNA methylation empirically determined using whole genome bisulfite sequencing (WGBS) is inversely correlated with CpG density. In addition, genes with low CpG density show greater conservation across Metazoa than genes in the high CpG density group.This work provides insights into the evolution of DNA methylation as an epigenetic modification and its correlation with cross-taxa gene conservation.

A centralized, versioned, community-driven gene annotation for the *Nematostella vectensis* 2.0 Genome

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Nematostella vectensis has been established for over a decade as a model outgroup organism to study the evolution of Bilateria. It continues to provide insight into origins of post-Cambrian body plans, cell type diversity and regulatory complexity. Yet to date, there has been a lack of community-wide consensus on a set of gene models which best characterize the *N. vectensis* proteome. As a part of the *N. vectensis* genome 2.0 project, we developed a pipeline to re-annotate the genome, leveraging multiple sources of evidence, including over 50 GB of paired-end RNA-Seq data from various studies and protein alignments from Bilataria, Cnidaria, Ctenophora, Porifera, Placozoa and Choanoflagellata. We summarized all predicted loci into 22,093 models of protein-coding genes. A manual inspection of over 300 genes shows an improvement of exon-intron and transcriptional boundary accuracy. In recognition of the fact that all automated pipelines and even manual analysis sometimes yield inaccurate models, we developed an accession and versioning system which will track all model updates via mandatory logging and provide forwarding for deprecated accession numbers. The models will be made available on a permanent resource in the form of a genome browser and a BLAST database at the Stowers institute, including correspondence of the current model set to past accessions. Update and revision of the models will be an ongoing effort, and all changes will be announced and visible. We hope this will encourage community-driven updates via the interactive Apollo gene editing plugin. We envision this as a central citable reference to advance the profile of *N. vectensis* as a model organism.

Targeted immune cell cascade gene expression analysis in a beneficial symbiosis under modeled microgravity conditions

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Space flight imposes numerous physical challenges to the human body including the dysregulation of the immune system. Although considerable progress has been made in delineating the mechanisms associated with this problem, one area of research that has received little attention is the role that non-pathogenic microbes play in maintaining the healthy immune function of astronauts in space. To address this, we used the symbiosis between the bioluminescent bacterium, *Vibrio fischeri*, and the bobtail squid, *Euprymna scolopes,* as a model system. During colonization, exogenous *V. fischeri* peptidoglycan triggers the migration of host hemocytes into the blood sinus of the squid light organ, the site of the symbiosis. Previous research found that hemocyte migration is delayed in simulated microgravity suggesting a dysregulation of the host immune response in microgravity. In this study, targeted genes relating to a putative peptidoglycan recognition receptor protein (PGRP) pathway that may be facilitating hemocyte migration were examined for differential expression from host light organs at important developmental timepoints in simulated microgravity and gravity conditions. There was increased expression of peptidoglycan receptors in symbiotic animals compared to aposymbiotic (i.e., without symbiont) animals under both gravity and simulated microgravity treatments. However, only two receptor PGRP genes, PGRP1 and PGRP2, followed an expression pattern that could explain the delay of hemocyte migration, indicating other receptor genes may be important in initiating hemocyte migration. Several cascade genes also follow an initial pattern for hemocyte delay at early time points. Current work is focused on assessing significant differential expression of these immune cell genes at additional time points in microgravity.

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Prolific origination of eyes in Cnidaria with co-option of non-visual opsins

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Animal eyes vary considerably in morphology and complexity, and are thus ideal for understanding the evolution of complex biological traits. While eyes evolved many times in bilaterian animals with elaborate nervous systems, image-forming and simpler eyes also exist in cnidarians, which are ancient non-bilaterians with neural nets and regions with condensed neurons to process information. How often eyes of varying complexity, including image-forming eyes, arose in animals with such simple neural circuitry remains obscure. Here, we produced large-scale phylogenies of Cnidaria and their photosensitive proteins and coupled them with an extensive literature search on eyes and light-sensing behavior to show that cnidarian eyes originated at least eight times, with complex, lensed-eyes having a history separate from other eye types. Compiled data show widespread light-sensing behavior in eyeless cnidarians and comparative analyses support ancestors without eyes that already sensed light with dispersed photoreceptor cells. The history of expression of photoreceptive opsin proteins supports the inference of distinct eye origins via separate co-option of different non-visual opsin paralogs into eyes. Overall, our results show eyes evolved repeatedly from ancestral photoreceptor cells in non-bilaterian animals with simple nervous systems, co-opting existing precursors, similar to what occurred in Bilateria. Our study underscores the potential for multiple, evolutionarily distinct visual systems even in animals with simple nervous systems.

Species delimitation and the evolution of freshwater tolerance in the invasive hydrozoan *Cordylophora* using phylogenetic, population genomic and environmental evidence

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The study of invasive species can provide us with new insight into ecological and evolutionary processes by presenting “natural experiments” as species adapt to new environments and native species respond to the invaders. For cnidarians, the evolutionary transition from a marine to freshwater habitat enables them to expand their potential range. An excellent system for investigating this evolutionary transition is the invasive hydrozoan *Cordylophora caspia* as it can be found in freshwater and brackish habitats. Previous studies suggest it may be a species complex comprising lineages of exclusively brackish, freshwater and euryhaline colonies. Given that the lineages have distinct, experimentally-determined salinity tolerance ranges, it is predicted that relative salinity would dictate population genetic structure and potentially contribute to isolation between species. Here we provide a more detailed phylogenomic study of the complex in order to examine possible patterns of species delimitation, including sampling along estuarine salinity gradients where we may expect the different lineages to come into contact. Additionally, we map native salinities of the sampled genotypes onto our phylogeny to confirm that they are separated by salinity and to reconstruct the possible evolutionary history of this trait in the group as it transitioned from marine to freshwater. The pattern of evolution we uncover in this species complex may in fact provide insight into the transition from marine to freshwater in general - the full adaptation to freshwater of single clades from within a euryhaline species complex, and then subsequent isolation of a freshwater clade to the point of speciation.

