The colonial cnidarian *Hydractinia*

Uri Frank\(^1\)*, Matthew L. Nicotra\(^2\) and Christine E. Schnitzler\(^3,4\)

**Abstract**

*Hydractinia*, a genus of colonial marine cnidarians, has been used as a model organism for developmental biology and comparative immunology for over a century. It was this animal where stem cells and germ cells were first studied. However, protocols for efficient genetic engineering have only recently been established by a small but interactive community of researchers. The animal grows well in the lab, spawns daily, and its relatively short life cycle allows genetic studies. The availability of genomic tools and resources opens further opportunities for research using this animal. Its accessibility to experimental manipulation, growth- and cellular-plasticity, regenerative ability, and resistance to aging and cancer place *Hydractinia* as an emerging model for research in many biological and environmental disciplines.

**Keywords:** Cnidaria, Hydrozoa, Allorecognition, Regeneration, Stem cells, CRISPR

**Natural habitat and lifecycle**

*Hydractinia symbiolongicarpus* and *H. echinata* are sister species of colonial hydrozoan cnidarians. *H. symbiolongicarpus* occurs along the eastern coast of North America, from Maine to South Carolina \([1]\). *H. echinata* is found along North European Atlantic coasts \([2]\). In the field, they are found exclusively on gastropod shells occupied by hermit crabs (e.g., *Pagurus longicarpus*). Colonies consist of polyps specialized for feeding, reproduction, or defense, which grow from a sheet of tissue called the stolonal mat (Fig. 1a). Unlike many of its hydrozoan relatives, *Hydractinia* does not produce a free-living medusa stage (jellyfish). Instead, gametes mature in a rudimentary medusoid that remains attached to sexual polyps (Fig. 1b). All polyps within a colony are clonally derived and therefore genetically identical. The mat consists of two epidermal cell layers, which sandwich a network of gastrodermal canals connecting polyps to each other and forming a gastrovascular system. Colonies grow by expanding the edge of the mat or by elongating individual stolons, extensions of gastrovascular canals encased in a thin, chitinous integument called the periderm. Colonies are dioecious and spawn about 90 min after first light. Eggs sink to the bottom after fertilization and develop into a planula larva within 2–3 days (Fig. 1b). Mature larvae latch onto a passing hermit crab shell by firing nematocysts located in their posterior ends \([3]\). Once on the shell, the larvae metamorphose into a primary polyp in response to a bacterial cue \([4]\). The juvenile colony then grows as described above, frequently covering the entire shell.

**Lab culture and field collection**

*Hydractinia* can be cultured in the lab with supplies available at most aquarium stores (Fig. 1c). A typical setup is a 39-L glass aquarium filled with any commercial artificial seawater (29–32 ppt) and maintained at 18–22 °C. Colonies grow best with ample water movement, thus a power head (usually one designed for a 110 L tank) is attached to the side of the tank. *Hydractinia* is sensitive to the accumulation of ammonia and nitrites. Biological filtration is therefore provided with an external filter or an internal sponge filter and second power head. Phosphates can also inhibit colony growth but are controlled by placing small bags of phosphate absorbing media in each tank. With this in place, a weekly 25% water change is enough to maintain healthy colonies.

\(^*\)Correspondence: uri.frank@nuigalway.ie

\(^1\) Centre for Chromosome Biology, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland

Full list of author information is available at the end of the article

© The Author(s) 2020. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
To establish a *Hydractinia* culture from a field-collected animal, a piece of the colony bearing several feeding polyps is excised from its shell with a sharp blade and then tied with thread to a standard 25 × 75 mm glass microscope slide. The thread is removed after the explant attaches to the slide. This colony can then be propagated indefinitely by explanting it onto new slides. The slides can be stored in a histology slide box with the cover removed and the bottom cut out, which is placed at the bottom of the tank.

*Hydractinia* can also be bred in the lab. This is done by keeping them on a regular dark:light cycle (e.g., 8 h:16 h). Approximately an hour and a half after turning on the lights, eggs and sperm are spawned and can be combined in artificial seawater in a Petri dish, where fertilized eggs begin embryonic development. After 3–4 days, the resulting larvae may be induced to settle by incubating them for 2–3 h in 100 mM CsCl and subsequently placing them on a new microscope slide. There, larvae metamorphose into a primary polyp, which is competent to feed within 1–3 days post-metamorphosis. Despite the predictable, light-induced spawning, spawning may occasionally occur at other times. It is therefore advisable to keep male and female colonies in separate tanks to prevent the uncontrolled production of larvae.

