Published in final edited form as:

Trends Genet. 2011 January ; 27(1): 7–13. doi:10.1016/j.tig.2010.10.002.

A genomic view of 500 million years of cnidarian evolution

Robert E. Steele^{*}, Charles N. David[#], and Ulrich Technau⁺

^{*} Department of Biological Chemistry and the Developmental Biology Center, University of California, Irvine, Irvine, CA 92697

[#] Department Biologie II, Ludwig-Maximilians-Universität, D-82152 Planegg-Martinsried, Germany

⁺ Department for Molecular Evolution and Development, Centre for Organismal Systems Biology, Faculty of Life Sciences, University of Vienna, Althanstrasse 14, 1090 Wien, Austria

Abstract

Cnidarians (corals, anemones, jellyfish, and hydras) are a diverse group of animals of interest to evolutionary biologists, ecologists, and developmental biologists. With the publication of the genome sequences of *Hydra* and *Nematostella*, whose last common ancestor was the stem cnidarian, we are beginning to see the genomic underpinnings of cnidarian biology. Cnidarians are known for the remarkable plasticity of their morphology and life cycles. This plasticity is reflected in the *Hydra* and *Nematostella* genomes, which differ to an exceptional degree in size, base composition, transposable element content, and gene conservation. We now know what cnidarian genomes are capable of doing given 500 million years; the next challenge is to understand how this genomic history has led to the striking diversity we see in cnidarians.

Why cnidarian genomics?

The phylum Cnidaria contains ~9,000 species with a remarkably rich diversity of form and function, ranging from the inconspicuous freshwater *Hydra* to 100 foot long marine jellyfish and massive coral reefs. The genetic basis of this diversity is of interest for understanding the evolution of animal form and is largely unexplored. With the sequencing of the *Nematostella vectensis* [1] and *Hydra magnipapillata* genomes [2], efforts are now underway to understand how cnidarian evolution is reflected in the content, organization, and activity of the genome.

Cnidaria is divided into five classes (Fig. 1): Anthozoa (corals, sea anemones, and sea pens); Hydrozoa (hydras and marine hydrozoans); Cubozoa (box jellyfish); Scyphozoa (true jellyfish); Staurozoa (stalked jellyfish) [3]. Fortuitously, the first two cnidarian genomes to be sequenced come from Anthozoa (*Nematostella*) and Hydrozoa (*Hydra*); the last common ancestor of these two classes was the stem cnidarian (Fig. 1). A sequence phylogeny based on 337 orthologous proteins indicates that the split between the *Hydra* and *Nematostella* lineages is ancient, having occurred at about the same time as the split leading to protostomes (flies and worms) and deuterostomes (vertebrates) [1].

The genomes of *Nematostella* and *Hydra* were sequenced primarily because of the phylogenetic placement of Cnidaria as a sister group to bilaterian animals. In addition,

Corresponding author: Steele, R.E. (resteele@uci.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Nematostella has recently been adopted as a model for studying the evolution of animal embryonic development [4,5], and *Hydra* has long been used as a model for studying pattern formation [6], regeneration [7–9], and stem cell biology [10,11]. As expected from their last common ancestor being the stem cnidarian, *Hydra* and *Nematostella* are remarkably different cnidarians. *Nematostella* is marine and, being an anthozoan, lacks a medusa stage in its life cycle. *Hydra* is one of the few cnidarians to live in freshwater and, atypical for a hydrozoan, lacks both the planula larva and medusa stages. Thus in comparing these two organisms' genomes, we are sampling an extraordinarily large swath of cnidarian evolutionary change. Here we highlight interesting findings that have come from comparison of the *Hydra* and *Nematostella* genomes, particularly findings that provide insight into the evolution of cnidarians.

Basic features of the two genomes

The basic features of the *Nematostella* and *Hydra* genomes differ dramatically, in keeping with the length of time that has passed since these two lineages separated from their common ancestor (Table 1). The *H. magnipapillata* genome is at least twice as large as the genome of *Nematostella*. However, both species have the same number of chromosomes ($2n=30$). Interestingly, the genome of the “green” *Hydra viridissima* (which contains algal symbionts) is also $2n=30$, but only about 0.38 Gb in size [12]. The last common ancestor of *H. magnipapillata* and *H. viridissima* was the stem *Hydra*, and it has been estimated that these two species diverged 46–61 Myrs ago [13]. Thus there have clearly been dramatic changes in genome size in the *Hydra* lineage. These changes have been accompanied by bursts of transposon expansion and the occurrence of transposon-specific ESTs indicates that many transposon families are currently active in the *Hydra* genome [2]. By comparison, *Nematostella* has far less repetitive DNA than *Hydra* and there is no evidence for bursts of transposon expansion similar to those in *Hydra* [2].

The *Hydra* and *Nematostella* genomes also differ greatly in base composition, with the *Hydra* genome being much more AT-rich (71% AT) than the *Nematostella* genome (59.4 % AT). Both genomes undergo methylation of cytosines in CpG dinucleotides [14,15]. This finding suggests that cnidarians may be fertile ground for investigating the evolution of epigenetic regulation of animal genomes by DNA methylation.

Given the association between telomere maintenance and aging [16], telomere maintenance in cnidarians is of considerable interest because of the apparent absence of senescence in *Hydra* [17]. *Hydra* and *Nematostella* chromosomes contain typical animal telomeres [2,18]. Evidence that telomeres in cnidarians are maintained in a canonical fashion is the presence of a gene encoding Telomerase Reverse Transcriptase (TERT) in both species (*Hydra* genome browser gene model Hma2.231798; *Nematostella* genome scaffold ABAV01000437). Thus the absence of senescence in *Hydra* is not due in any immediately obvious way to changes in the telomere maintenance machinery.