Gap junctions disruption in cnidarians

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Cnidaria the phylum including sea anemones, jellyfish, and their relatives is the sister lineage to bilaterians. As multicellular animals, cnidarians possess various cell types including neurons though unlike bilaterians, they do not have a centralized neural system and instead, have nervous network. Accordingly, surveys into cnidarian genetic repertoires can provide a unique window illuminating into the origin and evolution of the CNS in the common ancestor of cnidarians and bilaterians. One aspect of neuronal communication is electrical conduction facilitated by gap junction (GJ) proteins such as connexins and innexins. The phylogenetic distribution of connexins are exclusive to chordates, while innexins composed of innexins and pannexins are exclusive to invertebrates and some invertebrates and some chordates, respectively. In cnidarians, gap junctions show a polarizing distribution with a single surveyed anthozoan *Nematostella vectensis* (sea anemones, corals, and relatives) possessing 1 copy and surveyed medusozoans possessing multiple copies. Accordingly, here we test 91 cnidarian species, including Staurozoa, Myxozoa, and Cubozoa members for the first time, for the distribution of GJ proteins. We find pannexins exclusively in anthozoans and innexins exclusively in medusozoans and propose alternative gene losses within cnidarians of GJ proteins.

A quick fuse: melded trees highlight progress and opportunities in anemone biology

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A decade of effort to resolve anemone evolutionary relationships has led to a major reorganization of our understanding of relationships and evolutionary trajectories within this lineage. While this represents a significant advance, the reconstruction of the phylogeny of actiniarian sea anemones has proceeded along three parallel tracks: analyses of highly conserved DNA sequences to discern the major lineages and their interrelationships, analyses of more variable genes to assess relationships within these lineages, and analyses of morphology to determine the affinity of species for which DNA is not available. I present a synthesis of these lines of evidence as a single tree, highlighting major insights and points of instability, describing the phylogenetic context for model taxa, and showcasing ways in which broad and integrative phylogenetic perspective can guide inferences about sea anemone biology and evolution.

Investigating neuronal subtype development in *Nematostella vectensis*

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We are interested in understanding how cnidarian nerve nets are patterned during development. Previous efforts identified neurogenic programs that act broadly to specify *N.vectensis* neural progenitors and promote neuronal differentiation. While this work has improved our understanding of cnidarian neurogenesis, little progress has been made in understanding how neural subtypes fates are imparted in developing nerve nets. Our goal is to expand the number of known neural subtypes within *N.vectensis,* and to improve our understanding of the developmental mechanisms that control neuronal fate acquisition. We identified 18 putative transcription factors that are likely to play a role in neuronal subtype patterning. We determined that 11/18 genes are downstream of *NvAth-like* confirming their neurogenic function. We are using shRNA mediated gene knockdown to assess the roles of these genes in neuronal subtype specification. In addition to this, we hypothesize that spatial domains established by axial patterning cues contribute to neuronal patterning. To address this we are knocking down the aboral domain specifier *Nvsix3/6* and assessing whether aboral neurons are lost. To identify neuronal subtypes we characterized the role of acetylcholine as a modulator of tentacular contractions. Inhibition of sodium channels by lidocaine is sufficient to block acetylcholine mediated tentacular contractions, which implies acetylcholine activates neurons rather than muscles. mRNA *in situ* localization of the acetylcholine receptors investigated to date supports the model that cholinergic neurons modulate tentacular contractions, but that acetylcholine does not directly activate tentacular muscles. We are currently determining which cholinergic cells promote muscular contraction. To date we have identified neurogenic transcription factors that act downstream global cues, begun assessing the impact of spatial cues on neuronal fates, and identified that the role for cholinergic neurons in the tentacular motor circuit.

Investigation of nervous system dynamics in *Nematostella vectensis*

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Here we utilize our *NvLWamide-like::mCherry* transgenic reporter line to investigate growth, maintenance, and regeneration of the nerve net in *Nematostella vectensis.* Two subtypes described by this transgenic reporter line are the longitudinal and tripolar neurons. They are distributed along the oral-aboral axis and their numbers positively correlate to body size. We found that longitudinal and tripolar neurons are significantly reduced during starvation in sexually immature adults. Conversely, these neurons significantly increase in number in fed animals. Both the increase and decrease were reversible by switching fed animals to starvation and vice versa. These data suggest that the *Nematostella* nervous system is highly dynamic.