Laboratory cultures of *Hydractinia* fare well on a diet of 4-day-old *Artemia* nauplii, which they receive three times per week. When many embryos are required for an experiment, we have found it beneficial to supplement this diet with pureed oysters twice a week. Colonies receiving this diet release gametes more reliably, in greater quantities, and with higher quality.

Today, most laboratories studying *Hydractinia symbioticargus* work with strains derived from a single population in New Haven Harbor, Connecticut. The primary strain is a male colony, called 291-10, which is particularly vigorous in laboratory culture and, for this reason, was the animal chosen for the *Hydractinia* genome project (see below). Several female strains (e.g., 295-8) are also in use and their genome sequencing is in progress. Transgenic/mutant strains, derived from crossing 291-10 to a female strain, have also been established. All strains are available by request from our labs. Some European researchers use *H. echinata*, for which a full genome sequence has been generated as well; however, no selected laboratory strains exist for this species and its maintenance is more challenging.

**Major interests and research questions**

Cnidarians are an interesting and highly diverse group of animals. This phylum diverged from the lineage leading to bilaterian animals (that includes flies, worms, and vertebrates) at least 600 million years ago [5], providing sufficient time for substantial diversification within the cnidian lineage (Fig. 2). Most extant cnidarians share a body wall consisting of an epithelial bilayer, a gastric cavity, and a unique cell type—the stinging cell or cnidocyte (also known as nematocyte) from which the phylum name derives. Cnidarians are phylogenetically positioned as the sister group to bilaterians [6]; therefore, studying biological phenomena in cnidarians can provide insight into their origin and how they have changed over evolutionary time between
and within phyla. The past two decades have brought substantial progress in cnidarian molecular biology and genetics, enabling functional genetic studies at least in some cnidarian representatives [7]. Overall, cnidarians’ relative morphological simplicity, sequenced genomes [8–10], amenability to genetic manipulation [11–13], and phylogenetic position promise a fruitful future in research on these animals that will inform areas spanning all the way from evolutionary biology to biomedical sciences.

Current research on *Hydractinia* focuses on a number of topics, including embryonic development [14], neurogenesis [15, 16], stem cells, germ cells, and regeneration [17–20], allorerecognition [21], metabolism [22], immunity [23], and natural product chemistry [24]. Allore cognition refers to the ability to discriminate ‘self’ from ‘non-self’ within the same species, a phenomenon observed in most colonial cnidarians, but not in *Hydra* or *Nematostella*, the two most commonly used cnidarian model systems for molecular work. At present, *Hydractinia* is the only cnidarian in which genes controlling allore cognition have been identified and functionally characterized [25].

Other areas of interest are stem cells and regeneration. These topics have been well studied in *Hydra* [26, 27] and are emerging topics for *Nematostella* researchers too [28, 29]. Interestingly, data published to date suggest that both stem cell behavior and the mode of regeneration differ substantially between cnidarian species [18, 28, 30]. For example, hydrozoan neuronal cells derive from migratory i-cells, whereas in anthozoans, neural progenitor cells are epithelial [16]. As to regeneration modes, *Hydra* can reform the main head structures following decapitation in the absence of cell proliferation whereas in *Hydractinia* and *Nematostella* cell proliferation is essential for regeneration [18]. These findings highlight the importance of studying more than one animal in order to prevent false conceptual generalizations and underestimation of the complexity underlying biological phenomena.

*Hydractinia* does not show any evidence for age-related deterioration [31], is highly resistant to ionizing irradiation [18], and develops tumors only very rarely following genetic manipulation [19] but not spontaneously. These features are consistent with high genomic stability in this animal, a feature that remains to be investigated.

**Experimental approaches**

Manipulating gene expression has so far only been established in four cnidarians: *Hydra, Nematostella, Hydractinia*, and *Clytia* [11–13, 32]. This can be done either by permanent modification of the animal’s genome or by transient interference with specific gene products. Both approaches have their pros and cons and their usage depends on the type of experiment being conducted and availability of appropriate protocols for a given species and life stage.