Comparisons of the organization of genes in the *Nematostella* genome relative to gene organization in the human genome led to the unexpected finding of extensive conservation of linkage groups [1]. Traces of this conservation of gene organization are also apparent in the *Hydra* genome [2]. These findings are in contrast to the situation in the more recently branching *Caenorhabditis elegans* and *Drosophila* genomes, which show no trace of gene linkage conservation relative to the human genome using the same criteria [1]. Comparisons of gene structure and sequence between *Hydra*, *Nematostella*, and humans show that the *Hydra* genome has clearly been on a faster evolutionary track than the *Nematostella* genome. *Hydra* has lost more of the introns that were in homologous locations in the stem cnidarian and humans than has *Nematostella*. *Hydra* genes also show a greater rate of amino

acid substitution relative to humans than do those of *Nematostella*. The forthcoming genome sequences of another hydrozoan, *Clytia hemisphaerica*, and another anthozoan, *Acropora millepora*, will indicate whether this is a general feature of anthozoans versus hydrozoans.

Cnidarian novelties based on gene invention and gene loss

It was to be expected that novel genes specific to the phylum Cnidaria would have evolved and, in view of the ancient split between anthozoan and medusozoan lineages, that there would be lineage-specific genes as well. And indeed a recent comparison of *Hydra* and *Nematostella* gene predictions indicates that 15% of the genes in each genome are lineage-specific [19]. In contrast, *Clytia*, a hydrozoan with a medusa stage in addition to polyp and larval stages, has an estimated 25% lineage-specific genes [19]. This estimate is based on EST data and thus may change when the complete genome sequence becomes available. Conversely, each of the three species has lost significant numbers of conserved RefSeq hits present in the other two species [19]. These results indicate that evolution of the diverse cnidarian forms and life cycles has been accompanied by invention of novel genes and by gene loss. Novel genes are of particular interest since they presumably encode proteins that function in biological processes or cell types unique to cnidarians.

Nematocytes, or stinging-cells, are a unique cnidarian-specific cell type used for prey-capture. As expected, the great majority of nematocyte-specific genes identified so far have orthologs in *Hydra* and *Nematostella*, but no homologues in other metazoan phyla [20,21]. Several of these genes, e.g. those encoding minicollagens, form structural components of the nematocyst capsule [22,23]. These gene families also show ongoing evolution within the phylum: anthozoans have a small set of nematocyst types and only five minicollagen genes while hydrozoans have more nematocyst types and accordingly *Hydra* has seventeen minicollagen genes [24]. Similarly, the gene for nematogalectin – a nematocyst tubule-specific protein is – present in only one copy in anthozoans while hydrozoans have two copies, one of which forms a novel structure found only in hydrozoan nematocysts [25]. While minicollagens and nematogalectin contain ancient protein domains, their novel domain combinations (e.g. collagen and galectin) occur only in cnidarians. Finally, nematocilin is a novel intermediate filament protein that forms the central filament in the cnidocil (sensory cilium) of hydrozoan nematocytes [26]. A central filament is not present in the cnidocil of anthozoan nematocytes. Consistent with this, there are two nematogalectin genes in *Hydra* and none in the anthozoan *Nematostella*. Sequence comparisons indicate that the nematocilin gene evolved following duplication of the nuclear lamin gene in the hydrozoan lineage.

Comparison of the *Hydra* and *Nematostella* genomes with those from bilaterians has also revealed the presence of so-called taxon-restricted genes (TRGs) [27] in individual lineages. One such domain, called SWT, was initially identified in the extracellular portions of two *Hydra* receptor tyrosine kinases [28,29], one called *Sweet Tooth* (hence the SWT designation for the domain). The SWT domain is absent from *Nematostella*, but present in a large number of *Hydra* proteins, both receptors and secreted proteins [2]. It is also present in ESTs from the hydrozoan *Clytia* [2]. Thus it appears that a novel protein domain, potentially playing a role in signal transduction, evolved after the anthozoan/medusozoan split.

Another class of novel genes in cnidarians encodes so-called epitheliopeptides [30]. These peptides, identified biochemically and by subtractive hybridization approaches in *Hydra* [31,32], play roles in patterning and cell differentiation [30]. The 301 peptide family is uniquely found in *Hydra*, differentially expressed in the head region and involved in regulating tentacle morphogenesis [32,33]. Given that tentacle formation is common to all

cnidarians, it is surprising that 301 family peptides appear to be absent from the *Nematostella* genome.

In addition to taxon-restricted genes, there are cases of lineage-specific expansions of gene families in *Hydra* and *Nematostella*. An interesting example is the genes encoding innexins, the proteins that form gap junctions in flies, worms, and other protostomes [34]. *Hydra* has seventeen innexin genes while *Nematostella* has only one [2]. *Clytia* has at least eight innexin genes based on ESTs [2], suggesting that expansion of the innexin gene family is a feature of hydrozoans. In *Hydra*, the increase in innexin genes is accompanied by electron microscopic evidence of gap junctions between several cell types, including epithelial and nerve cells [35,36]. That the innexin genes in *Hydra* form gap junctions has been demonstrated by expression of the *Hydra Innexin-1* gene in epithelial cells [37]. By comparison, gap junctions have not been observed in anthozoans [38]. Gap junctions between nerve cells are electric synapses enabling rapid bidirectional communication, and they appear to constitute the cellular substrate enabling pacemaker activity, which coordinates behavior in *Hydra* [39–41]. These findings lead to the unexpected hypothesis that nervous system organization differs in fundamental ways between hydrozoans and anthozoans.

The genes encoded by two allorecognition loci (*alr1* and *alr2*) from the colonial hydrozoan *Hydractinia symbiolongicarpus* were reported recently [42,43]. These genes encode transmembrane receptors with highly variable extracellular Ig-related domains and are responsible for the fusion-rejection capability of *Hydractinia* colonies [44]. Homologues of these genes are absent from both the *Hydra* and *Nematostella* genomes, and are unrelated to the genes responsible for allorecognition in vertebrates and urochordates, which themselves are unrelated to each other. This leads to the striking conclusion that the *alr* genes and their associated function evolved recently in the class Hydrozoa.

Hox, Parahox and NK class homeobox genes arose from a megacluster in early animal evolution [45], traces of which are still detectable in a number of gene linkages in the *Nematostella* genome [46]. In *Hydra*, massive loss of genes arising from the megacluster has occurred (Fig. 2), and overall *Hydra* has retained only about half of the homeobox genes found in *Nematostella* [46]. Some of these losses are apparently relatively recent since the *Emx* and *Eve* genes are present in other hydrozoans but absent from *Hydra*. Interestingly, the loss of these two genes correlates with the absence of a larval stage in *Hydra*, suggesting that these losses either contributed to or are a consequence of the loss of the larva.