Cnidarians have the ability to regenerate following injury, and are therefore useful models to investigate regenerative processes. The morphological events that occur during oral regeneration in *Nematostella* have been established. However, what happens to individual neuronal subtypes during regeneration has not been investigated. The numbers of *NvLWamide-like* neurons were quantified before amputation, immediately following bisection, 24 hours post amputation (hpa), and at completion of regeneration. Both the longitudinal and tripolar neurons decrease in number between 0-24 hpa. Longitudinal neurons increase in number between 24 hpa and completion of regeneration, but interestingly only in smaller animals. Larger animals did not appear to regenerate their longitudinal neurons following the loss at 24 hpa, suggesting these animals may be employing different modes of regeneration based on their size. However, we cannot rule out that the location of the amputation site impacts the amount of neuronal regeneration, and we are currently testing this possibility.

A phase transition in activity of *Hydra's* nervous system as it reassembles from individual cells

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A growing body of evidence suggests that neural circuits in the cortex and other parts of the brain may operate at a state of self-organized criticality, a concept used to describe the complexity that emerges in systems attracted to dynamics on the verge of a phase transition. Despite the observation of these dynamics and their accompanying power-law scaling in various preparations, there is little *in vivo* experimental evidence for any proposed function of this transition state in the nervous system.

To explore this issue, we work with the small Cnidarian *Hydra vulgaris*, a representative of some of the earliest nervous systems in evolution. *Hydra's* simple nervous system of 300-2,000 neurons is organized in two independent nerve nets in its ectoderm and endoderm and is distributed through the body of the animal without any cephalization or ganglia. Moreover, under the right conditions *Hydra* can reassemble itself into a normal animal after complete dissociation into individual cells. Using transgenic Hydra which express the calcium sensor GCaMP6s in every neuron (Dupre and Yuste, 2017) we have imaged the neuronal activity of dissociated preparations as they re-aggregate over a period of several days. Our data show how the subcritical activity of dissociated cells transitions to the supercritical state of the reassembled circuitry of the intact animal. The phase transition of *Hydra's* nervous system through a critical regime of activity during the process is reminiscent of the scale-free dynamics of the constantly fluctuating activity of neuronal ensembles of the mammalian cortex. With experimental manipulations to alter the profile of critical dynamics of the process in *Hydra*, we provide *in vivo* experimental evidence in support of the proposed roles of criticality in extending the dynamic range and information capacity of susceptible neural circuits as they form and process information.

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A cnidocyte-specific gene regulatory network from *Nematostella vectensis*

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Cnidocytes (stinging cells) are the defining characteristic of cnidarians; yet, little is known about how cnidocyst diversity arises. While amounting evidence suggests cnidocytes share a common origin with neurons, the network of genes responsible for conferring the unique identity of cnidocytes has not been fully described. Using a CRISPR-mediated knockdown strategy, we characterize the molecular development of cnidocytes following their differentiation from a SoxB2-expressing progenitor cell lineage in the sea anemone, *Nematostella vectensis*. We demonstrate that both conserved and cnidarian-specific transcription factors are necessary for the development of cnidocytes. Interestingly, many of the transcription factors known to be critical for cnidocyte development in *Hydra* are unaffected by SoxB2 knockdown in *N. vectensis,* suggesting that cnidocyte development in these two lineages of cnidarians involves distinct signaling pathways. Using a provisionally assembled cnidocyte gene regulatory network for *N. vectensis*, we explore commonalities in the developmental pathways of this unusual cell type across cnidarian lineages.

Third Allodeterminant found in *Hydractinia* allorecognition complex

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Allorecognition is the ability to distinguish self tissues from those of conspecifics via cell-cell contact. Among cnidarians, the molecular basis of allorecognition is best understood in *Hydractinia.* Previously, isogeneic and congenic lines of *Hydractinia* were used to map a genomic region controlling allorecognition, called the allorecognition complex (ARC). Partial sequencing of the ARC revealed two genes, *Allorecognition 1* (*Alr1*) and *Allorecognition 2 (Alr2)*, which encode transmembrane proteins with highly polymorphic ectodomains. The sequences of these ectodomains predict allorecognition responses in inbred strains such that colonies sharing alleles fuse, while those that do not reject. In outbred colonies, however, *Alr1* and *Alr2* alone could not predict alloresponses. This observation, along with the presence of *Alr*-like sequences flanking *Alr1* led us to hypothesize that the ARC may contain additional allorecognition genes. Here, we report the assembly and annotation of three ARC haplotypes. Each span >12 megabases (Mb) and contain >60 *Alr*-like sequences. These ARC haplotypes contain at least ten *Alr* genes (*Alr1-Alr10*). Mapping alloresponses in an F1 population produced by crossing two outbred colonies has allowed us to identify a 0.4 Mb region containing at least one new allodeterminant. This region was not mapped in previous experiments because inbreeding had rendered it monomorphic in laboratory strains. Our work reveals that *Hydractinia* allorecognition is controlled by a complex genomic region containing at least three genes that serve to distinguish self from non-self.

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Cellular And Genomic Immunity of *Pocillopora damicornis*

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The innate immune system is the primary defense used by metazoans to defend against foreign pathogens. Cnidarians, and in particular corals have an incredibly complex innate immune repertoire which is only just beginning to be understood. To better understand this complexity my laboratory has been developing methods such as fluorescent activated cell sorting (FACS), *in situ* hybridization and using genome sequencing for the coral *Pocillopora damicornis.* Through this work, we have identified primary cell types including immune cells, and stem cells; characterized the genomic signature of the *P. damicornis* immune system, and are starting to understand the spatial expression of candidate immune genes. We have found that the genome of *P. damicornis* has limited gene family expansion in comparison to Scleractinians, and that many *P. damicornis* specific genes have GO annotations for immune-like genes, such as “activation of NF-κB inducing kinase activity,” indicating that the innate immune repertoire in *P. damicornis* may be highly specialized. Additionally, we have found that *P. damicornis* possess 12-cell populations. Using FACS we have identified ALDH positive cells, indicating a population of cells with stem cell-like qualities and we have identified phagocytic cells using fluorescently labeled beads. Future studies will focus on further characterizing these cells through cell culture assays and sequencing.