The most common approach in *Hydractinia* is microinjection of nucleic acids and/or proteins into the zygote. *Hydractinia* spawning is light-induced without the need for any further induction [33]. Eggs are not embedded in jelly and can be directly microinjected upon fertilization [12]. Electroporation techniques are currently being developed in the authors’ labs with promising results. Circular plasmids readily integrate into the *Hydractinia* genome [12]. The site of integration is unknown, but the process is highly efficient; in excess of 80% of injected embryos become transgenic in the hands of experienced researchers. This approach has been used to create fluorescent reporter lines for many developmental genes and cell type-specific markers (e.g., Fig. 3). A more targeted way to genetically manipulate the animals is provided by CRISPR–Cas9 technology. In *Hydractinia*, this is performed by microinjecting site-specific short guide
RNAs (sgRNA) together with recombinant Cas9 to generate loss-of-function mutations [16, 20]. Adding to the injecting cocktail a plasmid including a fragment of DNA, flanked by two homology arms, can be used for targeted knock-in of fragments [34]. As with all plasmids, this DNA could also integrate randomly into the genome. Designing the injected DNA such that it must rely on the promoter of the target gene limits the likelihood that it would be expressed if integrating non-specifically.

Gene expression manipulation without genetic alteration can be achieved by injecting short hairpin RNA (shRNA) [20] or morpholino oligonucleotides [35] to lower expression of genes, or synthetic RNA to overexpress them (Török et al. unpublished data). Finally, incubating polyps in seawater containing double stranded RNA (dsRNA) transiently lowers the expression of the corresponding gene, albeit with low efficiency [36].

Hydractinia is also unique among model cnidarians for being the only species in which a forward genetic approach has been used to identify the genetic basis of a phenotype. The reasons for this are almost entirely logistical. First, Hydractinia colonies can produce hundreds of embryos per day, making it possible to quickly generate large populations of banded animals. Second, the animals grow as encrustations on a surface that can be labeled, making it possible to co-culture large populations of genetically distinct animals in a small number of tanks. To date, forward genetic approaches have been used to identify genes responsible for allorecognition [37–39] and sex determination (Nicotra, unpublished data). Given the availability of a sequenced genome and the cost efficiency of high-throughput genotyping, it seems feasible to consider mutagenesis screens as well.

An additional experimental approach in Hydractinia is grafting of tissues. This can be done for, e.g., introducing transgenic cells into a colony [20]. Grafting of tissues from genetically distinct individuals requires at least partial matching of allorecognition alleles to prevent allogeneic rejection [25].

Single-cell RNA sequencing methods are also under development in our labs with the first single-cell sequencing libraries giving encouraging results. Our current goal is to develop a robust cellular atlas to define major cell types and subtypes in Hydractinia and to identify marker genes for all cell types as was recently done in Hydra and Nematostella [40, 41]. With a robust genome and cellular atlas in place, Hydractinia will be poised to answer biological questions in a more comprehensive way. Flow cytometry and fluorescence activated cell sorting (FACS) protocols are available [20], and together with many transgenic reporter strains it allows for generating cell type-specific transcriptomes following FACS-sorting of defined cell populations.

As with any model organism, Hydractinia has limitations. Perhaps most obvious one is that it lacks a medusa stage, so researchers interested in this feature must look elsewhere, notably to the hydroid Clytia and the scyphozoan Aurelia. The existing Hydractinia research community also remains small compared to that for Hydra and Nematostella, so the availability of shared reagents and techniques is somewhat more limited. This concern is increasingly mitigated by additional labs beginning to study Hydractinia, and an upsurge in crosstalk between researchers.

Research community and resources
The Hydractinia research community is relatively small but growing as Hydractinia is gaining recognition as a tractable cnidarian research model. A recent NSF Enabling Discovery through Genomic Tools (EDGE) grant has been awarded to the authors, ensuring that the genetic toolkit and community of Hydractinia researchers will continue to blossom and grow. Current resources include high-quality genomes and transcriptomes from both Hydractinia symbiolongicarpus and H. echinata. Draft Illumina genome and transcriptome assemblies are publicly available through the Hydractinia Genome Project Portal (https://research.nhgri.nih.gov/hydractinia/), and long-read PacBio genome assemblies for both species are forthcoming (Schnitzler et al. unpublished data).

With an estimated genome size of 774 Mb for H. echinata and 514 Mb for H. symbiolongicarpus, the Hydractinia genomes are larger than the genome of Nematostella (329 Mb) but smaller than that of Hydra (1086 Mb). Annotated reference genomes and transcriptomes can be used for mapping standard RNA sequencing data [20]. Laboratory selected, fast-growing strains are available to anyone. We are developing a community portal at www.hydractinia.org to be completed in the coming months, which will link to written and video-based protocols and to a community forum, and provide an online form to request animals. Newcomers to the field are encouraged to attend the two biennial research conferences, the American Cnidofest [42] and the European Tutzing meeting [43].

