First Biennial North American Meeting of Hydroid Biologists

Bodega Marine Laboratory
Bodega Bay, CA
September 23-25, 2016

Co-Organizers:
Celina Juliano
Christy Schnitzler

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Any opinions, findings, conclusions, or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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About Hydroidfest

Hydrozoan model organisms are being used to make fundamental discoveries in many fields of research including neurobiology, stem cells, regeneration, aging, development, allorecognition, symbiosis, and evolution.

The first studies of *Hydra* date back to Abraham Trembley's 1774 memoirs, which documents the amazing regenerative capabilities of this hydroid. Hydroids, particularly *Hydra* and *Hydractinia*, are well understood at a morphological level due to a rich body of literature collected over the past forty years.

Hydroids are members of the phylum Cnidaria, which are sister to all bilaterians and therefore sit at an informative position for evolutionary analysis and discovery of conserved biological processes in animals. In addition, many hydroids are translucent and thus well-suited for live imaging. This is very powerful when combined with recent advances in transgenic technology, such as with tracking fluorescently labeled cells.

Further, transgenic technology has also allowed knockdown studies using RNAi and cell sorting to identify the mRNA and small RNA populations of particular cell lineages. As we collect more cell type-specific reporters, these studies will become more refined; understanding the transcriptomic signature of every cell type in hydroids is not a distant dream, but a very manageable goal.

Thanks to rapidly advancing technologies, the hydroid 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 the rise of single-cell sequencing technologies will help us to refine these data sets further in the near future. These data, in combination with CRISPR/Cas9 for gene editing open grand new experimental avenues that will allow us to use hydroids to answer fundamental biological questions.

A number of North American laboratories, both well-established and newly established, have recognized the great potential of hydroid models and are now using these animals to address fundamental biological questions. Hydroid research in North America is clearly on the rise.

Our long term goal is to hold our US-based hydroid meeting every two years, alternating between the Bodega Marine Laboratory in California and the Whitney Marine Laboratory in Florida, thus balancing travel burdens for west coast and east coast scientists.

Follow Hydroidfest on Twitter: @hydroidfest
Use the hashtag #Hydroidfest for all of your tweets!

Organizing Committee

Meeting Co-Organizers

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

Celina is the on-site co-organizer. Celina is using *Hydra* as a model to study stem cells and aging. She is also utilizing gene editing technologies to conduct her research. You can read more about Celina and her research at juliano.faculty.ucdavis.edu

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

Christy is one of our co-organizers and will host the next meeting at Whitney labs. Christy uses evolutionary and functional genomics in *Hydractinia* to explore fundamental biological processes, such as stem cell-mediated regeneration, innate immunity, and cellular senescence. Read more about Christy at whitney.ufl.edu/christineschnitzler/

Organizing Committee

Committee Members

Dr. Robert Steele
Professor
University of California, Irvine
Twitter: @robthehoosier

Rob is our wise advisor. Work in Rob's lab addresses how communication between cells is used to regulate the composition and pattern of tissues in *Hydra*. You can read more about Rob and his research at faculty.uci.edu/profile.cfm?faculty_id=2387

Mr. Christophe Dupre
PhD Candidate
Columbia University

Chris is our graduate student representative and meeting report writer. He is using *Hydra* to understand how a nervous system creates behavior and is using state of the art live imaging techniques to make these observations. You can read more about Chris at columbia.edu/cu/biology/faculty/yuste/

Dr. Juris Grasis
Post-Doctoral Fellow
San Diego State University
Twitter: @JurisGrasis

Juris is our post-doc representative and meeting website and social media administrator. He is using *Hydra* as a model for host-holobiont interactions, with a focus on viral interactions. You can read more about Juris and his research at jurisgrasis.com

Hydroidfest 2016 Schedule At A Glance

Friday, September 23

11:00am – 1:00pm	Registration Check-in
1:00 – 1:15pm	Welcome
1:15 – 2:30pm	Session I: Modeling and Calcium Imaging
2:30 – 3:00pm	Coffee Break
3:00 – 4:15pm	Session II: Sensing, Behavior, and Neurogenesis
4:30 – 6:00pm	Keynote Address by Dr. Charles David
6:00 – 6:30pm	Drinks/Appetizers
6:30 – 8:00pm	Dinner
8:00 – 8:30pm	Lightning Session I
8:30 – 10:00pm	Poster Session I
10:00 – Midnight	Know about each other in the bar

Saturday, September 24

7:00 – 9:00am	Breakfast
9:00 – 10:15am	Session III: Regeneration and Aging
10:15 – 10:45am	Coffee Break
10:45am – 12:00pm	Session IV: Genes and Development
12:00 – 1:00pm	Lunch
1:00 – 2:15pm	Session V: Immunology
2:15 – 2:45pm	Coffee Break
2:45 – 4:00pm	Session VI: Symbiosis
4:00 – 4:15pm	Hydroidfest 2016 Group Photo
4:15 – 6:00pm	Hiking/Exploring Bodega Bay
5:30 – 6:00pm	Drinks/Appetizers
6:00 – 8:00pm	BBQ Dinner
8:00 – 8:30pm	Lightning Session II
8:30 – 10:00pm	Poster Session II
10:00 – Midnight	Know about each other in the bar – Movie Night: “The Birds”

Sunday, September 25

7:00 – 9:00am	Breakfast
9:00 – 9:50am	Technology Workshop
9:50 – 10:05am	Coffee Break
10:05 – 10:20am	Awards Ceremony
10:20 – 12:00pm	Technology Workshop
12:00pm	Lunch and Depart

Hydroidfest 2016 Program

Friday, September 23

11:00am – 1:00pm	Registration Check-in
1:00 – 1:15pm	Welcome
1:15 – 2:30pm	Session I: Modeling and Calcium Imaging Session Chair: Yuki Noro Christophe Dupre (p. 11) – Calcium imaging in the nervous system of *Hydra* Shuting Han (p. 12) – Machine Learning Mapping of Behavior in *Hydra vulgaris* Adrienne Fairhall (p. 13) – Building a biomechanical model of *Hydra*
2:30 – 3:00pm	Coffee Break
3:00 – 4:15pm	Session II: Sensing, Behavior, and Neurogenesis Session Chair: Rafa Yuste James Gahan (p. 14) – Notch signalling is required for tentacle patterning but dispensable for neurogenesis in *Hydractinia echinata* David Plachetzki (p. 15) – Cnidarian polymodal sensory-motor neurons as a model for cell type subfunctionalization in the origins of bilaterian complexity Hiroshi Shimizu (p. 16) – Peduncle nervous system of *Hydra* functions as central nervous system
4:30 – 6:00pm	Keynote Address Charles David (p. 17) Spontaneous contractile behavior in *Hydra* and what controls it: where's the "brain" in a nerve net?
6:00 – 6:30pm	Drinks/Appetizers
6:30 – 8:00pm	Dinner
8:00 – 8:30pm	Lightning Session I
8:30 – 10:00pm	Poster Session I 1 – Krishna Badhiwala (p. 18) 2 – Sofia Barreira (p. 19) 3 – Jack Cazet (p. 20) 4 – Aiden Huene (p. 21) 5 – Adolfo Lara (p. 22) 6 – Tiffany Locke (p. 23) 7 – Robert Monroy (p. 24) 8 – Catriona Munro (p. 25)
10:00 – Midnight	Know about each other in the bar

Hydroidfest 2016 Program

Saturday, September 24

7:00 – 9:00am	Breakfast
9:00 – 10:15am	Session III: Regeneration and Aging Session Chair: Diane Bridge Stefan Siebert (p. 26) – Piwi-piRNA pathway function in somatic stem cells of *Hydra* Paulyn Cartwright (p. 27) – Comparison of the role of the Runx gene during regeneration in *Nematostella, Hydra* and *Hydractinia* Daniel Martínez (p. 28) – The transcriptome of inducible aging in *Hydra oligactis*
10:15 – 10:45am	Coffee Break
10:45am – 12:00pm	Session IV: Genes and Development Session Chair: Rob Steele Olivier Cochet-Escartin (p. 29) – Physical mechanisms of cell sorting during *Hydra* regeneration from aggregates Masha Brooun (p. 30) – Fat and Dachsous cadherins in *Hydra* development Christine Schnitzler (p. 31) – Comparative genomics of *Hydractinia* and *Hydra*
12:00 – 1:00pm	Lunch
1:00 – 2:15pm	Session V: Immunology Session Chair: Matt Nicotra Steven Sanders (p. 32) – Investigating the *in vivo* function of *Hydractinia* allorecognition proteins using CRISPR/Cas9 genome editing Kristin Michel (p. 33) – Does *Hydra* remember its foes? Jung Shan Hwang (p. 34) – Cellular Membrane Binding and Cytolytic Function of *Hydra* HALT-1
2:15 – 2:45pm	Coffee Break
2:45 – 4:00pm	Session VI: Symbiosis Session Chair: John Pringle Trevor Tivey (p. 35) – Host and symbiont cell division in the symbiotic anemone *Aiptasia pallida* Philip Cleves (p. 36) – Developing Transgenic Tools to Study Cnidarian-Dinoflagellate Symbiosis in a Sea-Anemone Model System Juris Grasis (p. 37) – The Interdependence of Viruses and the Holobiont