Cnidarian Toll-like Receptor Signaling

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Toll-like receptors (TLRs) are primary pathogen detectors that elicit innate immune responses in organisms from insects to vertebrates. Upon pathogen binding, membrane-bound TLRs initiate downstream signaling to transcription factor NF-κB, which then directs the expression of immune effector genes. TLRs also have roles in development in many species. We have been studying TLRs in *Nematostella vectensis* (*Nv*) and the endangered coral *Orbicella faveolata* (*Of*). Both cnidarians have TLRs that are structurally similar to vertebrate TLRs, as well as homologs of most vertebrate TLR-to-NF-κB pathway proteins. We show that Nv-TLR can activate canonical NF-κB signaling in human cells and this activation is abolished by removal of the intracellular TIR domain. Furthermore, the TIR domains of Nv-TLR and Of-TLR can interact directly with the human TLR adapter proteins, MAL and MYD88. The coral pathogen *Vibrio coralliilyticus* causes a rapidly lethal disease in *Nv*, and heat-inactivated *V. coralliilyticus* and bacterial flagellin can activate a reconstituted Nv-TLR-to-NF-κB pathway in human cells. Nv-TLR is expressed in a subset of cnidocytes, many of which also express Nv-NF-κB. Additionally, the nematosome expresses mRNAs for predicted TLR-to-NF-κB pathway homologs, and is capable of engulfing *V. coralliilyticus*. Morpholino knockdown of Nv-TLR results in early gastrulation defects, indicating a role for Nv-TLR in embryonic development. Treatment of *Of* tissue with lipopolysaccharide, a ligand for mammalian TLRs, causes gene expression changes consistent with NF-κB pathway mobilization. Ongoing studies seek to show the direct interaction of flagellin with cnidarian TLRs, and to identify cells in *Of* that are capable of pathogen recognition. Overall, these results 1) represent the first identification of a bacterial pathogen of *Nv*, 2) suggest that the nematosome, a *Nematostella*-specific structure, is a circulating immune organ, and 3) suggest that innate immune signaling is highly conserved from cnidarians to humans.

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Chromatin dynamics enable transcriptional rhythms in the non-symbiotic cnidarian *Nematostella vectensis*

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The genomic potential relies not only on the DNA sequence. Epigenetic factors such as chromatin accessibility can be rapidly modified, and may thus represent mechanisms for fast acclimation to a changing environment. Therefore, the chromatin accessible landscape of the genome reveals valuable information about the active *cis*-regulatory elements (CREs) and the TFs that bind them, and enables to study the relationship between CREs and gene expression in varied taxa. To further study the insight into the accessible chromatin, we choose to work with the new basal cnidarian model organism *Nematostella vectensis* (NV). Here, we compared animals kept under two different light regimens and based our investigation on previous RNA-seq acquired from our group. Using ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing), We show for the first time that sensitive measuring of chromatin accessibility in a whole NV enables to detect dynamic changes and identify potential TF binding sites with only a small subset of samples. Increasing the sequencing focus on the euchromatin, highlights regulatory genomic regions and adds another layer of understanding gene expression data of NVs rhythmicity. Pairing the DNA accessibility profile with motif analysis allows assessing which TFs are active and localize their binding sites. This work opens a new window into dynamic chromatin by showing the high reproducibility of the protocol and integrating the analysis with published data. By using a cnidarian model we uncover gene regulation elements which provide an additional tool, other than transcriptomic, that lead to a better understanding of this important group.

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Viruses as Intimate Partners in the *Hydra* Holobiont

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Multicellular organisms in functional association with their microbiome, consisting of prokaryotes, eukaryotes, and viruses are referred to as “holobionts”. Deciphering these holobiont associations has proven to be both difficult and controversial. In particular, holobiont association with viruses has been of debate even though these interactions have been occurring since cellular life began. The controversy stems from the idea that all viruses are parasitic, yet their associations can also be beneficial. To determine viral involvement within the holobiont, it is necessary to identify and elucidate the function of viral populations in symbiosis with the host. We are using *Hydra* as a laboratory system to explore the holobiont, with a particular focus on *Hydra*-virus interactions. Like all animals, *Hydra* have epithelia covered by microbial and viral communities. Previous studies found that the viral DNA communities associating with *Hydra* are species-specific and affect holobiont metabolism. We are currently exploring the RNA and eukaryotic-specific viral communities associating with *Hydra*, and the bacteriophages from *Hydra*-associated bacterial isolates. Together, these projects combine for a comprehensive animal-associated viral metagenome (virome). In separate studies, we are determining how this basal metazoan, with an environmentally exposed epithelium and only an innate immune system, deals with viral presence and how these interactions are maintained. *Hydra* are hyper-responsive to foreign viruses, even in germ-free conditions. These data suggest viral discrimination in the basal metazoan *Hydra* requires a complex recognition network dependent upon bacterial PAMPs. The purpose of these projects is to develop a model system to identify how an animal selects for its viral partners, uses viruses as a microbiome regulation parameter, and bases this selection using a well-conserved innate immune system.