Transcription factor gene families have been subjected to both loss and expansion in the *Hydra* and *Nematostella* lineages. *Hydra* has lost more transcription factor families than *Nematostella* [46]. Transcription factor subfamilies in *Nematostella* have undergone independent expansions by gene duplications, with the resulting paralogs showing divergent expression patterns. For instance, in the SoxB group, *Nematostella* has five paralogs with distinct expression patterns [47]. Thus, although considered a slowly evolving cnidarian, *Nematostella* clearly has derived features.

By comparison, most of the major signaling factor subfamilies have been preserved in both *Hydra* and *Nematostella*. The best studied class of signaling molecules in cnidarians are the Wnt genes, of which *Nematostella* has 12 and *Hydra* has 9 out of 13 subfamilies [48,49]. This impressive diversity in both cnidarians sharply contrasts with the situation in sponges, which have only three Wnt genes that cannot be reliably placed in specific Wnt subfamilies [50]. Notably, several of these Wnt subfamilies have been lost in the main ecdysozoan model systems. Thus, the diversity of Wnt subfamilies arose at the base of the Eumetazoa and has been largely maintained in cnidarians and vertebrates. Most of the homologous Wnt

subfamilies in *Nematostella* and *Hydra* have similar expression patterns around the blastopore and its derivative, the hypostome [48,49], suggesting that their function was established in the stem cnidarian. Wnt signaling is clearly involved in axial development and regeneration in cnidarians [51–53]. It is tempting to speculate that the high regenerative capacity of cnidarians is correlated with the conservation of Wnt subfamilies, whereas transcription factors governing the region- or cell type-specific differentiation have been subject to lineage-specific duplications and losses.

Horizontal gene transfer – another route to novelty

The significance of horizontal gene transfer (HGT) as a source of novelty in metazoan evolution is a much-debated and still unresolved question [54]. This question has been addressed recently in cnidarians using ESTs and genome sequences. From an analysis of ESTs from the coral *Acropora* and *Nematostella* [55] it was concluded that most putative cases of HGT in these two organisms were best explained by ancient origins followed by multiple secondary losses. This conclusion has not been revisited since the *Nematostella* genome was sequenced. By comparison, analysis of the *Hydra* genome has identified 71 genes as candidates for horizontal gene transfer from prokaryotic donors [2]. A particularly interesting case is what appears to be horizontal transfer into the *Hydra* genome of the three genes needed to produce the activated heptose that serves as a precursor for synthesizing the inner core of the lipopolysaccharide of Gram-negative bacteria, a uniquely prokaryotic structure. All three of these *Hydra* genes lack introns, and homologues have not been found in any other sequenced metazoan genome [2].

The major issue confronting hypotheses of HGT is the difficulty of envisioning a route by which the gene could enter the germline of the recipient animal, which it would have to do in order to be transmitted to subsequent generations. This is particularly problematic in metazoans, since the germline is often segregated early in development. *Hydra* is, by comparison, an attractive organism in which to search for examples of HGT. Its germline is not segregated [56], sexual reproduction occurs infrequently relative to asexual reproduction by budding, all of its cells are in direct or near direct contact with its aquatic environment [57], and it contains a significant bacterial flora on both its external surface and between its cells [58]. Although one also expects *Nematostella* to have an associated microbiome, this has not yet been examined.

Regulation of gene expression in cnidarians: alternative-splicing, trans-splicing, and miRNAs

Metazoans have evolved a variety of mechanisms to control gene expression and to increase genetic complexity without increasing gene number. With genome sequences in hand, we are beginning to explore how these mechanisms are used by cnidarians. Alternative splicing occurs in over 95% of all transcribed genes in humans, yielding a significant expansion of the proteome [59]. From EST data sets and specific gene studies it is clear that alternative splicing occurs in both *Hydra* and *Nematostella*, yet the impact of alternative splicing on the proteome in cnidarians is still unclear, as no systematic analysis has been reported.

Trans-spliced leader addition is a process whereby a small RNA is spliced in trans to the 5' end of mRNAs. In cnidarians, trans-spliced leader addition was first shown to occur in the genus *Hydra* [60] and subsequently in six other hydrozoan species [2,61]. No evidence of trans-spliced leader addition has been found in EST datasets from *Nematostella* or in three other anthozoans (*Acropora*, *Metridium*, and *Anemonia*) [62]. Trans-spliced leader addition occurs sporadically across the metazoan tree [61] making it difficult to determine with certainty whether this process evolved early and has been lost multiple times or has evolved

multiple times independently [63,64]. Parsimony analyses [62] and arguments regarding the difficulty of reversing the use of spliced leaders [63] favor multiple independent gains of trans-spliced leader addition in metazoans. If this scenario is correct, transspliced leader addition in cnidarians would have evolved at some point following the divergence of anthozoans and medusozoans. Organisms that carry out trans-spliced leader addition have the capacity, unusual for metazoans, to organize genes into operons [63]. Bioinformatic analysis suggests that *Hydra* has operons, but this awaits experimental confirmation.

A third important way for controlling gene expression is through miRNAs, a class of 21-24 bp small non-coding RNAs, which bind in a sequence-specific manner to mRNAs, usually to the 3' UTR and lead to the degradation of the mRNA or the inhibition of translation. Over 600 different miRNAs are present in the human genome, but only 40 have been identified in *Nematostella* by deep sequencing of small RNAs [65]. From the *Hydra* genome only 17 mRNAs could be detected so far [2], but this is likely an underestimate. To date, nothing is known about the biological role of miRNAs in cnidarians, but interestingly, only one of the *Nematostella* miRNAs is conserved in bilaterians [65].

An independent origin of stem cells within Cnidaria?