Hydroidfest 2016 Program

4:00 – 4:15pm	Hydroidfest 2016 Group Photo
4:15 – 5:30pm	Hiking/Exploring Bodega Bay
5:30 – 6:00pm	Drinks/Appetizers
6:00 – 8:00pm	BBQ Dinner
8:00 – 8:30pm	Lightning Session II
8:30 – 10:00pm	Poster Session II
	9 – Casandra Newkirk (p. 38)
	10 – Yuki Noro (p. 39)
	11 – David Plachetzki (p. 40)
	12 – Charles Sebasta (p. 41)
	13 – Noemie Sierra (p. 42)
	14 – Bryan Teefy (p. 43)
	15 – Cawa Tran (p. 44)
	16 – Rui Wang (p. 45)
10:00 – Midnight	Know about each other in the bar
	Movie Night: "The Birds" viewing in the lounge

Sunday, September 25

7:00 – 9:00am	Breakfast (Make Bag Lunch)
9:00 – 9:50am	Technology Workshop
	'Omics
	Dan Rokhsar – Computational Genome Analysis and Comparative Developmental Biology
9:50 – 10:05am	Coffee Break
10:05 – 10:20am	Awards Ceremony
10:20 – 12:00pm	Technology Workshop
	Live Imaging
	Sara Abrahamsson – Fast multicolor 3D imaging using aberration-corrected multifocus microscopy
	Genome Editing
	Gaurav Varshney – Development of Novel CRISPR-based Strategies for Functional Genomics
12:00pm	Lunch and Depart

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Please note that the recording of oral or poster sessions by audio, video, or still photography is strictly prohibited except with the advance permission of the author(s) and the notification of the organizers.

Speaker Abstracts – Friday, September 23

Calcium imaging in the nervous system of *Hydra*

Christophe Dupre, Sanjana Salwi, Stephen Leong and Rafael Yuste

Department of Biological Sciences, Columbia University, New York, NY-10027

Hydra is representative of the most primitive nervous systems. Yet, it turns out that it does not function like its structure (a nerve net) would suggest and there might be more to learn about neurons in *hydra* than previously thought. This cnidarian offers a convenient preparation for calcium imaging, especially because it is possible to image the entire animal simultaneously. Our experiments using such technique revealed multiple groups of neurons (or conduction systems) that are anatomically distinct and that fire simultaneously, together with a series of neurons that seem to fire independently. Accordingly, we are interested in dissecting the nervous system of hydra and answering the following questions: How many different circuits are there in hydra? What type of computation does each of these circuits do, and to what extent are these circuits dependent on each other? Answering these questions will help understand the properties of the most primitive nervous systems and how they produce the appropriate behavioral response to a given situation.

Funding acknowledgement: NIH Grant DP1EY024503; Army Grant ARO W911NF-12-1-0594 (MURI)

Machine Learning Mapping of Behavior in *Hydra vulgaris*

Shuting Han[1], Ekaterina Taralova[1], Rafael Yuste[1]

[1]Neurotechnology Center, Dept. Biological Sciences, Columbia University, New York, NY.

Animal behaviors have been studied for centuries, but there are still few efficient methods available to automatically identify and classify all behaviors of an animal quantitatively. Studies of animal behavior have been limited by the subjective and often imprecise nature of human observation, the intrinsic limitation of the properties of human visual system and the slow speed of annotating behavioral data. Our group recently generated a *Hydra* strain with neuronal transgenic genetically encoded calcium indicators (GCaMP6s), which allows simultaneous optical recording from the entire nervous system while the animal is behaving (Dupre and Yuste, in review). *Hydra* is evolutionally close to the first species that developed a nervous system, therefore studying the relation between behavior and their underlying neural mechanism in *Hydra* could provides a unique opportunity of understanding the most basic rules of how nervous systems compute and organize behaviors. For this purpose, we developed an automatic behavior identification and classification method for *Hydra* using machine learning algorithms. We imaged freely moving *Hydra*, extracted motion and shape features from the resulting videos, and constructed an analysis dictionary of all movements. We identified 10 behavior types using unsupervised embedding analysis methods based on this dictionary, and trained classifiers with manual labels for each behavior type. These findings were confirmed with independent manual annotation. We conclude that machine learning can be use as a general platform for systematic study of *Hydra* behavior quantitatively.

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Building a biomechanical model of *Hydra*

Adrienne Fairhall

University of Washington

Notch signalling is required for tentacle patterning but dispensable for neurogenesis in *Hydractinia echinata*

James M Gahan[1], Christine E Schnitzler[2], Liam B Doonan[1], Justyna Kanska[1], Sofia Barreira[2], Kerry Thompson[3], Philipp Schiffer[4], Andreas D Baxevanis[2], Uri Frank[1]

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[3] Centre for Microscopy and Imaging, Discipline of Anatomy, School of Medicine, National University of Ireland, Galway, Galway, Ireland
[4] Genetics Environment and Evolution, University College London, London, UK

Notch signalling is an evolutionarily conserved juxtacrine cell signalling system which is utilized in diverse contexts such as regulation of boundary formation, neural development, and stem cell-progeny communication. Notch signalling has been studied in both *Nematostella* and *Hydra*, but a consensus on pathway function in cnidarians remains unclear. Pharmacological inhibition of γ-secretase, an essential component of the pathway, has revealed a putative role for Notch signalling in tentacle patterning in both of these cnidarians. Genetic and pharmacological manipulation of the pathway in *Nematostella* has revealed a bilaterian-like role in the development of the nervous system. In *Hydra* polyps, on the other hand, γ-secretase inhibition shows no effect on neurons, but causes defects in nematocyte differentiation. We have utilised pharmacological and genetic approaches in *Hydractinia echinata* to address the role of Notch signalling in both tentacle patterning and neurogenesis. We show that Notch is required for correct tentacle patterning during normal development and regeneration, representing the first genetic evidence for such a role. Neurogenesis, on the other hand, is unaffected by Notch manipulation in both embryogenesis and in the adult. However, we do observe a severe defect in nematocyte differentiation upon γ-secretase inhibition that is not linked to Notch signalling. This loss of Notch function may be related to the evolution of mesenchymal, migratory neural progenitor cells, or i-cells, in hydrozoans from epithelial type neural progenitors, such as those in *Nematostella* and most bilaterian groups. We hypothesize that loss of Notch signalling in neural progenitor cells either facilitated this transition or is a consequence of it.

Cnidarian polymodal sensory-motor neurons as a model for cell type subfunctionalization in the origins of bilaterian complexity

David Plachetzki

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Taste 1 receptors (T1R) are class C G-protein-coupled receptors that mediate sweet and savory taste perception in vertebrates. Current evidence suggests that T1Rs originated in the lineage leading to gnathostomes, but are absent in all agnathan and invertebrate lineages examined to date. Here we present evidence from phylogenomic analyses of 36 holozoan whole genome sequences that orthologs of T1Rs are present in several non-bilaterian genomes including cnidarians, demonstrating a pre-bilaterian origin for this sensory gene family. A corollary of this finding is that T1Rs were independently lost in several lineages including those leading to protostomes, cephalocordates and agnathans. We also describe gene expression studies of a polymodal sensory-motor (PSM) neuronal cell type that coordinates cnidocyte discharge in *Hydra magnipapillata*. We show that transcripts of T1Rs and opsins co-localize to PSM neurons in the *hydra*. In addition, studies of cnidocyte discharge behavior demonstrate that T1Rs and opsins play opposing roles in mediating cnidocyte discharge where T1R signaling is excitatory and opsin-mediated phototransduction is inhibitory. Our findings reorder the current view of the evolutionary history of T1Rs and suggest that this sensory gene family was in fact present prior to the major diversification events in animals, but lost in several lineages independently. In addition, the nature of cnidarian PSM neurons, where both T1Rs and opsins contribute to function, suggests novel hypotheses for the origins of animal sensory neurons and their transduction cascades.

Peduncle nervous system of *Hydra* functions as central nervous system (CNS)

Hiroshi Shimizu, Yukihiko Noro, Katsuhiko Mineta, Takashi Gojobori

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

It has been a general understanding that nervous system started as a diffuse nerve net as we find in *Hydra* and that evolution of body plan from radial symmetry to bilateral symmetry triggered the concentration of neurons as ganglia evolutionarily leading to central nervous system (CNS). Here we present evidence against this common understanding by showing that the nervous system of *Hydra* in the peduncle region shows characteristic features of CNS. Locomotion of *Hydra* takes place in two steps, 1) wobbling movement, 2) subsequent discharge of nematocytes (Nc) termed isorhizas to a distant position thereby changing the position of the animal. We first found that both wobbling movement and discharge of Nc on the tentacles are regulated by the peduncle nervous system. Secondly, we found evidence that *Hydra* shows negative geotaxis (favoring upward locomotion) and that the gravity-sensing organ is located in the head region. Since the gravity altered the pattern of wobbling, it is highly likely that the gravity signal was transmitted to the peduncle nervous system, followed by the processing of the signal there and finally activating the upward locomotion. These observations demonstrate that the peduncle nervous system 1) received gravity signal from the head as an input, 2) emitted motor signal into the peduncle itself and the tentacles as an output. Meanwhile, CNS is functionally defined by a nervous system that receives environmental signal as the input and emits motor signal as the output. These comparisons quite naturally suggest that the peduncle nervous system of *Hydra* is functionally comparable to CNS of bilaterians. Peduncle nervous system includes subsets of Hym-176 group neuropeptides. To examine the involvement of those peptides, functional assay by synthetic peptides is being undertaken. To add to it, anatomical identification of gravity-sensing structure in the head is underway.