The possible role of C-type lectins in establishing specificity in Cnidarian-*Symbiodinium* symbiosis

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Many members of the phylum Cnidaria form a mutualism with endosymbiotic dinoflagellate algae of the genus *Symbiodinium*. There are nine recognized divergent lineages of *Symbiodinium*, clades A-I, with diverse host ranges and functional properties (1). Glycans and/or glycoproteins on the algal cell surface may contribute to recognition by host Cnidarians (2,3). Using the small sea anemone *Aiptasia*, we are investigating how Cnidarians recognize and select for compatible symbiotic algae and, more specifically, the possible role of C-type lectin proteins during initial contact. C-type lectins are sugar-binding proteins that are utilized by organisms for the recognition of various microbial compounds termed microbe-associated molecular patterns (MAMPs), such as glycans.

By transcriptomic analyses we identified several putative Aiptasia lectins that are expressed at much higher levels in aposymbiotic than in symbiotic anemones. We confirmed the sequences of the corresponding genes by Sanger sequencing, cloned them into a GST expression vector, and purified recombinant GST-lectin proteins. We then interrogated the ability of the lectins to recognize various axenic clonal *Symbiodinium* strains using *in vitro* binding assays and fluorescence microscopy. Surprisingly, we observed that one of the lectins displayed more binding to incompatible than to compatible algal strains. Analysis of a second lectin is in progress.

References: (1) Ponchon and Gates, 2010, *Mol. Phylogenet. Evol.* (2) Wood-Charlson et al., 2006, *Cell. Microbiol*. (3) Parkinson et al., 2018, *Front. Microbio*.

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Transcription factor NF-κB is Modulated by Symbiotic Status in the Sea Anemone Aiptasia

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In addition to the immune system's role in pathogen recognition and clearance, the immune system has a role in managing beneficial microbes and supporting mutualistic microbial symbioses. Some cnidarians such as corals and sea anemones undergo symbiosis with *Symbiodinium*, a genus of photosynthetic algae. To explore the interaction of host immunity and symbiosis, we characterized the NF-κB transcription factor pathway in Aiptasia, a commonly used sea anemone model for cnidarian symbiosis and loss of symbiosis (bleaching). The NF-κB pathway has been widely studied for its roles in a variety of immune-related processes, as well as diseases, in animals ranging from insects to mammals. In Aiptasia, NF-κB protein levels, DNA-binding activity, and tissue expression increase when loss of *Symbiodinium* is induced by heat or menthol treatment. Kinetic analysis of NF-κB levels following loss of symbiosis show that NF-κB levels increase only after *Symbiodinium* is cleared. Moreover, introduction of *Symbiodinium* into naïve Aiptasia larvae results in a decrease in NF-κB expression, however the introduction of non-compatible *Symbiodinium* strains does not decrease NF-κB. These results suggest that *Symbiodinium* suppresses NF-κB in order to enable establishment of symbiosis in Aiptasia. Moreover, decreased NF-κB expression in aposymbiotic anemones is correlated with decreased survival in response to infection with the pathogenic bacterium *Serratia marcescens*, suggesting that NF-κB is important for the immune response to pathogens. These results are the first to characterize NF-κB expression in relation to symbiotic status and to correlate immune competence with symbiotic status in Aiptasia. Current studies are aimed at identifying the mechanism by which *Symbiodinium* down-regulates NF-κB, and whether NF-κB is also affected in corals that undergo obligate symbiosis with *Symbiodinium*.

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Acquisition and proliferation of algal symbionts in polyps of the upside-down jellyfish

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The mutualistic relationship between dinoflagellates in the genus *Symbiodinium* (a.k.a. 'zooxanthellae') and animals in the phylum Cnidaria make up one of the most well-known symbioses in the marine environment. Use of the model system *Cassiopea xamachana* a scyphozoan that can be reared throughout its entire life cycle in a laboratory setting, allows for the study of the cnidarian-algal symbiosis without many of the constraints involved when working with corals and other cnidarians. The polyp stage of *C. xamachana* was used in this study to visualize the acquisition of algal cells by endodermal cells of host animals, a requisite phenomenon for the metamorphosis to a free living medusae (strobilation). Polyps infected with algae from environmental sources prior to experimentation were subjected to high temperatures to initiate the expulsion of algae and to obtain polyps that were completely free of symbionts (aposymbiotic). Aposymbiotic polyps were subsequently introduced to low numbers of *Symbiodinium*, and the intrinsic growth rate of algae calculated based on count data generated at 7 day intervals. *Symbiodinium* used for reintroduction in experimental trials were obtained from three sources: (1) cultured zooxanthellae (strain A194), (2) zooxanthellae that had been expelled from their host tissues following heat-induced bleaching, and (3) freshly isolated zooxanthellae. *Symbiodinium* cells were acquired by *C. xamachana* polyps in all three treatment groups, although strain A194 exhibited the slowest mean intrinsic growth rate once they were housed within endodermal tissues. Mean times to strobilation was accordingly slow for those polyps harboring strain A194 symbionts. Strobilation was determined to be independent of the absolute number of symbionts within a polyp, suggesting that strobilation is ultimately stimulated by other triggers.