Hydra has been a model for the study of stem cells for ~30 years [11,66]. Of particular interest is the multipotent stem cell of the interstitial cell lineage, the lineage that produces nerve cells, nematocytes, secretory cells, and germ cells [11,66]. An adult *Hydra* polyp has on the order of 3000 such stem cells [67], and they have been defined at the molecular level by expression of the *nanos*-related gene *Cnnos1* [68]. Until the availability of the *Hydra* and *Nematostella* genome sequences, it was impossible to determine what, if any evolutionary relationship the stem cells of *Hydra* had with the stem cells of other animals and whether the genes required for pluripotency in vertebrates are present in cnidarians. The *Hydra* genome was queried for *myc*, *nanog*, *klf4*, *Oct4*, and *Sox2* genes – the genes that confer pluripotency on mammalian cells in culture. *Hydra* has four *myc* homologues [2] and one of these is expressed in the interstitial lineage stem cell [69]. Notably absent from both the *Hydra* and *Nematostella* genomes is *nanog*. In addition to being present in mammals, *Nanog* is present in the chicken [70], the axolotl [71], and apparently in fish [72], but it has not been reported outside of vertebrates. No clear homologue of *klf4* is present in *Hydra* or *Nematostella*, although members of the Krüppel-like family are present. *Oct4* is a member of class V of the POU family. POU family genes are present in *Hydra* and *Nematostella*, but class V POU family members *per se* appear to be absent. The evolutionary relationship of cnidarian Sox B group genes to vertebrate *Sox2* genes is not yet clear [73,74]. Thus it is currently difficult to make a definitive statement regarding *Sox2* in cnidarians. Taken together, these results suggest that the molecular circuitry responsible for establishing and maintaining multipotent stem cells in *Hydra* either has evolutionary origins independent from vertebrates or that it has diverged to the extent that its evolutionary history is obscured. Multipotent interstitial cells have been identified in several hydrozoans in addition to *Hydra* [75–78], but have not been found in anthozoans [79], scyphozoans [80], and cubozoans [81]. This is consistent with multipotent stem cells of hydrozoans arising after the diversification of cnidarians. It will be of great interest to understand this independent evolutionary experiment in producing stem cells, which may have much to tell us about the molecular logic underlying “stemness.”

Concluding remarks

With the sequencing of the *Hydra* and *Nematostella* genomes we can see the end results of two ~500 million year long experiments in cnidarian genome evolution. It is now clear that cnidarian genomes are much more plastic than we might have anticipated. The genes that

underlie cnidarian biology are starting to be identified, and of particular interest are those involved in synthesis and function of the nematocyte, the defining cnidarian feature. How this fascinating organelle evolved and how it operates will be the object of increased attention now that the genes involved are becoming accessible.

But we are still missing the historical record written in the pages of other cnidarian genomes, including those from three of the five cnidarian classes. We obviously need to see more cnidarian genome sequences, and we will not have to wait long for this. The genomes of the hydrozoan *Clytia hemisphaerica* [82] and the anthozoan *Acropora millepora* [83] are currently being sequenced using next generation methods. The two species are of interest because *Clytia* has a complete hydrozoan life cycle and because *Acropora* is a reef-building coral. As sequencing continues to get less expensive, we will undoubtedly see a number of other cnidarian genome sequences and deep sequencing of cnidarian transcriptomes in the not-too-distant future.

In addition to providing a wealth of data for bioinformatics mining, the *Hydra* and *Nematostella* genome sequences provide us with the raw material for carrying out hypothesis-driven studies of gene function to address questions of evolution within Cnidaria and Metazoa. Such studies will be greatly facilitated by: (1) robust methods for generating stably transgenic *Hydra* [84] and *Nematostella* [85] that are now available, and (2) RNAi-mediated knockdown of gene expression. This has been achieved in *Nematostella* by soaking the animals in double-stranded RNA [86] and in *Hydra* by electroporation [32,33,87–90] and feeding with bacteria producing double-stranded RNA [52,91,92].

The ability to make transgenic animals combined with the availability of genome sequences also provides opportunities for studying cis-regulation of genes and dissecting the logic of gene regulation in cnidarians. With many fewer cell types and tissues than bilaterians, cnidarians are attractive systems for such studies. In addition, functional studies of gene regulation in diverse cnidarians will provide insight into how changes in cnidarian morphology and life cycle are tied to changes in regulatory circuits.

Acknowledgments

We are grateful to the Department of Energy Joint Genome Institute, the J. Craig Venter Institute, the Genome Sequencing Center at Washington University, the National Human Genome Research Institute, and the National Science Foundation for funding and carrying out the sequencing of the *Hydra* and *Nematostella* genomes and EST libraries. We especially appreciate the rapid release and public access policies that allowed the free exchange of information and fostered the collaborative spirit associated with both of these genome projects. We are grateful to Dr. Ira Blitz for his insightful comments on a draft of this article and to Dr. Yehu Moran for help with the genomic comparisons. Research in the Steele lab is supported by grant 1R24GM080537-01A1 from the National Institute of General Medical Sciences. Research in the Technau lab is supported by grants from the Marie-Curie Initial Training Network EVONET, the Austrian National Science Foundation (FWF), and the Norwegian Research Council.

References

1. Putnam NH, Srivastava M, Hellsten U, Dirks B, Chapman J, Salamov A, Terry A, Shapiro H, Lindquist E, Kapitonov VV, et al. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science*. 2007; 317:86–94. [PubMed: 17615350]
2. Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T, Rattei T, Balasubramanian PG, Borman J, Busam D, et al. The dynamic genome of *Hydra*. *Nature*. 2010; 464:592–596. [PubMed: 20228792]
3. Collins AG, Schuchert P, Marques AC, Jankowski T, Medina M, Schierwater B. Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. *Syst Biol*. 2006; 55:97–115. [PubMed: 16507527]