Keynote Speaker Abstract – Friday, September 23

Spontaneous contractile behavior in *Hydra* and what controls it: where's the "brain" in a nerve net?

Charles N. David

Ludwig-Maximilians-University, Munich, Germany

Behavior in *Hydra* is controlled by nerve cells organized in a "nerve net" throughout the body column. Classic experiments by Passano and McCullough (1963) associated neural activity with contractile behavior. However, the architecture of the nerve net has been less well investigated due to the absence of a good neural marker. Cloning of the *hydra* cadherin gene by Holstein and Hobmayer (unpublished) and generation of an antibody to the intracellular domain has now permitted high resolution imaging of the nerve net. These results show that there are two distinct nerve nets, one in the endoderm and one in the ectoderm and that, in both nerve nets, nerve cells and nerve cell processes are closely associated with epithelial muscle fibers. The results show, furthermore, that the endodermal and ectodermal nerve nets are not interconnected and that, unexpectedly, that there are no nerve cells in the endoderm of tentacles. Models for the neural control of behavior, in particular the dramatic tentacle behavior associated with feeding, need to include these facts.

Neuroscience on a chip with microfluidic *Hydra* interrogation chambers

K. N. BADHIWALA[1], D. L. GONZALES[2,3], D. G. VERCOSA[2,3], C. DUPRE[4], R. YUSTE[4], J. T. ROBINSON[1,2,3,5]

[1]Bioengineering, [2]Applied Physics Program, [3]Electrical and Computer Engin., Rice Univ., Houston, TX; [4]Biol. Sci. and Neurosci., Columbia Univ., New York, NY; [5]Neurosci., Baylor Col. of Med., Houston, TX

The ability to observe the activity of every neuron as an organism interacts with its environment would reveal more about how the nervous system drives animal behavior – a fundamental goal of neuroscience. To reach this goal, we are developing a scalable microfluidic platform that will allow us to immobilize and interrogate the transparent cnidarian *Hydra*, which has a spiking network of few hundred to thousand of distributed neurons that can be individually imaged using optical microscopy. A major advantage of *Hydra* as a model system for neuroscience is the fact that these millimeter scale invertebrates can be confined to quasi-two-dimensional recording chambers that can be arrayed on a chip. This quasi-2D geometry allows us to perform high-speed volumetric optical imaging and implement a range of microfabricated technologies to control the local environment and record cellular-scale electrical activity. In particular, we show that our platform can combine the high-spatial resolution optical imaging with the high-temporal resolution electrophysiology to track contraction bursts (CB) and tentacles pulses (TP) as they correlate with behavior. Moreover, arrays of nanofabricated electrodes allow us to measure action potential propagation or deliver local electrical stimuli to provoke behavioral responses. Together, the spiking neural network of the *Hydra* combined with the microfabricated interrogation chambers provides a scalable "neuroscience on a chip" for studying the relationship between behavior and whole-brain activity at a single cell level.

Genomics of *Hydractinia*: Characterizing and Determining the Biological Relevance of Highly Repetitive Regions

Sofia Barreira[1], Andreas D. Baxevanis[1]

[1]NHGRI, NIH

Repetitive elements represent around two-thirds of the human genome. Large tandem repeats such as ribosomal genes (44kb), segmental duplications (<130 kb), and telomeric repeats comprise the short arms of acrocentric chromosomes. Importantly, these sequences are usually missing from any genome reference. Establishing their organization and distribution is crucial to fully understand cellular function. To extend these regions, careful approaches are necessary to ensure accurateness of sequence information, orientation, and placement. Using these kinds of approaches, we have successfully extended the sequence content after the last human ribosomal gene (rDNA) repeat on the telomere side by 550 kb. Currently, our group is sequencing and assembling the genomes of two *Hydractinia* species, *Hydractinia echinata* and *Hydractinia symbiolongicarpus*. *Hydractinia*, a colonial marine, initially studied for early developmental processes, is positioning itself as a valuable model organism for regenerative medicine research. *Hydractinia* stem cells are pluripotent and many genes associated with the ability to self-renew and differentiate have been identified, reinforcing its value in the study of regeneration. *Hydractinia* also enables *in vivo* experiments, is easy to grow and maintain in the lab, and is not hindered by ethical concerns. Since the overall repeat and AT-content of *Hydractinia* is quite high (47% and 65%), we intend to apply similar strategies as those used with the human genome sequence to identify important repetitive regions such as centromeres, telomeres, and rDNA in these *de novo* assemblies. This will enable us to offer a more complete assembly than those of any other available model organism and provide a foundation for better understanding development, genetic variation, and key metabolic pathways. *Hydractinia* presents the opportunity to advance basic research efforts focused on a variety of human diseases and the development of new clinical approaches that improve human health.

Loss of PIWI-piRNA Pathway Function in *Hydra* Results in the Upregulation of Early Regeneration Genes

Jack Cazet[1], Stefan Siebert[1], and Celina Juliano[1]

[1]Department of Molecular and Cellular Biology, University of California Davis, Davis, California

The small cnidarian polyp *Hydra vulgaris* is well known for its remarkable ability to regenerate virtually any part of its body; however, the molecular mechanisms underlying *Hydra*'s exceptional healing capacity are not well understood. The PIWI-piRNA pathway is a highly conserved mechanism for repressing gene expression that is most commonly associated with germ cells, but is expressed in both germline competent and somatic stem cells in Hydra. Recent studies have shown that some animals typically lacking somatic piwi expression transiently induce piwi expression in regenerating tissue, suggesting that piwi expression may play a conserved role in the regeneration process. Currently, a mechanistic understanding of how the PIWI-piRNA pathway contributes to regeneration is elusive. To better understand the role of the PIWI-piRNA pathway in hydra, we performed differential gene expression (DGE) analysis on 4-day old wild type and piwi knock down hydra hatchlings. We found that piwi knock down results in the upregulation of over 700 transcripts, including at least 25 transcripts known to be associated with the early regeneration response in both planarians and *Hydra*. The DGE analysis also revealed that these ~700 upregulated genes have lower relative expression in tissues expressing piwi, consistent with the hypothesis that they are downregulated via the PIWI-piRNA pathway. Because piwi genes are downregulated early in regeneration in both *Hydra* and *S. mediterranea*, and because several genes that are upregulated during regeneration are also upregulated in hywi KD animals, we hypothesize that the PIWI-piRNA pathway may have a conserved function in regulating regeneration through the direct targeting of transcripts required for the early stages of the regeneration response. Future research will aim to characterize the pathway through which the PIWI-piRNA pathway modulates the expression of regeneration genes.

Detecting protein-protein interactions between *Hydractinia* allorecognition proteins with an ELISA-based assay

Aidan L. Huene, Matthew L. Nicotra

University of Pittsburgh, Pittsburgh, PA

Allorecognition is the ability to distinguish between self and genetically distinct members of the same species. In the hydroid *Hydractinia*, allorecognition determines whether colonies aggressively compete for space or fuse to form a single colony when they encounter each other as they grow. Previous studies in inbred lines of *Hydractinia* have determined that allorecognition is controlled by a genomic region called the Allorecognition Complex (ARC) which contains at least two allorecognition genes. These genes (Alr1 and Alr2) are highly polymorphic, encode transmembrane proteins and have extracellular domains similar to IgSF-like genes. Using an *in vitro* assay, we have examined four Alr1 and six Alr2 alleles and determined each selectively binds across opposing cell membranes to itself or a nearly identical isoform. In nature, more than 180 allelic isoforms of Alr2 have been documented, and Alr1 is expected to be similarly diverse. If the majority of these isoforms are similarly capable of isoform-specific homophilic binding, and if this binding is required for colonies to fuse, it would provide a mechanistic explanation for the very low rates of fusion (~2%) observed in wild-type colonies. A related question is how sequence diversity contributes to unique binding specificities. By testing isoforms in a pairwise format, the binding specificity locales can be identified. To efficiently screen the binding properties of these known Alr1/2 alleles, and to identify the regions of the Alr ectodomains that contribute to binding specificity, we are developing an ELISA-like high-throughput assay that relies on recombinant proteins expressed in either *Drosophila* or mammalian cell lines. Here we describe preliminary data on the production and purification of these proteins and optimization of the binding assay.

Cnidarian Chatter: Implications of Potassium Channel Evolution in Early Neural Networks

Adolfo Lara[1], Jason Macrander[2], Marymegan Daly[2], and Joseph Ryan[3]

[1]Department of Invertebrate Zoology, Richard Gilder Graduate School at the American Museum of Natural History, New York, USA
[2]Museum of Biological Diversity, The Ohio State University, Ohio, USA
[3]Whitney Laboratory for Marine Bioscience, University of Florida, USA

Cnidarians, a phylum including sea anemones, jellyfish, and their relatives, are the sister lineage to bilaterians (a group representing 99% of all described animal species). Unlike bilaterians, which usually have a centralized nervous system (CNS), most cnidarians have an organized neural network and therefore provide a unique window illuminating into the origin and evolution of the CNS in animals. Extensive nervous network and system studies are available for bilaterians such as roundworms, fruit flies, and mice, but there is far less information available about the nervous networks of cnidarians. With this in mind, we applied bioinformatic techniques to survey a combination of published and newly generated transcriptomic and genomic cnidarian data, representing 56 species, to phylogenetically describe the distribution and variety of potassium channels within Cnidaria. We identified 3 out of 4 representatives of potassium channel families, in addition to pseudogenes, in these 56 species (Shal, Shaw and Shaker). Given their role in neuron repolarization following an action potential, the implications of potassium channel evolution regarding early cnidarian and cnidarian-bilaterian ancestor neural network properties will be discussed.