Keynote Speaker Abstract – Dr. Virginia Weis

Symbiotic sea anemones as model systems for the study of corals: Easier, faster, softer

Virginia Weis

Oregon State University, Corvallis, Oregon, USA

Corals engage in a mutualistic symbiosis with intracellular photosynthetic dinoflagellates. This intimate partnership forms the trophic and structural foundation of coral reef ecosystems. In this presentation, I will discuss the use of the sea anemone Aiptasia (*Exaiptasia pallida)* as a model system for the study of coral-dinoflagellate symbiosis. I will talk about both advances and barriers to success of this model system in moving the understanding of coral biology forward, in a time of deep crisis for corals and coral reef survival. I will discuss studies in the Weis Lab over the past 22 years that have aimed to characterize the cellular and molecular mechanisms underlying the establishment, maintenance and breakdown of the symbiosis in cnidarian-dinoflagellate partnerships. Host innate immunity and symbiont strategies for modulating this immune response are central to the stability of the symbiosis. During onset and maintenance of symbiosis these mechanisms include, lectin-glycan signaling, upregulation of the immunosuppressive TGFβ pathway and changes in the sphingolipid rheostat and complement pathway. Coral bleaching, a severe threat to the health of reefs worldwide, is caused by global warming and results from dysbiosis: the collapse of the symbiosis. I will present evidence that cnidarian bleaching is a host innate immune response to a compromised and stressed symbiont. This includes increased nitric oxide levels, and host cell apoptosis and autophagy in heat-stressed animals, all well-known immune mechanisms in other systems to eliminate detrimental microbial invaders. Finally, I will discuss the international effort to rapidly advance Aiptasia-*Symbiodinium* genetic techniques and develop new tools for the field while cooperatively growing this model system community and helping to save corals.

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Variation in transcription and protein-protein interactions of Hsp70 in the anemone Nematostella vectensis

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Heat shock protein 70s (Hsp70s) are a highly conserved class of molecular chaperones that are involved in diverse cellular processes, including stress response, homeostatic maintenance, and cell cycle progression, in all organisms. Hsp70 can also be effective biomarkers to determine mechanisms of physiological acclimation and evolutionary adaptations to environmental stress. However, despite the number of cnidarian species from which Hsp70 has been studied, there remains limited data on the differences in expression for the numerous homologs present in a genome and virtually no data on the protein-protein interactions for these essential chaperones. Nematostella vectensis is an estuarine anemone that has emerged a model species to characterize molecular responses to physiological stressors due to its exposure to diverse, extreme abiotic conditions such as temperature, UV radiation, salinity, and pollutants. We report the transcriptional differences for the three cytosolic Hsp70s (NvHsp70) for N. vectensis from different geographic locations exposed to acute and chronic thermal stress. We compare this dynamic expression with the expression of these Hsp70s when anemones are exposed to other environmental variation (salinity, light). We will also summarize similarities and differences of protein-protein interactions for these three Hsp70s from mass spectrometry data when expressed in yeast cells under heat shock conditions. Hsp70 homologs interact with 100s of proteins, only half of which are shared for all three anemone proteins. Together, these data show gene-specific transcriptional patterns and a rich, diverse set of interacting proteins for cnidarian Hsp70 homologs that suggest novel biomarkers to characterize the response of cnidarians to their rapidly changing environments.

Plasticity in parental effects confers rapid thermal tolerance to *Nematostella vectensis* larvae

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Parental effects increase offspring survivorship under environmental conditions similar to the parents'[1]. Adjusting parental effects to new environmental conditions can facilitate rapid acclimatization and survival across generations[2,3]. We investigated parental effects in the anemone *Nematostella vectensis* by exposing clonal parental populations to heat stress and testing larval thermal tolerance. Parental preconditioning yields a consistent shift in larval thermal tolerance (temperature at which 50% of larvae survive: LT50). Exposure of both parents confers a larger shift than maternal/paternal exposure alone, suggesting a contribution of both sexes to larval survival. Ensuing larval cohorts return to baseline LT50s, indicating a highly responsive mechanism for ensuring larval survival. Preconditioning also increased adult survival four-fold following a subsequent severe heat stress. Previous studies have shown N. vectensis populations are thermally adapted to local environments across their wide latitudinal range[4]. LT50 shifts due to parental effects exceed differences between Massachusetts and North Carolina larvae reared in common garden conditions. Furthermore, hybrid larvae show intermediate LT50s, indicating that larval thermal tolerance is influenced by both long-term adaptive differences and short-term epigenetic mechanisms. Our results show protective parental effects can provide substantial thermal resistance to offspring. Further understanding the mechanisms responsible for these responses can better elucidate the fate of thermally sensitive marine ectotherms in a rapidly changing thermal environment.

1. T. Uller (2008) *Trends Ecol. Evol.* 23, 432–438
2. D.J. Marshall, T. Uller (2007) *Oikos.* 116, 1957–1963
3. H.M. Putnam, R.D. Gates (2015) *J. Exp. Biol.* 218, 2365–2372
4. A.M. Reitzel et al. (2013) *Mar. Ecol. Prog. Ser*. 484, 115–130

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Ceriantharia (Cnidaria) as an alternative model to study evolution and life cycles

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Our understanding of complex life cycles reflecting on how they have evolved in different groups is the research focus of several investigators around the world. Even though Cnidaria are considered “simple” animals, their developmental trajectory in an evolutionary context is not so simple. That is the case of several clades in Cnidaria, especially within Anthozoa. In this scenario, Ceriantharia is an outstanding theme with tremendous variation occurring across different life cycles despite having relatively few species previously characterized. Some new data are revealing the influence of environment in the life cycle (long-living x short-living larvae), but the molecular mechanism that signaling these variations is completely unknown. Ceriantharia may be an insightful lineage for determining the mechanisms for life cycle evolution in cnidarians, including the origin of the medusa as well as adaptations in larval stages. Early investigations of the molecular biology and genomics of Ceriantharia have shown surprising differences in this lineage when compared with other anthozoans, including what appears to be a linear and segmented mitochondrial genome and toxin gene assemblages that are incongruent with other cnidarians. The ease of lab maintenance for ceriantharian specimens is not difficult, presenting them as an opportune lineage to perform experiments in the lab related to variations on developmental plasticity, toxin diversity, and overall ecology really attractive.