4. Darling JA, Reitzel AR, Burton PM, Mazza ME, Ryan JF, Sullivan JC, Finnerty JR. Rising starlet: the starlet sea anemone, *Nematostella vectensis*. *Bioessays*. 2005; 27:211–221. [PubMed: 15666346]
5. Genikhovich G, Technau U. The starlet sea anemone *Nematostella vectensis*: an anthozoan model organism for studies in comparative genomics and functional evolutionary developmental biology. *CSH Protoc*. 2009; 2009 pdb emo129.
6. Bode HR. Axial patterning in hydra. *Cold Spring Harb Perspect Biol*. 2009; 1:a000463. [PubMed: 20066073]
7. Bode HR. Head regeneration in Hydra. *Dev Dyn*. 2003; 226:225–236. [PubMed: 12557201]
8. Bosch TC. Why polyps regenerate and we don't: towards a cellular and molecular framework for Hydra regeneration. *Dev Biol*. 2007; 303:421–433. [PubMed: 17234176]
9. Holstein TW, Hobmayer E, Technau U. Cnidarians: an evolutionarily conserved model system for regeneration? *Dev Dyn*. 2003; 226:257–267. [PubMed: 12557204]
10. Bosch TC. Hydra and the evolution of stem cells. *Bioessays*. 2009; 31:478–486. [PubMed: 19274660]
11. David CN, Murphy S. Characterization of interstitial stem cells in hydra by cloning. *Dev Biol*. 1977; 58:372–383. [PubMed: 328331]
12. Zacharias H, Anokhin B, Khalturin K, Bosch TCG. Genome sizes and chromosomes in the basal metazoan *Hydra*. *Zoology*. 2004; 107:219–227. [PubMed: 16351940]
13. Martínez DE, Iñiguez AR, Percell KM, Willner JB, Signorovitch J, Campbell RD. Phylogeny and biogeography of *Hydra* (Cnidaria: Hydridae) using mitochondrial and nuclear DNA sequences. *Mol Phylogenet Evol*. 2010; 57:403–410. [PubMed: 20601008]
14. Hassel M, Cornelius MG, Vom Brocke J, Schmeiser HH. Total nucleotide analysis of Hydra DNA and RNA by MEKC with LIF detection and 32P-postlabeling. *Electrophoresis*. 2010; 31:299–302. [PubMed: 20084629]
15. Zemach A, McDaniel IE, Silva P, Zilberman D. Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science*. 2010; 328:916–919. [PubMed: 20395474]
16. Stewart SA, Weinberg RA. Telomeres: cancer to human aging. *Annu Rev Cell Dev Biol*. 2006; 22:531–557. [PubMed: 16824017]
17. Martínez DE. Mortality patterns suggest lack of senescence in hydra. *Exp Gerontol*. 1998; 33:217–225. [PubMed: 9615920]
18. Traut W, Szczepanowski M, Vitkova M, Opitz C, Marec F, Zrzavy J. The telomere repeat motif of basal Metazoa. *Chromosome Res*. 2007; 15:371–382. [PubMed: 17385051]
19. Foret S, Knack B, Houliston E, Momose T, Manuel M, Queinnec E, Hayward DC, Ball EE, Miller DJ. New tricks with old genes: the genetic bases of novel cnidarian traits. *Trends Genet*. 2010; 26:154–158. [PubMed: 20129693]
20. Hwang JS, Ohyanagi H, Hayakawa S, Osato N, Nishimiya-Fujisawa C, Ikeo K, David CN, Fujisawa T, Gojobori T. The evolutionary emergence of cell type-specific genes inferred from the gene expression analysis of *Hydra*. *Proc Natl Acad Sci U S A*. 2007; 104:14735–14740. [PubMed: 17766437]
21. Milde S, Hemmrich G, Anton-Erxleben F, Khalturin K, Wittlieb J, Bosch TC. Characterization of taxonomically restricted genes in a phylum-restricted cell type. *Genome Biol*. 2009; 10:R8. [PubMed: 19161630]
22. Adamczyk P, Meier S, Gross T, Hobmayer B, Grzesiek S, Bachinger HP, Holstein TW, Ozbek S. Minicollagen-15, a novel minicollagen isolated from Hydra, forms tubule structures in nematocysts. *J Mol Biol*. 2008; 376:1008–1020. [PubMed: 18206162]
23. Kurz EM, Holstein TW, Petri BM, Engel J, David CN. Mini-collagens in hydra nematocytes. *J Cell Biol*. 1991; 115:1159–1169. [PubMed: 1955459]
24. David CN, Ozbek S, Adamczyk P, Meier S, Pauly B, Chapman J, Hwang JS, Gojobori T, Holstein TW. Evolution of complex structures: minicollagens shape the cnidarian nematocyst. *Trends Genet*. 2008; 24:431–438. [PubMed: 18676050]
25. Hwang JS, Takaku Y, Momose T, Adamczyk P, Ozbek S, Ikeo K, Khalturin K, Hemmrich G, Bosch TCG, Holstein TW, et al. Nematogalectin, a nematocyst protein with GlyXY and galectin