How ECM dynamics influence *Hydra* regeneration from cellular aggregates

Tiffany Locke[1], Olivier Cochet-Escartin[2], Rui Wang[3], Robert E. Steele[4], and Eva-Maria S. Collins[1,2]

[1]Division of Biological Sciences, University of California, San Diego
[2]Department of Physics, University of California, San Diego
[3]Department of Bioengineering, University of California, San Diego
[4]Department of Biological Chemistry, and Developmental Biology Center, University of California, Irvine

The extracellular matrix (ECM) is a fundamental component for the interplay between mechanical cues and molecular signaling for pattern formation in development and regeneration. However, little is known about how the precise timing of ECM formation and ECM-cell communication influences cell behaviors. *Hydra* is a unique model of study the role of the ECM for pattern formation because its regenerative capacities allow for the observation of *in vivo* pattern formation in the simple context of cellular aggregates. In addition, *Hydra* is anatomically simple and consists of two epithelia separated by an experimentally accessible ECM layer. We therefore use Hydra cellular aggregates to study the dynamics of cell-ECM interactions and their effect on morphogenesis during cell sorting and axis formation. Through the generation of transgenic laminin-GFP *Hydra* lines and usage of 2-photon time-lapse microscopy and immunohistochemistry, we test the hypothesis that ECM formation causes a "phase transition" in cellular aggregates from a liquid-like to a solid-like state, which is indispensable for successful regeneration.

This research is funded by NSF grant CMMI-1463572 and the Research Corporation for Science Advancement.

Characterizing expression patterns of PIWI-piRNA pathway genes in *Hydra* stem cell

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Hydra vulgaris has an extremely long life span, with adult stem cells showing no evidence of senescence. *Hydra* contains three cell lineages that are each supported by a specific stem cell population. The two somatic epithelial lineages, endoderm and ectoderm, are each supported by a unipotent stem cell population. By contrast, the interstitial cell lineage, which gives rise to both somatic cells and germ cells, is supported by a multipotent stem cell population. The PIWI-piRNA pathway functions in all three stem cell types. The pathway has a conserved function in the germline as a transposon repressor, however the pathway function in the somatic stem cells of any animal is still not well understood. We find that the PIWI-piRNA pathway is essential in the epithelial lineages of *Hydra*. In order to shed light on PIWI-piRNA pathway function in both epithelial stem cells and multipotent interstitial stem cells in *Hydra*, we aim to identify the expression patterns of conserved PIWI-piRNA pathway genes via in situ hybridization. Studying the expression patterns of these genes will elucidate both similarities and differences between PIWI-piRNA pathway function in somatic vs. germline-competent stem cells. In addition, we are establishing CRISPR as a genome editing tool in *Hydra*. With CRISPR, we will be able to manipulate and test the function of the genes involved in the PIWI-piRNA pathway. Our ultimate goal is to better understand how the PIWI-piRNA pathway functions in *Hydra* stem cells and how it may contribute to its long-lived life span.

Gene expression patterns in siphonophore zooids

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Siphonophores are pelagic colonial hydrozoans that consist of genetically identical zooids that are thought to be homologous to solitary polyps and medusae. We are interested in identifying evolutionary shifts in differential gene expression within Siphonophora, to identify expression that is associated with zooid identity within species, as well as between species. Here we present preliminary results from a comparative gene expression study, looking at expression differences between different zooid types (at different developmental stages) between multiple species. Within species, we find significant expression similarities between hypothesised polyp-like and medusa-like zooids.

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Piwi-piRNA pathway function in somatic stem cells of *Hydra*

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PIWI proteins are central players in a RNA regulatory pathway that is largely specific to the germ line and stem cells. The function of the PIWI-piRNA pathway and the identity of the pathway target genes in stem cells is, however, not well understood. Here we study the function of the *Hydra* PIWI homolog *hywi* in stem cell maintenance within somatic stem cells. Knocking down *hywi* in *Hydra* epithelial stem cells is lethal. RNA-seq and differential gene expression analysis comparing transcript levels between 4-day old *hywi* RNAi juveniles and age-matched wild type siblings indicates that the PIWI-piRNA pathway represses RNA expression in these stem cells. Tissue and lineage specific RNAseq reveals that a significant fraction of the upregulated genes in RNAi animals are expressed within the differentiated cells of wild type animals. These findings lend support to the hypothesis that *hywi* is required to maintain somatic stem cells by repressing differentiation genes. Furthermore, the upregulated genes include many that have been identified as injury response genes in regeneration studies. This suggests that *hywi* acts upstream of a gene set that is activated during cell differentiation in wild type animals and that can be ectopically triggered by regeneration cues.

Comparison of the role of the Runx gene during regeneration in *Nematostella*, *Hydra* and *Hydractinia*

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Cnidarians have traditionally been used as a model system to study regeneration. Regeneration in *Hydra* has been well characterized and often cited as a classic case of morphallaxis, in which regeneration occurs through the re-differentiation of existing tissue and in the absence of cell proliferation (Morgan, 1901). By contrast, the sea anemone *Nematostella* appears to undergo regeneration by epimorphosis, which involves the development of an undifferentiated blastema that undergoes cell proliferation, followed by cellular differentiation (Passamaneck and Martindale, 2012). The colonial cnidarian, Hydractinia appears to regenerate by utilizing cell proliferation (epimorphosis) in conjunction with cell migration (Bradshaw et al., 2015). Given their diverse patterns of regeneration, *Nematostella*, *Hydractinia*, and *Hydra* are ideal models to investigate, in a comparative context, the respective roles, regulation, and timing of cell proliferation, cell migration and differentiation during regeneration. It has been noted that in many animal systems, activity of the conserved transcriptional factor Runx increases during developmental transitions from cell proliferation to differentiation (Coffman, 2003). To investigate whether the differences in regeneration processes between the three model species involve evolutionary changes in Runx activity, we treated regenerating animals with the Runx inhibitor Ro5-3335 and founds that it appears to delay regeneration in *Nematostella* and *Hydractinia*, whereas it has no apparent effect in *Hydra*. This is consistent the role of Runx in regulating cell proliferation during the transition to cellular differentiation. Using the CRISPR/Cas9 system, I am generating conditional mutants and reporter lines to further investigate the role of Runx in *Nematostella* regeneration.

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The transcriptome of inducible aging in *Hydra oligactis*

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Our work on induced aging in *H. oligactis* has established that 40% of induced animals do not age but instead revert to a non-aging phenotype. To look at the molecular mechanisms that regulate aging we have analyzed transcriptomes of both groups (aging versus non-aging, revertant hydra). Our analyses identified 1357 transcripts differentially expressed between revertant and aging *hydra*. A total of 42 transposon-associated sequences are upregulated in aging *hydra*, representing numerous transposon families, including Gypsy, Line-1, Tcb1, IS4, Tigger, and Piggybac. Transposon activation has been associated with aging in diverse taxa, including *Drosophila*, mice, and worms. The dramatically higher expression of transposon sequences in aging hydra may suggest differences in the chromatin organization of the aging and the non-aging animals.

Physical mechanisms of cell sorting during *Hydra* regeneration from aggregates

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How patterns emerge from an initially homogenous cluster of cells is a fundamental question in developmental biology. *Hydra* is a powerful model for studying mechanisms driving pattern formation because of its ability to regenerate after dissociation into individual cells. The first step of regeneration from cell aggregates involves segregation of the heterogeneous mixture of two epithelial cell types into their respective tissue layers, a process referred to as cell sorting.

Two competing theories have been proposed to explain this sorting: the differential cell migration hypothesis and the differential adhesion hypothesis (DAH). The first attributes cell sorting to differences in cell motility of the two tissue types, the second to differences in effective surface tensions of the two tissues, similar to the breaking up of an emulsion of immiscible liquids. Using 2-photon time-lapse microscopy of 3D cellular aggregates, we show that no difference exists in the motility of the two types of epithelial cells, rejecting the differential cell migration hypothesis. In contrast, our data favor the DAH since we find that (a) cell aggregates exhibit fluid-like behaviors on long time scales, justifying the concept of tissue surface tension, and (b) cell sorting dynamics seem to mimic the breaking up of an emulsion. Finally, quantification of effective surface tensions using tissue rheology allows us to directly test the DAH as the underlying mechanism for *Hydra* cell sorting.

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Fat and Dachsous cadherins in *Hydra* development

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Planar cell polarity (PCP) is a form of tissue organization of cells within the plane of a tissue, perpendicular to the apical-basal axis. Fat and Dachsous (Ds) are atypical cadherins and the major components of Fat/Ds PCP pathway. We have identified hydra homologues of Fat-like (HyFat) and Ds (HyDs) proteins and cloned their intracellular domains (ICD). Polyclonal antibodies against the ICD of HyFat show that HyFat is localized at apical junctions of ectodermal epithelial cells throughout the animal. The protein is more abundant in the head and in the body column regions and less abundant in the foot. We have generated transgenic *hydras* (shFat9) bearing shRNA for hyFat. In situ hybridization and Western analysis show the decrease of both hyFat mRNA and HyFat protein expression in the transgenic animals. shFat9 hydras have several features: elongated shape of the body column, irregular shape of both ecto- and endo- epithelial cells, high content of interstitial cells, increased inductive capacity of the body column. That suggests that hyFat has a role in patterning process in hydra. Currently, we are investigating whether shFat9 *hydras* have defects in PCP as well.