Acknowledgements
We thank members of our lab for lively discussions.

Authors’ contributions
UF, MLN, and CES wrote the paper. All authors read and approved the final manuscript.

Funding
The authors are jointly funded by the NSF program “Enabling Discovery through Genomics tools—EDGE” (Grant No. 1923259). MLN is also funded by NSF (Grant No. 1557339). UF is a Wellcome Trust Investigator (Grant No. 210722/Z/18/Z, co-funded by the SFI-HRB-Wellcome Biomedical Research Partnership).
References

1. Buss LW, Yund PO. A sibling species group of hydractinia in the North-Eastern United States. J Mar Biol Assoc. 1973;53(1):99–113.
2. Frank U, Leitz T, Müller WA. The hydroid Hydractinia: a versatile, informative cnidian representative. BioEssays. 2001;23(10):903–7.
3. Weis VM, Keene DR, Buss LW. Biology of hydactinid hydroids. 4. Ultrastructure of the planula of Hydractinia echinata. Biol Bull. 1985;168:403–18.
4. Müller WA, Buchal G. Metamorphoseinduktion bei Planulalarven. II. Der Einfluss von Monovalenten Kationen auf die Metamorphose von Hydractinia echinata. Wilhelm Roux Arch. 1973;173:122–35.
5. dos Reis M, Thawornwattana Y, Angeli X, Telford MJ, Donoghue PC, Yang Z. Uncertainty in the timing of origin of animals and the limits of precision in molecular timescales. Curr Biol. 2015;25(22):2939–50.
6. Dunn CW, Giribet G, Edgecombe GD, Hejnol A. Animal phylogeny and its evolutionary implications. Annu Rev Ecol Evol Syst. 2014;45(1):371–95.
7. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Consensus for publication. Not applicable.
8. Leclere L, Horin C, Chevalier S, Lapebie P, Dru P, Peron S, Jager M, Condon CH. Consent for publication. Not applicable.
9. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
10. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
11. Wittlieb J, Khalturin K, Lohmann JU, Anton-Erxleben F, Bosch TCG. From coast to coast: a gene expression database to compare embryogenesis and regeneration in the sea anemone Nematostella vectensis. Development. 2018. https://doi.org/10.1242/dev.162867.
12. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Moving from coast to coast: a gene expression database to compare embryogenesis and regeneration in the sea anemone Nematostella vectensis. Development. 2018. https://doi.org/10.1242/dev.162867.
13. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Consent for publication. Not applicable.
14. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
15. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
16. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Consent for publication. Not applicable.
17. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
18. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
19. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
20. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
21. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
22. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
23. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
24. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
25. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
26. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
27. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
28. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
29. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
30. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
31. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
32. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
33. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
34. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
35. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
36. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
37. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
38. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
39. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
40. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
41. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
42. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
43. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
44. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
45. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
46. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
47. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
48. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Ethical approval and consent to participate. Not applicable.
49. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T. Availability of data and materials. Not applicable.
39. Rosa SF, Powell AE, Rosengarten RD, Nicotra ML, Moreno MA, Grimwood J, Lakiss FG, DellaPorta SL, Buss LW. Hydractinia allodeterminant alr1 resides in an immunoglobulin superfamily-like gene complex. Curr Biol. 2010;20(12):1122–7.

40. Siebert S, Farrell JA, Cazet JF, Abeykoon Y, Primack AS, Schnitzler CE, Juliano CE. Stem cell differentiation trajectories in Hydra resolved at single-cell resolution. Science. 2019;365(6451):eaav9314.

41. Sebe-Pedros A, Saudemont B, Chomsky E, Plessier F, Mailhe MP, Renno J, Loe-Mie Y, Lifshitz A, Mukamel Z, Schmutz S, et al. Cnidarian cell type diversity and regulation revealed by whole-organism single-cell RNA-Seq. Cell. 2018;173(6):1520–1534.e1520.

42. He S, Grasis JA, Nicotra ML, Juliano CE, Schnitzler CE. Cnidofest 2018: the future is bright for cnidarian research. Evodevo. 2019;10(1):20.

43. Funayama N, Frank U. Meeting report on “At the roots of bilaterian complexity: insights from early emerging metazoans,” Tutzing (Germany) September 16–19, 2019. Bioessays. 2019. https://doi.org/10.1002/bies.201900236.

**Publisher’s Note**
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.