- domains, demonstrates nematocyte-specific alternative splicing in *Hydra*. Proc Natl Acad Sci U S A. 2010 in press.
26. Hwang JS, Takaku Y, Chapman J, Ikeo K, David CN, Gojobori T. Cilium evolution: identification of a novel protein, nematocilin, in the mechanosensory cilium of *Hydra* nematocytes. Mol Biol Evol. 2008; 25:2009–2017. [PubMed: 18635678]
 27. Khalturin K, Hemmrich G, Fraune S, Augustin R, Bosch TC. More than just orphans: are taxonomically-restricted genes important in evolution? Trends Genet. 2009; 25:404–413. [PubMed: 19716618]
 28. Bridge DM, Stover NA, Steele RE. Expression of a novel receptor tyrosine kinase gene and a paired-like homeobox gene provides evidence of differences in patterning at the oral and aboral ends of hydra. Dev Biol. 2000; 220:253–262. [PubMed: 10753514]
 29. Reidling JC, Miller MA, Steele RE. Sweet Tooth, a novel receptor protein-tyrosine kinase with C-type lectin-like extracellular domains. J Biol Chem. 2000; 275:10323–10330. [PubMed: 10744720]
 30. Takahashi T, Fujisawa T. Important roles for epithelial cell peptides in hydra development. Bioessays. 2009; 31:610–619. [PubMed: 19382229]
 31. Takahashi T, Muneoka Y, Lohmann J, Lopez de Haro MS, Solleder G, Bosch TC, David CN, Bode HR, Koizumi O, Shimizu H, et al. Systematic isolation of peptide signal molecules regulating development in hydra: LWamide and PW families. Proc Natl Acad Sci U S A. 1997; 94:1241–1246. [PubMed: 9037037]
 32. Khalturin K, Anton-Erxleben F, Sassmann S, Wittlieb J, Hemmrich G, Bosch TC. A novel gene family controls species-specific morphological traits in hydra. PLoS Biol. 2008; 6:e278. [PubMed: 19018660]
 33. Takahashi T, Hatta M, Yum S, Gee L, Ohtani M, Fujisawa T, Bode HR. Hym-301, a novel peptide, regulates the number of tentacles formed in hydra. Development. 2005; 132:2225–2234. [PubMed: 15829526]
 34. Phelan P. Innexins: members of an evolutionarily conserved family of gap-junction proteins. Biochim Biophys Acta. 2005; 1711:225–245. [PubMed: 15921654]
 35. Wood RL. The cell junctions of hydra as viewed by freeze-fracture replication. J Ultrastruct Res. 1977; 58:299–315. [PubMed: 850295]
 36. Wood RL, Kuda AM. Formation of junctions in regenerating hydra: gap junctions. J Ultrastruct Res. 1980; 73:350–360. [PubMed: 7230324]
 37. Alexopoulos H, Bottger A, Fischer S, Levin A, Wolf A, Fujisawa T, Hayakawa S, Gojobori T, Davies JA, David CN, et al. Evolution of gap junctions: the missing link? Curr Biol. 2004; 14:R879–880. [PubMed: 15498476]
 38. Mackie GO, Anderson PAV, Singla CL. Apparent absence of gap junctions in two classes of Cnidaria. Biol Bull. 1984; 167:120–123.
 39. Campbell RD, Josephson RK, Schwab WE, Rushforth NB. Excitability of nerve-free hydra. Nature. 1976; 262:388–390. [PubMed: 958390]
 40. Passano LM, McCullough CB. Pacemaker Hierarchies Controlling the Behaviour of Hydras. Nature. 1963; 199:1174–1175. [PubMed: 14072038]
 41. Shimizu H, Koizumi O, Fujisawa T. Three digestive movements in *Hydra* regulated by the diffuse nerve net in the body column. J Comp Physiol A. 2004; 190:623–630.
 42. Nicotra ML, Powell AE, Rosengarten RD, Moreno M, Grimwood J, Lakkis FG, Dellaporta SL, Buss LW. A hypervariable invertebrate allodeterminant. Curr Biol. 2009; 19:583–589. [PubMed: 19303297]
 43. Rosa SFP, Powell AE, Rosengarten RD, Nicotra ML, Moreno M, Grimwood J, Lakkis FG, Dellaporta SL, Buss LW. *Hydractinia* allodeterminant *alr1* resides in an invertebrate immunoglobulin superfamily-like gene complex. Curr Biol. 2010; 20:1122–1127. [PubMed: 20537535]
 44. Cadavid LF. Self-discrimination in colonial invertebrates: genetic control of allorecognition in the hydroid *Hydractinia*. Dev Comp Immunol. 2004; 28:871–879. [PubMed: 15183028]
 45. Garcia-Fernandez J. The genesis and evolution of homeobox gene clusters. Nat Rev Genet. 2005; 6:881–892. [PubMed: 16341069]

46. Chourrout D, Delsuc F, Chourrout P, Edvardsen RB, Rentzsch F, Renfer E, Jensen MF, Zhu B, de Jong P, Steele RE, et al. Minimal ProtoHox cluster inferred from bilaterian and cnidarian Hox complements. *Nature*. 2006; 442:684–687. [PubMed: 16900199]
47. Magie CR, Pang K, Martindale MQ. Genomic inventory and expression of Sox and Fox genes in the cnidarian *Nematostella vectensis*. *Dev Genes Evol*. 2005; 215:618–630. [PubMed: 16193320]
48. Kusserow A, Pang K, Sturm C, Hrouda M, Lentfer J, Schmidt HA, Technau U, von Haeseler A, Hobmayer B, Martindale MQ, et al. Unexpected complexity of the Wnt gene family in a sea anemone. *Nature*. 2005; 433:156–160. [PubMed: 15650739]
49. Lengfeld T, Watanabe H, Simakov O, Lindgens D, Gee L, Law L, Schmidt HA, Ozbek S, Bode H, Holstein TW. Multiple Wnts are involved in *Hydra* organizer formation and regeneration. *Dev Biol*. 2009; 330:186–199. [PubMed: 19217898]
50. Srivastava M, Simakov O, Chapman J, Fahey B, Gauthier ME, Mitros T, Richards GS, Conaco C, Dacre M, Hellsten U, et al. The *Amphimedon queenslandica* genome and the evolution of animal complexity. *Nature*. 2010; 466:720–726. [PubMed: 20686567]
51. Broun M, Gee L, Reinhardt B, Bode HR. Formation of the head organizer in hydra involves the canonical Wnt pathway. *Development*. 2005; 132:2907–2916. [PubMed: 15930119]
52. Chera S, Ghila L, Dobretz K, Wenger Y, Bauer C, Buzgariu W, Martinou JC, Galliot B. Apoptotic cells provide an unexpected source of Wnt3 signaling to drive hydra head regeneration. *Dev Cell*. 2009; 17:279–289. [PubMed: 19686688]
53. Gee L, Hartig J, Law L, Wittlieb J, Khalturin K, Bosch TC, Bode HR. beta-catenin plays a central role in setting up the head organizer in hydra. *Dev Biol*. 2010; 340:116–124. [PubMed: 20045682]
54. Andersson JO. Lateral gene transfer in eukaryotes. *Cell Mol Life Sci*. 2005; 62:1182–1197. [PubMed: 15761667]
55. Technau U, Rudd S, Maxwell P, Gordon PM, Saina M, Grasso LC, Hayward DC, Sensen CW, Saint R, Holstein TW, et al. Maintenance of ancestral complexity and non-metazoan genes in two basal cnidarians. *Trends Genet*. 2005; 21:633–639. [PubMed: 16226338]
56. Bosch TCG, David CN. Stem cells of *Hydra magnipapillata* can differentiate into somatic cells and germ line cells. *Dev Biol*. 1987; 121:182–191.
57. Campbell, RD.; Bode, HR. Terminology for morphology and cell types. In: Lenhoff, HM., editor. *Hydra: Research Methods*. New York: Plenum Press; 1983. p. 5-14.
58. Fraune S, Bosch TC. Long-term maintenance of species-specific bacterial microbiota in the basal metazoan *Hydra*. *Proc Natl Acad Sci U S A*. 2007; 104:13146–13151. [PubMed: 17664430]
59. Nilsen TW, Graveley BR. Expansion of the eukaryotic proteome by alternative splicing. *Nature*. 2010; 463:457–463. [PubMed: 20110989]
60. Stover NA, Steele RE. Trans-spliced leader addition to mRNAs in a cnidarian. *Proc Natl Acad Sci U S A*. 2001; 98:5693–5698. [PubMed: 11331766]
61. Derelle R, Momose T, Manuel M, Da Silva C, Wincker P, Houliston E. Convergent origins and rapid evolution of spliced leader trans-splicing in metazoa: insights from the ctenophora and hydrozoa. *RNA*. 2010; 16:696–707. [PubMed: 20142326]
62. Douris V, Telford MJ, Averof M. Evidence for multiple independent origins of trans-splicing in Metazoa. *Mol Biol Evol*. 2009; 27:684–693. [PubMed: 19942614]
63. Blumenthal T. Operons in eukaryotes. *Brief Funct Genomic Proteomic*. 2004; 3:199–211. [PubMed: 15642184]
64. Hastings KE. SL trans-splicing: easy come or easy go? *Trends Genet*. 2005; 21:240–247. [PubMed: 15797620]
65. Grimson A, Srivastava M, Fahey B, Woodcroft BJ, Chiang HR, King N, Degnan BM, Rokhsar DS, Bartel DP. Early origins and evolution of microRNAs and Piwi-interacting RNAs in animals. *Nature*. 2008; 455:1193–1197. [PubMed: 18830242]
66. Bode HR. The interstitial cell lineage of hydra: a stem cell system that arose early in evolution. *J Cell Sci*. 1996; 109:1155–1164. [PubMed: 8799806]
67. David CN, Gierer A. Cell cycle kinetics and development of *Hydra attenuata*. III. Nerve and nematocyte differentiation. *J Cell Sci*. 1974; 16:359–375. [PubMed: 4448826]