Comparative genomics of *Hydractinia* and *Hydra*

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Comparative genomic approaches applied to cnidarian genomes can provide a powerful framework for exploring fundamental questions about the mechanisms and evolution of complex biological processes such as embryonic development, regeneration, self-recognition, and aging. We have chosen to focus on the colonial cnidarian *Hydractinia*, a hydrozoan representative that has lost the medusa stage and produces gametes directly from sexual polyps known as gonozooids. *Hydractinia* forms colonies of clonal polyps interconnected through a stolonal network and, in nature, colonies are typically found on shells inhabited by hermit crabs. We formed an international consortium and, in collaboration with the NIH Intramural Sequencing Center, we generated high-quality, high-coverage genome assemblies for *H. echinata* and its sister species, *H. symbiolongicarpus*, using PacBio and Illumina sequence data. The estimated genome size is 774 Mb for *H. echinata* and 514 Mb for *H. symbiolongicarpus*. Similar to *Hydra*, both genomes are AT-rich (65%) and highly repetitive (at least 47%). We also assembled and annotated the transcriptomes of both species as a first step towards generating comprehensive gene models for these genomes. We are using a comparative genomics approach where we (1) assess differences in gene content when compared to a recently updated high-quality *Hydra* Dovetail genome assembly; (2) explore regions of possible synteny among the genomes; and (3) compare non-coding RNAs, classes of repetitive elements, and conserved gene families. Interesting themes have begun to emerge in each of these areas that will ultimately reveal both the conserved features and extensive evolutionary novelties contained within these model hydrozoan genomes.

Investigating the *in vivo* function of *Hydractinia* allorecognition proteins using CRISPR/Cas9 genome editing

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Allorecognition is the ability to distinguish self-tissues and those of conspecifics. Many marine invertebrates are capable of allorecognition. In the colonial marine hydroid *Hydractinia symbiolongicarpus*, the alloresponse occurs after two colonies grow into contact with one another. Colonies will either fuse with one another, reject and fight, or perform some intermediate of the two (known as a transitory fusion). These alloresponses are controlled by two linked, highly polymorphic genes, Alr1 (Allorecognition 1) and Alr2. Both Alr1 and Alr2 encode single-pass transmembrane proteins with a hyper-variable ectodomain containing two or three IgSF-like domains. These genes also contain putative signaling motifs along their rather long cytoplasmic tails (~150 and 220 amino acids for Alr1 and Alr2, respectively). Recent work using a heterologous in vitro assay has shown that these allorecognition proteins will only bind with histocompatible alleles across the cell surface (allele-specific homophilic binding). While this gives us a compelling model for how Alr1 and Alr2 might regulate allorecognition, there has yet to be any work on the function of these proteins *in vivo*. To begin testing the function of these genes in *Hydractinia*, we have generated functional knockouts of Alr2 in an attempt to change the phenotype of a given alloresponse. These knockouts were created using CRISPR/Cas9 genome editing. Alr2 alleles with targeted deletions of multiple exons were passed through the germline and bred into different haplotype backgrounds to generate hemi- and homozygous knockout lines to test for changes in histocompatibility. By studying these animals, we will be able to begin unraveling the *in vivo* function of these proteins in regulating *Hydractinia* allorecognition.

Does *Hydra* remember its foes?

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The ability to respond more efficiently to previously encountered infections has been detected in many metazoans that do not possess adaptive immune systems and solely rely on innate immune reactions. These phenomena are described under the umbrella term 'Trained Immunity'. They confer protection against secondary infection after a priming event, often cross-protecting against other infections uses mechanisms distinct from adaptive immunological memory. Two main mechanisms underlying Trained Immunity have been suggested, the first being a quantitative enhancement of the immune response at the time of reinfection, the second being qualitative due to somatic diversification. However, clear demonstrations of these mechanisms in invertebrates rarely exist in the literature, and these two broad mechanisms may act in concert.

In the current study we tested whether the most basically branched animal model for innate immunity, *Hydra vulgaris* employs trained immunity to overcome infections with the Gram-negative gamma-proteobacterium, *Pseudomonas aeruginosa*. Solely relying on an epithelial immune system, *Hydra* species, including *H. vulgaris* can mount a robust immune response to microbe-associated molecular patterns. However, *H. vulgaris* eventually succumbs to high doses ($1x10^{12}$ cells/ml) of *Pseudomonas aeruginosa*. *P. aeruginosa* infection causes pathology, and disease progresses in five distinct quantifiable stages starting with tentacle swelling, followed by tentacle shortening and tentacle loss, which progresses to loss of body shape, and ultimately tissue lysis and death of the polyp. Onset and speed of disease progression was highly repeatable, and positively correlated with initial infectious dose of *P. aeruginosa*. However, prior 24h-long exposure with a 5-fold lower dose of the same bacterial species significantly slowed disease progression between stage 2-4. In addition, extended median survival by one day (Gehan-Breslow-Wilcoxon test, P=0.0008). Priming did not require a live infection, as exposure to the equivalent dose with heat-killed *P. aeruginosa* equally extended the life span of *H. vulgaris*.

The data present the first evidence of trained immunity in *H. vulgaris*, and suggest that a recall response is an ancient component of the immune system of all animals. Future studies will focus on elucidating the mechanisms underlying trained immunity in *H. vulgaris*.

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Cellular Membrane Binding and Cytolytic Function of *Hydra* HALT-1

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Hydra is a venomous animal producing multiple toxins and uses these toxins to catch preys and defend itself from predators. Many toxins are stored in the stinging capsule named nematocyst. One of these nematocyst toxins is a small protein of 20 kDa with hemolytic and cytolytic activity. It is later known as the pore-forming toxin. A total of seven actinoporins, namely HALTs 1-7 (*Hydra* Actionoporin-Like Toxins 1-7), have been isolated from *Hydra*. Among HALTs, we primarily studied HALT-1 on its molecular mechanism of pore formation. Site-directed mutagenesis produced ten HALT-1 mutants and as compared to the wild-type which lysed 50% of human cells at 15 μg/mL, HALT-1 mutants reduced or lost their cytolytic effect even the concentration was raised to 30 μg/mL. This suggested two functionally important regions in HALT-1, the N-terminal domain and the aromatic amino acid cluster. Further investigation of HALT-1 using dot-blot assay and LC MS/MS gave intriguing results whereby, unlike other actinoporins which bind sphingomyelin, HALT-1 may have alternative binding targets on the human cell membrane. Due to its high affinity to human cells, HALT-1 can be an ideal therapeutic agent for anti-inflammatory treatment. We constructed an immunotoxin, which contains a toxin moiety (HALT-1) that works to lyse cells and an antibody moiety that targets a surface molecule of macrophages. Our approach was to utilize HALT-1 to kill inflammatory macrophages and thereby control the inflammatory progression in rheumatoid arthritis.

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Host and symbiont cell division in the symbiotic anemone *Aiptasia pallida*

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The cellular coordination of a host and its symbiont is a fundamental and understudied component of endosymbiosis. To understand coordination in the context of cnidarian-dinoflagellate symbioses, we examined cellular division concurrently in two partners, the host cnidarian *Aiptasia pallida* and its resident algal symbiont Symbiodinium B1. Within this partnership, we observed host and symbiont cell division as a response to different conditions of symbiosis, temperature, light, and nutrition. To look at host division, symbiotic and aposymbiotic (without symbionts) *Aiptasia* were labeled with S-phase marker ethynyl deoxyuridine (EdU), G2/M-phase marker histone H3pSer10, and Hoechst dye, then imaged using confocal microscopy. To measure symbiont division, Symbiodinium was isolated from *Aiptasia* and labeled using Propidium Iodide, then analyzed via flow cytometry. Aposymbiotic *Aiptasia* had significantly higher proportions of S-phase cells in both gastrodermis and epidermis, indicating that the presence of symbionts may slow cell proliferation but not overall host biomass. Symbiotic *Aiptasia* also responded to increased temperature but not to changes in light-dark cycles. *Aiptasia* subjected to 33C experienced a temporary decrease in mitotic cell division compared to 25C controls after 4 hours of treatment. Neither *Aiptasia* nor in hospite Symbiodinium cell division responded to changes in light in a manner consistent with a diel cycle, based on 1-day and 2-day experiments. Finally, we performed long-term nutrition experiments to compare fed and starved anemones. Feeding had a positive effect on host size and pedal laceration. Despite these phenotypes, there was no difference in cell division between fed and starved groups when normalized by area or total nuclei. In comparison, the percent of Symbiodinium undergoing S-phase was significantly higher in fed compared to starved *Aiptasia*. These data indicate a complex relationship where host and symbiont cell cycle modulation is but one response to basic environmental cues.