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Transcriptional And Microbial Characterization Of Scleractinian Coral Cell Populations Separated By Fluorescence-Activated Cell Sorting (FACS)

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Coral reefs are facing extinction due to climate change and other human-induced threats. With corals remaining vulnerable to these environmental changes, there is a critical need to have a complete understanding of their evolutionary history, cellular responses to stress, and symbiotic relationships with their microbial partners, which may aid in restoration efforts and policy-making. While there have been many studies focused on the coral-dinoflagellate symbiosis, little is understood about the diversity of coral cells, their functions, and the cell-specific microbial partnerships with viruses and bacteria. Here we use fluorescence-activated cell sorting (FACS) to parse out coral cells based on universal cell markers. Using RNA-Seq and 16s rRNA amplicon sequencing, we are investigating the gene expression and bacterial communities specific to cell populations. These cell populations have been parsed using markers for DNA, lysosomes, cnidocytes, and reactive oxygen species (ROS). This novel approach of using cellular biology techniques in corals has great potential for cell type characterization and functional characterization for coral cells. Understanding the roles of various cell types and their microbial communities would aid in identifying tolerant and adaptable genotypes for preserving genetic diversity on coral reefs.

Atypical cadherin Fat in hydra development.

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Fat is an atypical cadherin and the major component of the planar cell polarity (PCP) pathway. Its homologues, although not involved in PCP, play roles in cellular polarization by regulating actin cytoskeleton. In addition Fat molecules are involved in regulation of Hippo and Wnt pathways. Unlike other studied organisms hydra has single homologue of *Fat* (*HyFat*) gene. Analysis of the several lines of transgenic hydras showed *HyFat* affects actin polymerization in ectodermal epithelial cells: downregulation of *HyFat* causes mild misalignment of actin cables, and the ectopic expression of its intracellular domain (ICD) in ectodermal epithelial cells causes severe misalignment under certain conditions. This effect on actin cytoskeleton might be the reason for changes in the apical cell surface of ectodermal epithelial cells observed in the mutants. Our data also indicate that *HyFat* might be involved in Hippo and Wnt pathways in hydra. Therefore, the only homologue of Fat molecule in hydra, HyFat, might serve multiple functions that later in evolution had been split between several homologues.

Mechanical coupling coordinates mouth opening in *Hydra*

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Hydra lacks a permanent mouth. For feeding, it rips itself a new mouth opening in a continuous epithelium, which can be tens of cell diameters wide. Mouth opening is achieved entirely through coordinated cell deformation. We have previously shown that the area of the mouth opening as a function of time fits a sigmoidal curve [1] but what sets the timescales governing the process remains unknown.
Excision and wounding experiments within the hypostome region show that the length of the largest unit that contracts 'as one' is much smaller than the radius of the mouth. This suggests the need for communication between the units to generate the coordinated global mouth opening response. The high density of neurons and gap junctions in the hypostome make them prime suspects for facilitating this communication [2]. However, we found that blocking gap junctions using heptanol and octanol does not affect the mouth opening kinematics. Using nerve-free animals, we further investigated the role of the nerve-net as a means of long-distance communication. Preliminary data from electrical stimulation experiments on nerve-free *Hydra* indicate that while a nerve net is necessary for initiating mouth opening, continuous nerve signalling is not necessary to coordinate mouth opening. Together, these results point toward an interesting scenario where the coordination of an important and complicated physiological process depend entirely on mechanical properties of tissues and myonemes and their mechanical coupling.

[1] Carter, Jason A., Callen Hyland, Robert E. Steele, and Eva-Maria S. Collins. 2016. "Dynamics of Mouth Opening in *Hydra*." *Biophysical Journal* 110 (5): 1191–1201.
[2] Wood, R. L. 1979. "The Fine Structure of the Hypostome and Mouth of *Hydra*. II. Transmission Electron Microscopy." *Cell and Tissue Research* 199 (2): 319–38.

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Chitin prevalence and diversity of chitin synthase genes across Cnidaria

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The phylum Cnidaria is a clade of primarily marine aquatic animals displaying a diversity of body plans and ecological roles. All members of this clade possess an evolutionarily unique cell type – the cnidocyte – which produces the explosive harpoon-like organelle that gives cnidarians their famous sting. Cnidarians are usually gelatinous, being primarily composed of an extracellular gel-like collagenous tissue – the mesoglea. Some cnidarian species also possess chitinous structures like endoskeletons of some anthozoans or holdfast-like coatings on hydrozoan polyp stalks. However, genes coding for proteins involved in chitin synthesis have been putatively identified in two taxa with no known chitinous structures. We explore the prevalence and diversity of *chitin synthase* genes across Cnidaria using genomic and transcriptomic data. Our data show that the enzyme *chitin synthase* is present in all cnidarian classes, including in species or life stages with no known chitinous structures. Cnidarian *chitin synthase* proteins display diverse domain organizations, even within species, suggesting multiple roles for these enzymes. Chitin affinity histochemistry shows that chitin is present in the tissues of scyphozoans, hydrozoan medusae and polyps, and actinarian anthozoans. Chitin labeling is also present in some populations of cnidae. The genetic diversity of chitin synthesizing enzymes and widespread presence of the molecule chitin indicates an important role for chitin in the biology of these soft-bodied animals.