68. Mochizuki K, Sano H, Kobayashi S, Nishimiya-Fujisawa C, Fujisawa T. Expression and evolutionary conservation of nanos-related genes in *Hydra*. *Dev Genes Evol.* 2000; 210:591–602. [PubMed: 11151296]
69. Hartl M, Mitterstiller AM, Valovka T, Breuker K, Hobmayer B, Bister K. Stem cell-specific activation of an ancestral *myc* protooncogene with conserved basic functions in the early metazoan *Hydra*. *Proc Natl Acad Sci U S A.* 2010; 107:4051–4056. [PubMed: 20142507]
70. Cañón S, Herranz C, Manzanares M. Germ cell restricted expression of chick Nanog. *Dev Dyn.* 2006; 235:2889–2894. [PubMed: 16921504]
71. Dixon JE, Allegrucci C, Redwood C, Kump K, Bian Y, Chatfield J, Chen YH, Sottile V, Voss SR, Alberio R, et al. Axolotl Nanog activity in mouse embryonic stem cells demonstrates that ground state pluripotency is conserved from urodele amphibians to mammals. *Development.* 2010; 137:2973–2980. [PubMed: 20736286]
72. Camp E, Sanchez-Sanchez AV, Garcia-Espana A, Desalle R, Odqvist L, Enrique O'Connor J, Mullor JL. Nanog regulates proliferation during early fish development. *Stem Cells.* 2009; 27:2081–2091. [PubMed: 19544407]
73. Jager M, Queinnee E, Houlston E, Manuel M. Expansion of the SOX gene family predated the emergence of the Bilateria. *Mol Phylogenet Evol.* 2006; 39:468–477. [PubMed: 16439161]
74. Phochanukul N, Russell S. No backbone but lots of Sox: Invertebrate Sox genes. *Int J Biochem Cell Biol.* 2009;453–464. [PubMed: 19589395]
75. Denker E, Manuel M, Leclere L, Le Guyader H, Rabet N. Ordered progression of nematogenesis from stem cells through differentiation stages in the tentacle bulb of *Clytia hemisphaerica* (Hydrozoa, Cnidaria). *Dev Biol.* 2008; 315:99–113. [PubMed: 18234172]
76. Martin V, Archer W. Migration of interstitial cells and their derivatives in a hydrozoan planula. *Dev Biol.* 1986; 116:486–496.
77. Müller WA, Teo R, Frank U. Totipotent migratory stem cells in a hydroid. *Dev Biol.* 2004; 275:215–224. [PubMed: 15464584]
78. Rebscher N, Volk C, Teo R, Plickert G. The germ plasm component Vasa allows tracing of the interstitial stem cells in the cnidarian *Hydractinia echinata*. *Dev Dyn.* 2008; 237:1736–1745. [PubMed: 18489004]
79. Marlow HQ, Srivastava M, Matus DQ, Rokhsar D, Martindale MQ. Anatomy and development of the nervous system of *Nematostella vectensis*, an anthozoan cnidarian. *Dev Neurobiol.* 2009; 69:235–254. [PubMed: 19170043]
80. Nakanishi N, Yuan D, Jacobs DK, Hartenstein V. Early development, pattern, and reorganization of the planula nervous system in *Aurelia* (Cnidaria, Scyphozoa). *Dev Genes Evol.* 2008; 218:511–524. [PubMed: 18850237]
81. Stangl K, Salvini-Plawen L, Holstein TW. Staging and induction of medusa metamorphosis in *Carybdea marsupialis* (Cnidaria, Cubozoa). *Vie et Milieu.* 2002; 52:131–140.
82. Houlston E, Momose T, Manuel M. *Clytia hemisphaerica*: a jellyfish cousin joins the laboratory. *Trends Genet.* 2010; 26:159–167. [PubMed: 20227783]
83. Miller DJ, Ball EE. The coral *Acropora*: what it can contribute to our knowledge of metazoan evolution and the evolution of developmental processes. *Bioessays.* 2000; 22:291–296. [PubMed: 10684589]
84. Wittlieb J, Khalturin K, Lohmann JU, Anton-Erxleben F, Bosch TCG. Transgenic *Hydra* allow in vivo tracking of individual stem cells during morphogenesis. *Proc Natl Acad Sci U S A.* 2006; 103:6208–6211. [PubMed: 16556723]
85. Renfer E, Amon-Hassenzahl A, Steinmetz PR, Technau U. A muscle-specific transgenic reporter line of the sea anemone, *Nematostella vectensis*. *Proc Natl Acad Sci U S A.* 2010; 107:104–108. [PubMed: 20018670]
86. Pankow S, Bamberger C. The p53 tumor suppressor-like protein nvp63 mediates selective germ cell death in the sea anemone *Nematostella vectensis*. *PLoS ONE.* 2007; 2:e782. [PubMed: 17848985]
87. Lohmann JU, Bosch TC. The novel peptide HEADY specifies apical fate in a simple radially symmetric metazoan. *Genes Dev.* 2000; 14:2771–2777. [PubMed: 11069893]