DEVELOPING TRANSGENIC TOOLS TO STUDY CNIDARIAN-DINOFLAGELLATE SYMBIOSIS IN A SEA-ANEMONE MODEL SYSTEM

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Transcriptomic studies in corals and other cnidarians have identified many intriguing gene-expression changes that are correlated with the establishment and breakdown of symbiosis. However, the molecular bases of these processes remain poorly understood, in part because of the lack of a tractable genetic system to allow rigorous functional testing of candidate genes and pathways. Like corals, the small sea anemone *Aiptasia* is symbiotic with dinoflagellates in the genus Symbiodinium, and it has many experimental advantages, including a high – but as yet unrealized – potential for the needed genetic studies. As a first step in developing transgenic methods for *Aiptasia*, we have successfully expressed the photoconvertible Kaede fluorescent protein in larvae by microinjection of capped mRNA into 1-cell zygotes. This technique should allow both expression of tagged proteins for localization studies and the overexpression of candidate genes to analyze gain-of-function phenotypes. To analyze loss-of-function phenotypes in *Aiptasia*, we are taking two approaches. First, we have microinjected zygotes with translation-blocking morpholinos targeting the FGF1a gene, which is required for apical-tuft formation in a related cnidarian, as a proof-of-principle. Preliminary results show apparent loss of apical-tuft formation in successfully injected larvae, suggesting that the Fgf1a protein was effectively knocked down. In parallel, we are also using FGF1a for proof-of-principle experiments using CRISPR-Cas9 for gene knockout. If both gain-of-function and loss-of-function methods can be established, *Aiptasia* should become a uniquely powerful genetic model system for the study of cnidarian-Symbiodinium symbiosis.

We thank the Simons Foundation for supporting these studies.

The Interdependence of Viruses and the Holobiont

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Animals live in symbiosis with the microorganisms surrounding them. This symbiosis is necessary for animal health, while dysbiosis leads to a disease state. The functional symbiosis between the host, prokaryotes, eukaryotes, and viruses is called the holobiont. Deciphering these functional holobiont associations has proven to be both difficult and controversial. *Hydra* has shown to be an effective model to deconstruct these interactions with its simple body plan, limited number of microbial partners, and conserved immune regulatory pathways. Holobiont association with viruses is also controversial, though these interactions have been occurring since the dawn of animal life. The controversy derives from the idea that all viruses are parasitic, yet they can also be commensal and/or mutualistic. The goal of this project is to identify viral populations associating with different species of *Hydra* and to determine the function of these viruses on the *Hydra* holobiont. Viral metagenome (Virome) analyses allows the identification of the communities of eukaryotic and prokaryotic viruses that functionally associate with the *Hydra* holobiont. Transcriptional analyses of *Hydra* in response to viral presence allows the elucidation of how *Hydra* mediate these interactions. This research has found that different species of *Hydra* associate with distinct families of viruses, that these viral associations have both positive and negative impacts on the metabolism of *Hydra*, and that *Hydra* regulate viral interactions using its innate immune system. Ongoing research focuses on the genetic interactions between associated viruses and *Hydra* within the context of the holobiont.

A new model system for studying the causes of coral reef bleaching

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In the marine environment, there are many cnidarians (e.g. corals, sea anemones, and "jellyfish) that form a symbiotic relationship with dinoflagellates in the genus Symbiodinium. Among the most recognized group of cnidarians that maintain this relationship with these dinoflagellates, (aka 'zooxanthellae') are corals. Coral reefs are widely recognized as biodiversity "hotspots." Unfortunately, a wide variety of stressors threaten the existence of symbiotic relationships within cnidarians, due to a phenomenon known as bleaching. Bleaching is the expulsion of zooxanthellae out of the host species cells, and it is typically linked to a thermal stress or low light levels. Because working with corals can be problematic and many are already endangered species, we are developing a new bleaching model system, the scyphozoan species *Cassiopea xamachana* (the "upside down jellyfish). We can raise it through its entire life cycle in the lab, and are assessing the adaptive potential of these species in regards to a bleaching and evaluating how different stressors effect the quantity of symbiont cells in a host. The stressors that C. xamachana medusa will be subjected to are: 1) Cold shock 2) Complete darkness, and 3) Heat shock. Following bleaching, symbionts will be reintroduced to adult medusa while simultaneously introducing zooxanthellae to *C. xamachana* polyps which are aposymbiotic. Transcriptome comparisons of reintroductions between medusa and polyps will be conducted in future studies, along with experimental studies that focus on zooxanthellae that have been exposed to a stressor prior to reintroduction and their ability after exposure to infect *C. xamachana* hosts.

Functional Imaging of Compartmentalized Hym-176-related Peptidergic Neurons

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Understanding how the brain works is one of the most challenging goals in modern science. Although *Hydra* is believed to have no so-called brain, its simple nervous system and pattern of behaviors would be a good advantage to address this issue.

Our former studies have shown some basic behaviors of *hydra*; (1) peristaltic movements to digest food in the gastric region, (2) pumping movements to circulate nutrients in the gastrovascular cavity, and (3) wobbling behavior regulated by peduncle nervous system. However neurobiological bases that enable these locally regulated basic behaviors remain unknown at present.

On the other hand we also previously showed that the nerve net of *Hydra* is divided into several subpopulations, each of which expresses specific combination of the neuropeptide Hym-176 gene and its paralogues, and that they are aligned along the oral-aboral axis with clear boundaries. These features imply that the localized neuron subsets in *Hydra* are neural compartments and they behave as sort of functional units like those of higher organisms.

Therefore to test the possibility that different behaviors may be controlled by different subsets of neurons, we tried to see whether or how the Hym-176-related neuron subsets are functionally involved in the basic behaviors shown above by activating them with the corresponding synthetic neuropeptides, by suppressing their function with CRISPR, and by observing their activity through the functional imaging of the neuron subsets.

In this presentation we provide the latest updates of one of these trials, raising and characterizing of transgenic *hydra*, where calcium-sensing green fluorescent protein is expressed in each neuron subset of compartmentalized Hym-176-related peptidergic neurons. We hope these transgenic animals will be good tools to decode the evolutionally primitive neuron-behavior interaction expected in *Hydra*.

Gene Co-expression Modules Underlying Polymorphic and Monomorphic Zooids in the Colonial Hydrozoan, *Hydractinia symbiolongicarpus*

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Advances in sequencing technology have forced a quantitative revolution in Evolutionary Biology. One important feature of this renaissance is that comprehensive genomic resources can be obtained quickly for almost any taxon, thus speeding the development of new model organisms. Here, we analyze 20 RNA-seq libraries from morphologically, sexually, and genetically distinct polyp types from the gonochoristic colonial hydrozoan, *Hydractinia symbiolongicarpus* (Cnidaria). Analyses of these data using weighted gene co-expression networks highlight deeply conserved genetic elements of animal spermatogenesis and other organismal processes and demonstrate the utility of these methods in identifying modules of genes that correlate with different zooid types across various statistical contrasts. RNA-seq data and analytical scripts described here are deposited in publicly available databases.

Investigation of Mechanically Sensitive Ion Channels in *Hydra*

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Perception of mechanical stimuli has shown crucial roles in sensing pain, hearing, and touch as well as regulation of blood pressure and embryonic development. Mechanically sensitive ion channels are gated directly by force and convert the mechanical stimuli into an electrical signal that can propagate through the organism or trigger secondary messengers. The Cnidarian *Hydra* has a simple diffused nervous system enabling one to study the role of mechanically sensitive ion channels with minimal complexity. Additionally, *Hydra* demonstrates an easily observable contractile response to mechanical stimuli indicating presence of mechanotransduction sensors with large-scale behavioral effects.

Recent advances in transgenic modification, genetic interference, and in-situ hybridization of *Hydra* have enabled researchers to visualize and modulate expression patterns of specific genes within the organism. Based on mechanically sensitive ion channels described in other species and the transcriptome of *Hydra*, we aim to target proteins likely to demonstrate mechanotransduction in *hydra* and visualize their expression pattern throughout the animal. We can then modulate their expression to elucidate the importance of such proteins in *Hydra* behavior and response to mechanical stimuli. The simplistic nervous system of the *Hydra* enables us to also investigate the effect of mechanical stimuli in electrical signal propagation resulting in an observable contractile response.

Light modulated cnidocyte firing predates the evolution of eyes in Cnidaria (Metazoa)

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Massachusetts Institute of Technology, Cambridge
University of Queensland, Brisbane

Understanding the evolution of complex structures like eyes has long fascinated biologists. Although many assume that eyes derive from simpler precursors, this hypothesis has rarely been demonstrated with explicit phylogenetic methods. We examine the hypothesis that light sensing components of eyes in Cnidaria had an earlier history, and were used in simpler functions. Light sensing structures are common among medusozoans, in which they vary from pigment spots to pigment cup ocelli and complex eyes. Molecular, behavioral and pharmacological evidence indicate that light modulates cnidocyte discharge in the hydrozoan *Hydra vulgaris* Pallas, 1976. By surveying literature and incorporating new experimental data, we test two predictions of the hypothesis that light sensing functions of eyes had an earlier role in modulating cnidocytes. First, we predict that light modulated cnidocyte firing can be reconstructed as an ancestral state. Second, we predict that eyes assembled later in the history of Cnidaria. We show that three lineages of non-medusozoans (Actiniaria, Octocorallia, and Corallimorpharia) exhibit significant difference between the numbers of nematocysts discharged under two blue light (470 nm) intensities (dim, 0.1μW/cm^2; bright, 2.8μW/cm^2). Based on a working phylogenetic hypothesis, we also show that eyes and ocelli were absent from the cnidarian ancestor. Our results suggest that cnidocyte discharge regulation by light predates the emergence of light sensing organs, and both potentially use a homologous opsin-based phototransduction pathway. As such, this represents an empirical case in which an opsin-based behavior might have predated this pathway's function in light sensing organs as complex eyes.