Contractile actin rings suggest an engulfing role for ectodermal cells in *Hydra vulgaris*

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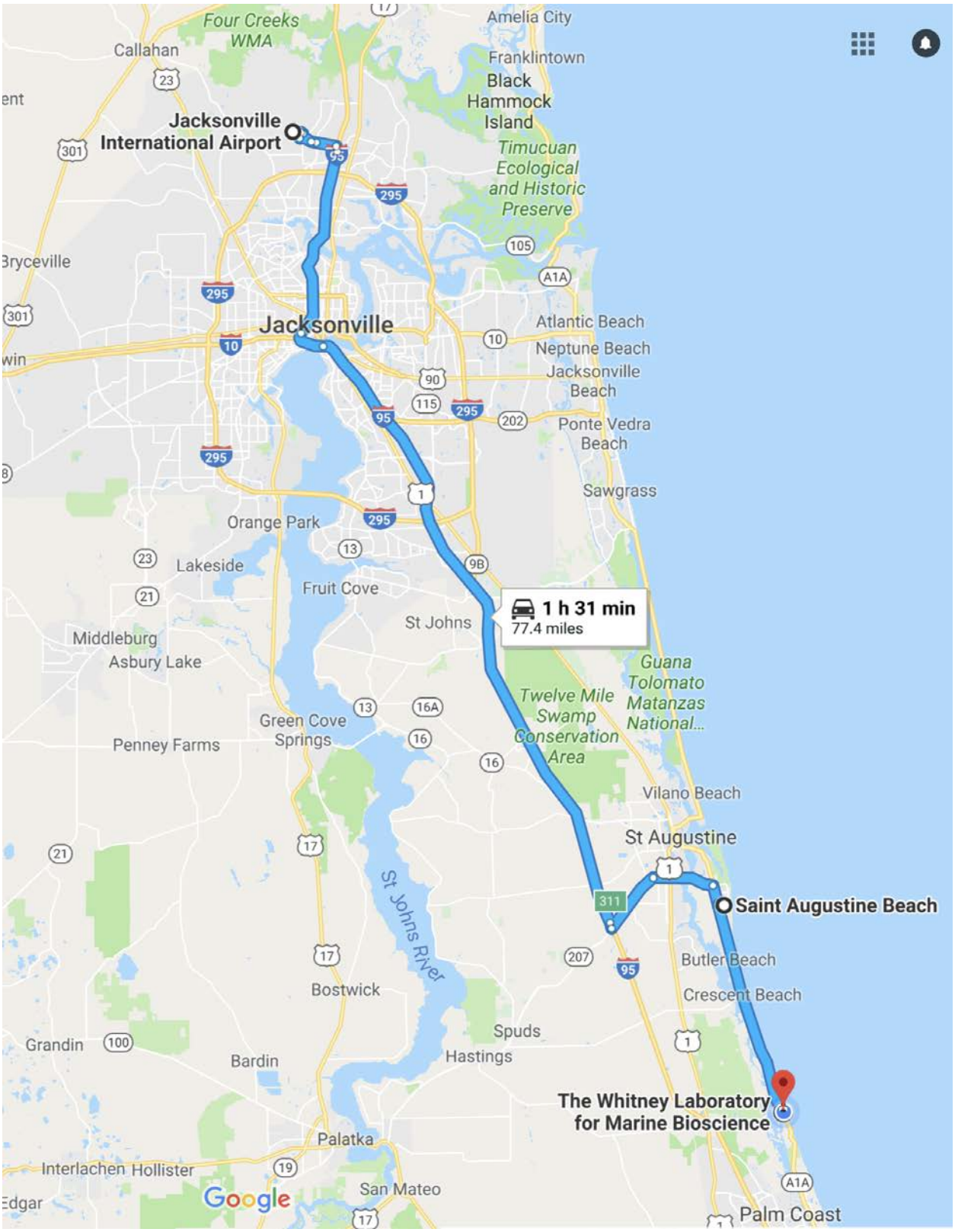
The actin cytoskeleton plays essential roles in cell- and tissue-scale functions through formation of numerous distinct structures that shape and deform the plasma membrane. It remains poorly understood how cells coordinate actin dynamics across subcellular compartments. Myoepithelial cells of *Hydra vulgaris* are characterized by a complex actin organization, including apical adhesion belts and basal myonemes, providing a powerful system to dissect the mechanisms of subcellular actin organization and compartmentalization. Investigating hydra's actin organization, we have identified a novel actin ring structure restricted to the apical surface of ectodermal cells. These actin rings distend the plasma membrane to form cup-shaped membrane protrusions with predictable cycles of expansion and contraction, suggestive of engulfing structures found in other cell types. Moving forward, we are defining the regulatory pathways involved in ectodermal actin ring formation and the biological function of these structures.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Guana Tolomato Matanzas National...
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