88. Lohmann JU, Endl I, Bosch TC. Silencing of developmental genes in Hydra. *Dev Biol.* 1999; 214:211–214. [PubMed: 10491269]
89. Smith KM, Gee L, Bode HR. HyAlx, an aristaless-related gene, is involved in tentacle formation in hydra. *Development.* 2000; 127:4743–4752. [PubMed: 11044390]
90. Cardenas MM, Salgado LM. STK, the src homologue, is responsible for the initial commitment to develop head structures in Hydra. *Dev Biol.* 2003; 264:495–505. [PubMed: 14651933]
91. Chera S, de Rosa R, Miljkovic-Licina M, Dobretz K, Ghila L, Kaloulis K, Galliot B. Silencing of the hydra serine protease inhibitor Kazal1 gene mimics the human SPINK1 pancreatic phenotype. *J Cell Sci.* 2006; 119:846–857. [PubMed: 16478786]
92. Miljkovic-Licina M, Chera S, Ghila L, Galliot B. Head regeneration in wild-type hydra requires de novo neurogenesis. *Development.* 2007; 134:1191–1201. [PubMed: 17301084]
93. Hemmrich G, Anokhin B, Zacharias H, Bosch TC. Molecular phylogenetics in Hydra, a classical model in evolutionary developmental biology. *Mol Phylogenet Evol.* 2007; 44:281–290. [PubMed: 17174108]
94. Fisher DA, Bode HR. Nucleotide sequence of an actin-encoding gene from Hydra attenuata: structural characteristics and evolutionary implications. *Gene.* 1989; 84:55–64. [PubMed: 2606361]
95. Medina M, Collins AG, Takaoka TL, Kuehl JV, Boore J. Naked corals: skeleton loss in Scleractinia. *Proc Natl Acad Sci U S A.* 2006; 103:9096–9100. [PubMed: 16754865]
96. Voigt O, Erpenbeck D, Worheide G. A fragmented metazoan organellar genome: the two mitochondrial chromosomes of Hydra magnipapillata. *BMC Genomics.* 2008; 9:350. [PubMed: 18655725]

Figure 1.

Cnidarian Phylogeny. The figure shows the evolutionary relationships among sponges, bilaterians (protostomes and deuterostomes), and the five classes in the phylum Cnidaria. Hydrozoa, Scyphozoa, Cubozoa, and Staurozoa belong to the subphylum Medusozoa. Staurozoa is a recently defined class that includes the stauromedusae [3]. Its evolutionary relationship to the other medusozoan classes is not certain, but the current evidence supports a basal branching within Medusozoa. The *Hydra* picture was taken by Peter Bryant and the *Nematostella* picture by Jens Fritzenwanker.

Figure 2.

Fate of Hox megacluster genes in *Hydra* and *Nematostella*. The figure shows the genes present in *Hydra* and *Nematostella* that derived from the Hox megacluster (top) present in the ancestor of cnidarians and bilaterians [45]. The megacluster contained the Hox and ParaHox genes, and genes of the NK cluster. Uncolored genes are present in both *Hydra* and *Nematostella*. Colored genes are missing from *Hydra*. Gene nomenclature and arrangements in the megacluster are from Garcia-Fernández [45]. Gene presence/absence data are from Chourrout et al. [46]. Note that some of the details of the current gene linkage patterns have been ignored for simplicity, e.g. the *Evx* gene in *Nematostella* is actually located between two Hox genes, and the *Mnx* gene is linked to the Hox/*Evx* cluster.

Table 1Comparison of the *Hydra magnipapillata* and *Nematostella vectensis* genomes

Genome Feature	<i>Hydra magnipapillata</i>	<i>Nematostella vectensis</i>	References
Haploid genome size	~1 Gb	~0.45 Gb ~0.22 Gb*	[1,2,93]
Diploid chromosome number	30	30	[1,12]
Base composition	71% A+T	59% A+T	[1,14,94]
Predicted protein coding genes	~20,000	~18,000	[1,2]
Transposable element content	~57%	~26%	[1,2]
Trans-spliced leader addition	Yes	No	[60,62]
Operons	Probably	No	[2]
Homeobox gene cluster	No	No	[46]
Telomere sequence	TTAGGG and TCAGGG repeats	TTAGGG repeats	[2,18]
Mitochondrial genome	two 8 kb linear molecules	single 16 kb circular molecule	[95,96]
Cytosine methylation	Yes	Yes	[14,15]
microRNAs	17	40	[2,65]

* The 0.45 Gb estimate is from the assembled genome sequence. The 0.22 Gb estimate is from Feulgen image analysis densitometry of embryo cells (T. R. Gregory, www.genomesize.com).