Mechanisms of PIWI-piRNA Pathway Function in *Hydra* Somatic Stem Cells

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The potency and self-renewal capacity of *Hydra* somatic stem cells remains undiminished over long periods of time. The PIWI-piRNA pathway, a small RNA pathway, is active in *Hydra* somatic stem cells. This pathway typically functions in animal germ lines, but also functions in the stem cells of some highly regenerative animals such as *Hydra*. However, the function of the PIWI-piRNA pathway in somatic stem cells remains poorly understood. At the core of the PIWI-piRNA pathway are PIWI proteins bound to small RNAs, called piRNAs. In the germline, the PIWI-piRNA complexes repress transposons transcriptionally in the nucleus and post-transcriptionally in the cytoplasm via base pairing between mRNAs and piRNAs. Recent data from our lab shows increased expression of non-transposon genes in *Hydra* somatic stem cells in response to piwi knockdown. Therefore, I hypothesize that the PIWI-piRNA pathway silences non-transposon mRNAs in *Hydra* somatic stem cells and that this is accomplished by PIWI-piRNA directed cleavage of mRNA targets. To test my hypothesis, I will use crosslinking, ligation, and sequencing of hybrids (CLASH) to directly identify PIWI-piRNA targets and piRNA recognition elements (piREs) within mRNA targets. I will then test whether the targets identified by CLASH are silenced by RNA cleavage using an in vitro cleavage assay. The goal of my research is to reveal the mechanisms by which the PIWI-piRNA pathway contributes to the extreme self-renewal capacity of *Hydra* somatic stem cells.

An apparent role for photosynthesis in symbiosis establishment and/or maintenance in cnidarians

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[2]Carnegie Institution for Science

The mechanisms by which cnidarians recognize and take up their Symbiodinium endosymbionts remain largely unknown and may involve the physical, chemical, and/or metabolic properties of the algae. When some Symbiodinium strains are grown in glucose-containing medium, they undergo a loss of peridinin and chlorophyll pigments, a reduction in photosynthetic activity, and alterations to the cell surface. In this study, we used the sea-anemone model system *Aiptasia* to ask if these changes in the algae affect their uptake by a host. In comparison to algae grown without glucose, the glucose-grown algae were taken up significantly more slowly, if at all, by both aposymbiotic adults and larvae, even when no glucose was present in the seawater during infection. To ask if this effect reflected the reduction in algal photosynthesis, we attempted to infect adult, aposymbiotic animals with algae that had been cultured in the light without glucose, but performing the infections either in the dark or in the presence of 25 μM DCMU, a specific inhibitor of photosystem II activity. In both cases, little or no algal uptake was observed. Similar results were obtained with larvae. Taken together, these results suggest that although other factors (such as recognition of algal cell-surface markers by host receptors) may also be involved, algal photosynthesis plays a key role in initiation, establishment, and/or maintenance of cnidarian-Symbiodinium symbiosis.

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Symmetry breaking during *Hydra* regeneration from aggregates

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The freshwater cnidarian *Hydra vulgaris* is capable of regenerating from spherical cell aggregates and tissue pieces ("spheres") which seem to lack axial polarity. This implies that *Hydra* spheres break symmetry *de novo*, but the mechanisms by which this is achieved on a cellular level are unknown. Symmetry breaking is linked to mechanical oscillations of the sphere, i.e. cycles of swelling and rupture, which are driven by osmotic pressure (Kuecken et al., 2008). It has been suggested that symmetry breaking and elongation in the direction of the future body axis are induced by rupture, but a direct correlation between rupture site and head induction remains to be shown. Using a custom-built imaging apparatus we are testing the hypothesis that the location of the rupture site is correlated with Wnt3 expression and hypostome formation. To control the direction of axis formation, we have adapted a published method of exposing *Hydra* spheres to a temperature gradient during regeneration, since it was shown that the oral-aboral axis forms along such a gradient (Soriano et al., 2006). By fixing the direction of axis formation, we are able to image rupture and head formation with high spatial resolution. Our instrument uses Peltier heaters to create a temperature gradient under tissue pieces in agarose wells. A camera and optics are mounted above the sample dish on a linear rail and are computer-controlled, enabling simultaneous imaging of multiple samples with both brightfield and fluorescence over the entire time of regeneration.

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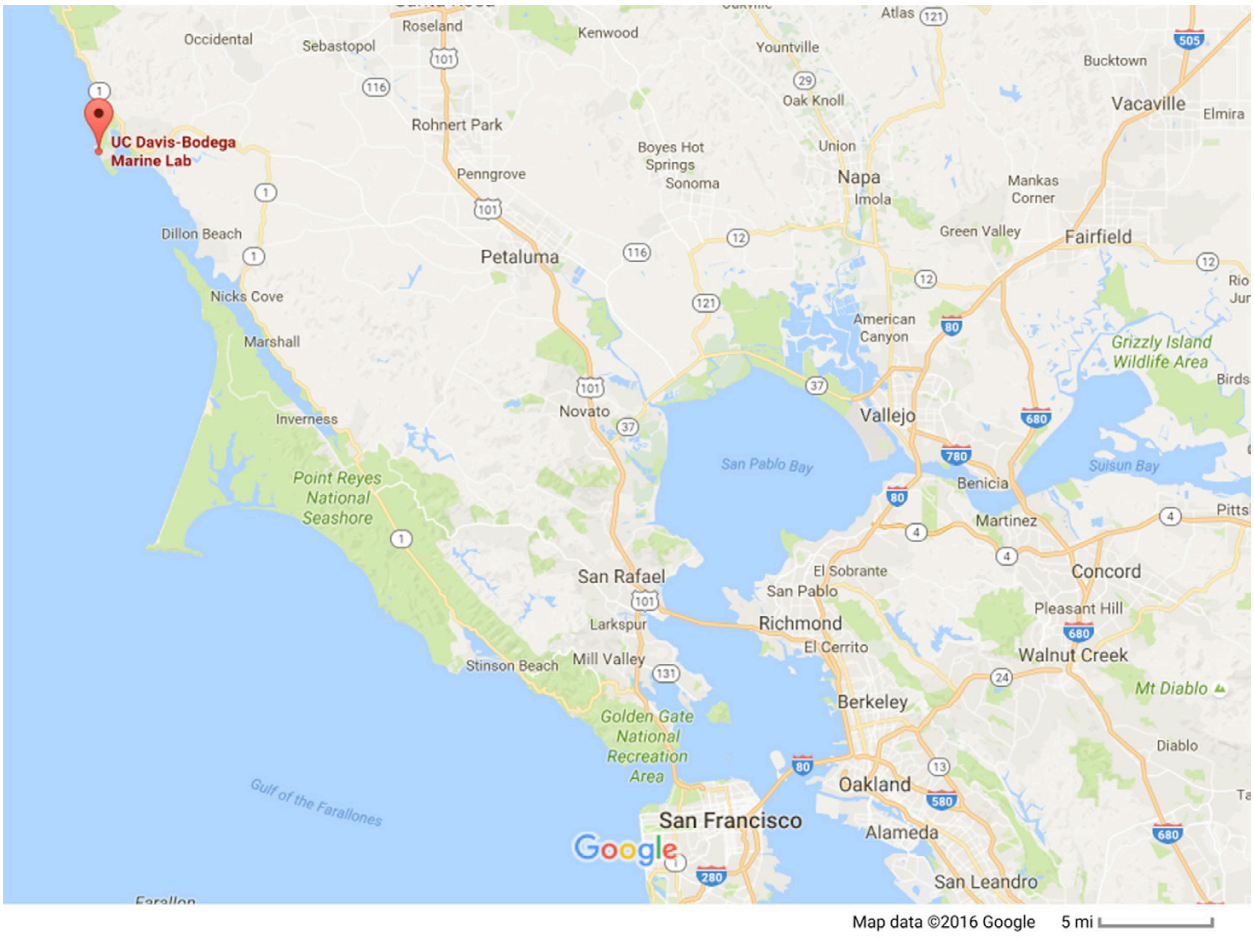
UC Davis-Bodega Marine Lab
Occidental
Sebastopol
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Kenwood
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Atlas
Bucktown
Vacaville
Elmira
Oak Knoll
Rohnert Park
Boyes Hot Springs
Sonoma
Union
Napa
Imola
Mankas Corner
Green Valley
Fairfield
Penngrove
Petaluma
Dillon Beach
Nicks Cove
Marshall
Inverness
American Canyon
Grizzly Island Wildlife Area
Novato
Vallejo
Point Reyes National Seashore
San Pablo Bay
Suisun Bay
Benicia
Martinez
Pitts
San Rafael
El Sobrante
San Pablo
Concord
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Larkspur
Richmond
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Walnut Creek
Stinson Beach
Mill Valley
Mt Diablo
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Gulf of the Farallones
Oakland
San Francisco
Alameda
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Google
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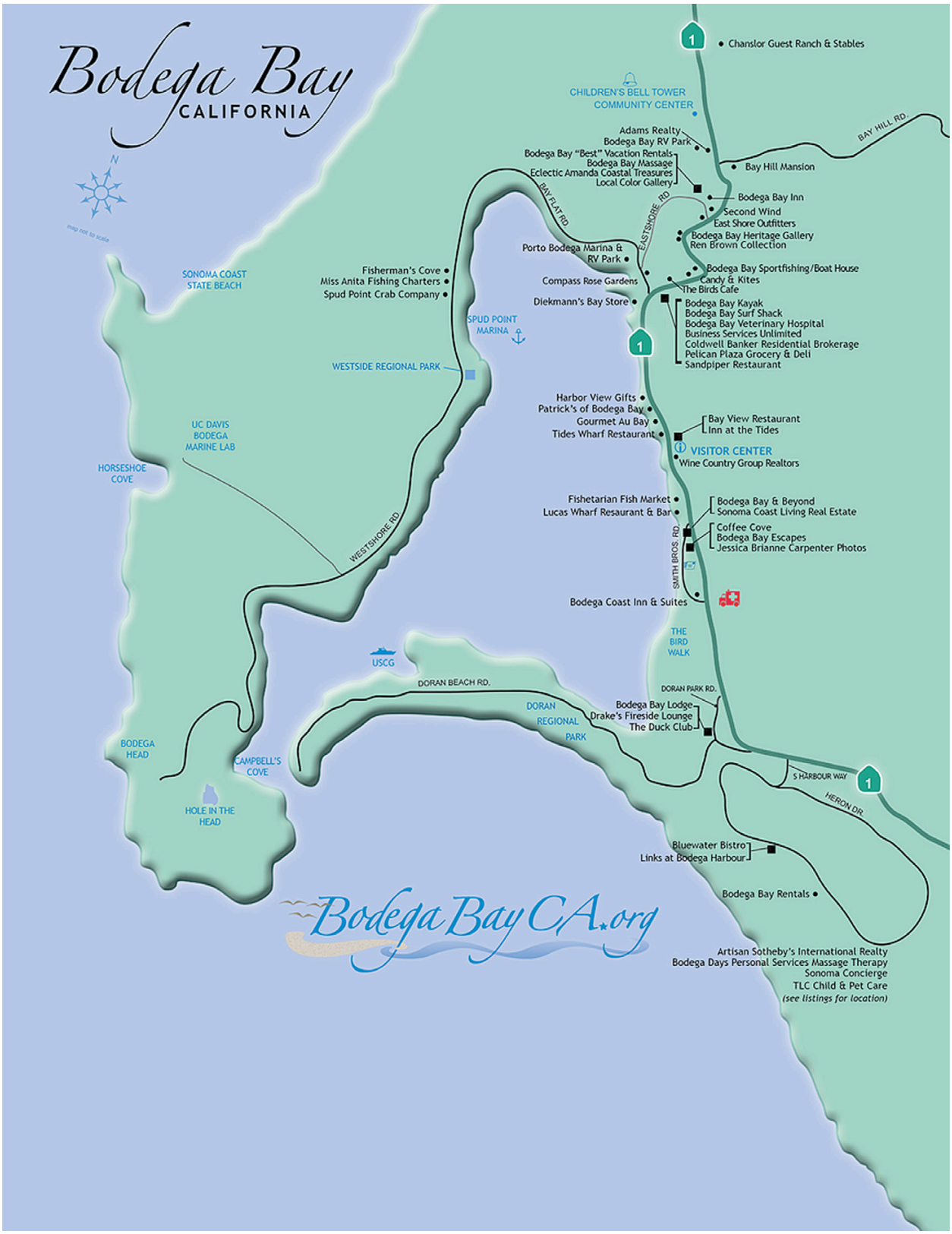
Bodega Bay
CALIFORNIA
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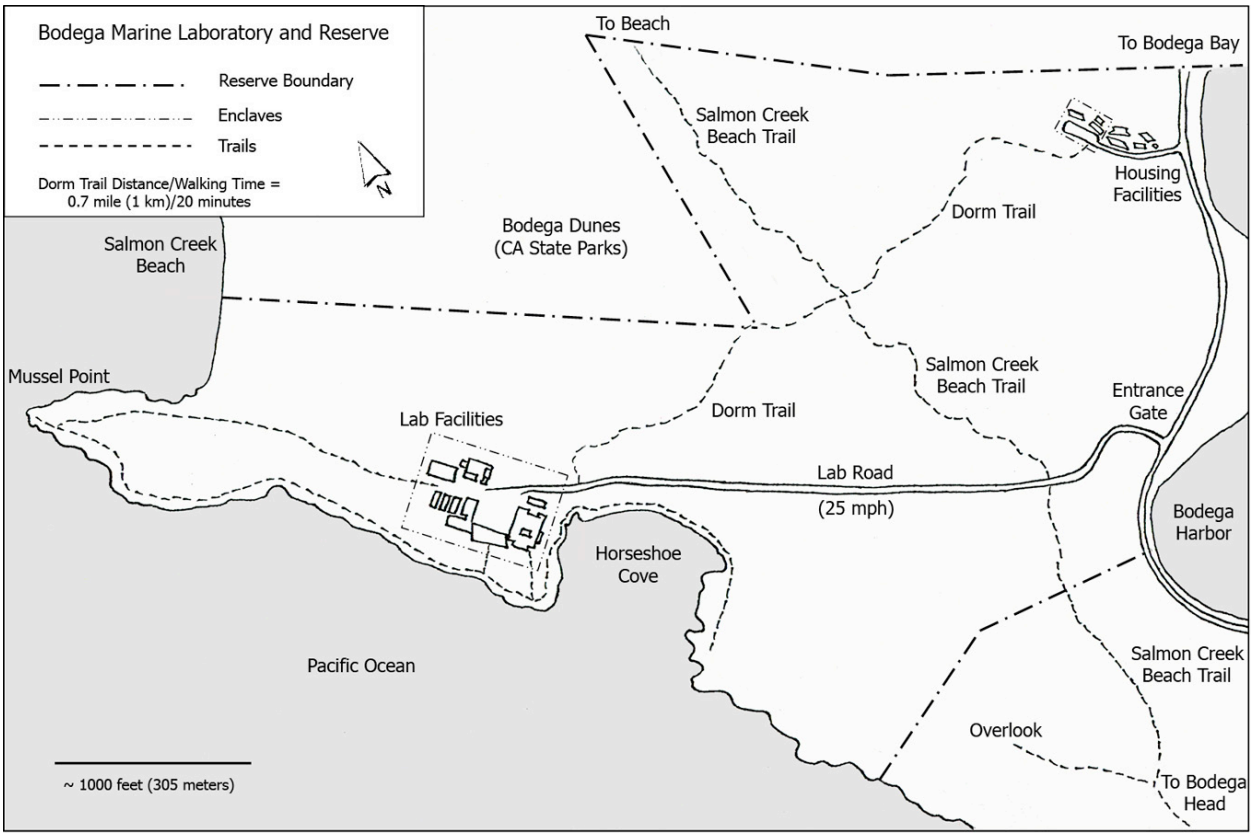
Chanslor Guest Ranch & Stables
CHILDREN'S BELL TOWER
COMMUNITY CENTER
Adams Realty
Bodega Bay RV Park
Bodega Bay "Best" Vacation Rentals
Bodega Bay Massage
Eclectic Amanda Coastal Treasures
Local Color Gallery
BAY HILL RD.
Bay Hill Mansion
BAY FLAT RD.
EASTSHORE RD.
Bodega Bay Inn
Second Wind
East Shore Outfitters
Bodega Bay Heritage Gallery
Ren Brown Collection
Porto Bodega Marina &
RV Park
Bodega Bay Sportfishing/Boat House
Candy & Kites
The Birds Cafe
SONOMA COAST
STATE BEACH
Fisherman's Cove
Miss Anita Fishing Charters
Spud Point Crab Company
Compass Rose Gardens
Diekmann's Bay Store
SPUD POINT
MARINA
Bodega Bay Kayak
Bodega Bay Surf Shack
Bodega Bay Veterinary Hospital
Business Services Unlimited
Coldwell Banker Residential Brokerage
Pelican Plaza Grocery & Deli
Sandpiper Restaurant
WESTSIDE REGIONAL PARK
Harbor View Gifts
Patrick's of Bodega Bay
Gourmet Au Bay
Tides Wharf Restaurant
Bay View Restaurant
Inn at the Tides
UC DAVIS
BODEGA
MARINE LAB
VISITOR CENTER
Wine Country Group Realtors
HORSESHOE
COVE
Fishetarian Fish Market
Lucas Wharf Resaurant & Bar
Bodega Bay & Beyond
Sonoma Coast Living Real Estate
Coffee Cove
Bodega Bay Escapes
Jessica Brianne Carpenter Photos
WESTSHORE RD.
SMITH BROS. RD.
Bodega Coast Inn & Suites
THE
BIRD
WALK
USCG
DORAN BEACH RD.
DORAN PARK RD.
DORAN
REGIONAL
PARK
Bodega Bay Lodge
Drake's Fireside Lounge
The Duck Club
BODEGA
HEAD
CAMPBELL'S
COVE
S HARBOUR WAY
HERON DR.
HOLE IN THE
HEAD
Bluewater Bistro
Links at Bodega Harbour
Bodega Bay Rentals
BodegaBayCA.org
Artisan Sotheby's International Realty
Bodega Days Personal Services Massage Therapy
Sonoma Concierge
TLC Child & Pet Care
(see listings for location)


Bodega Marine Laboratory and Reserve
Reserve Boundary
Enclaves
Trails
Dorm Trail Distance/Walking Time =
0.7 mile (1 km)/20 minutes
N
To Beach
To Bodega Bay
Salmon Creek
Beach Trail
Housing
Facilities
Dorm Trail
Bodega Dunes
(CA State Parks)
Salmon Creek
Beach
Mussel Point
Salmon Creek
Beach Trail
Entrance
Gate
Lab Facilities
Dorm Trail
Lab Road
(25 mph)
Bodega
Harbor
Horseshoe
Cove
Pacific Ocean
Salmon Creek
Beach Trail
Overlook
To Bodega
Head
~ 1000 feet (305 meters)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