Review

A genomic view of 500 million years of cnidarian evolution

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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, researchers 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. It is now known what cnidarian genomes, given 500 million years, are capable of; as we discuss here, the next challenge is to understand how this genomic history has led to the striking diversity seen in this group.

Why cnidarian genomics?

The phylum Cnidaria contains ~9000 species with remarkably diverse form and function, from the inconspicuous freshwater *Hydra* to 100-ft 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 (Figure 1): Anthozoa (corals, sea anemones and sea pens); Hydrozoa (hydras and marine hydrozoans); Cubozoa (box jellyfish); Scyphozoa (true jellyfish); and Staurozoa (stalked jellyfish) [3]. 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 (Figure 1). A sequence phylogeny based on 337 orthologous proteins indicates that the split between the *Hydra* and *Nematostella* lineages is ancient, having occurred at approximately 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, *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, by comparing the genomes of these two organisms, we are sampling a 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 that 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 ~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 million years ago [13]. Thus, there have 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 less repetitive DNA than does *Hydra* and there is no evidence for bursts of transposon expansion similar to those in *Hydra* [2].

The *Hydra* and *Nematostella* genomes also differ in their base composition, with the *Hydra* genome being more AT-rich (71% AT) than is the *Nematostella* genome (59.4% AT). Both genomes undergo methylation of cytosines in CpG dinucleotides [14,15]. This suggests that cnidarians are 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 interest because of the apparent absence of senescence in

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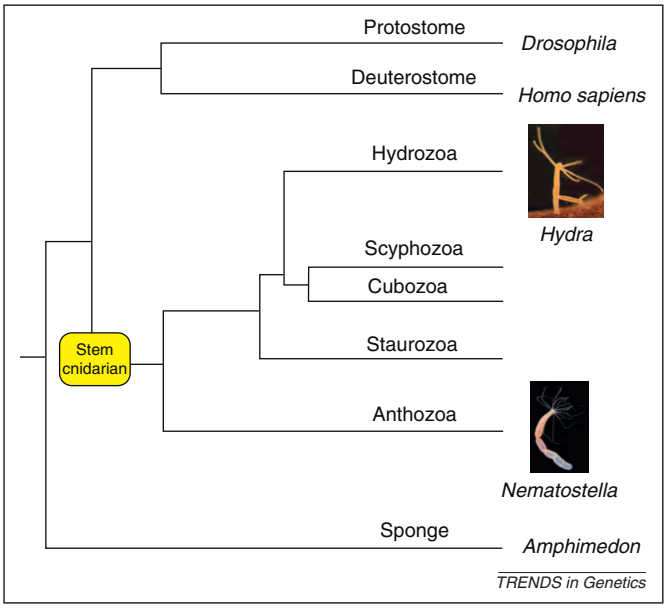

Figure 1. Cnidarian phylogeny. The figure shows the evolutionary relationships among sponges, bilaterians (protostomes and deuterostomes), and the five classes in the phylum Cnidaria. Hydrozoa, Scyphozoa, Cubozoa, and Staurozoa belong to the subphylum Medusozoa. Staurozoa is a recently defined class that includes the stauromedusae [3]. Its evolutionary relationship to the other medusozoan classes is not certain, but current evidence supports a basal branching within Medusozoa. Images reproduced with permission from Peter Bryant (*Hydra*) and Jens Fritzenwanker (*Nematostella*).

Hydra [17]. *Hydra* and *Nematostella* chromosomes contain typical animal telomeres [2,18]. Evidence that telomeres in cnidarians are maintained in a canonical fashion comes from 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 been on a faster evolutionary track than has 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 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 also be lineage-specific genes. A recent comparison of *Hydra* and *Nematostella* gene predictions indicates that 15% of the genes in each genome are lineage specific [19]. By 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, might change when the complete genome sequence becomes available. Conversely, each of the three species has lost significant numbers of conserved RefSeq hits that are 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 because they encode proteins that function in biological processes or cell types that are unique to cnidarians.

Nematocytes, or stinging-cells, are a unique cnidarian-specific cell type used for prey capture. Many nematocyte-specific genes identified so far have orthologs in *Hydra* and *Nematostella*, but no homologs 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, whereas hydrozoans have more nematocyst types and *Hydra* has 17 minicollagen genes [24]. Similarly, the gene encoding nematogalectin (a nematocyst tubule-specific protein) is present in only one copy in anthozoans, whereas

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

Genome feature	*Hydra magnipapillata*	*Nematostella vectensis*	Refs
Haploid genome size	~1 Gb	~0.45 Gb; ~0.22 Gb[a]	[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]

[a]The 0.4-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, http://www.genomesize.com).

hydrozoans have two copies, one of which forms a novel structure found only in hydrozoan nematocysts [25]. Although 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 genes encoding nematocilin in *Hydra* and none in the anthozoan *Nematostella*. Sequence comparisons indicate that the gene encoding nematocilin evolved following duplication of the gene encoding nuclear lamin 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 with 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], have roles in patterning and cell differentiation [30]. The 301 peptide family is uniquely found in *Hydra*, where it is differentially expressed in the head region and is 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 TRGs, 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 17 genes encoding innexin, whereas *Nematostella* has only one [2]. *Clytia* has at least eight genes encoding innexin based on ESTs [2], suggesting that expansion of the innexin gene family is a feature of hydrozoans. In *Hydra*, the increase in genes encoding innexin is accompanied by electron microscopic evidence of gap junctions between several cell types, including epithelial and nerve cells [35,36]. That the genes encoding innexin 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 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 variable extracellular Ig-related domains and are responsible for the fusion-rejection capability of *Hydractinia* colonies [44]. Homologs 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 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 several gene linkages in the *Nematostella* genome [46]. In *Hydra*, massive loss of genes arising from the megacluster has occurred (Figure 2) and, overall, *Hydra* has retained only approximately half of the homeobox genes found in *Nematostella* [46]. Some of these losses are apparently relatively recent given that 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 has *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* 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 is the Wnt genes, of which *Nematostella* has 12 and *Hydra* has nine out of 13 subfamilies [48,49]. This diversity in both cnidarians contrasts with the situation in sponges, which have only three Wnt genes, none of which can be placed reliably in specific Wnt subfamilies [50]. 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 involved in axial development and regeneration in cnidarians [51–53]. The high regenerative capacity of cnidarians might be 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], which has been addressed recently in cnidarians using ESTs and genome

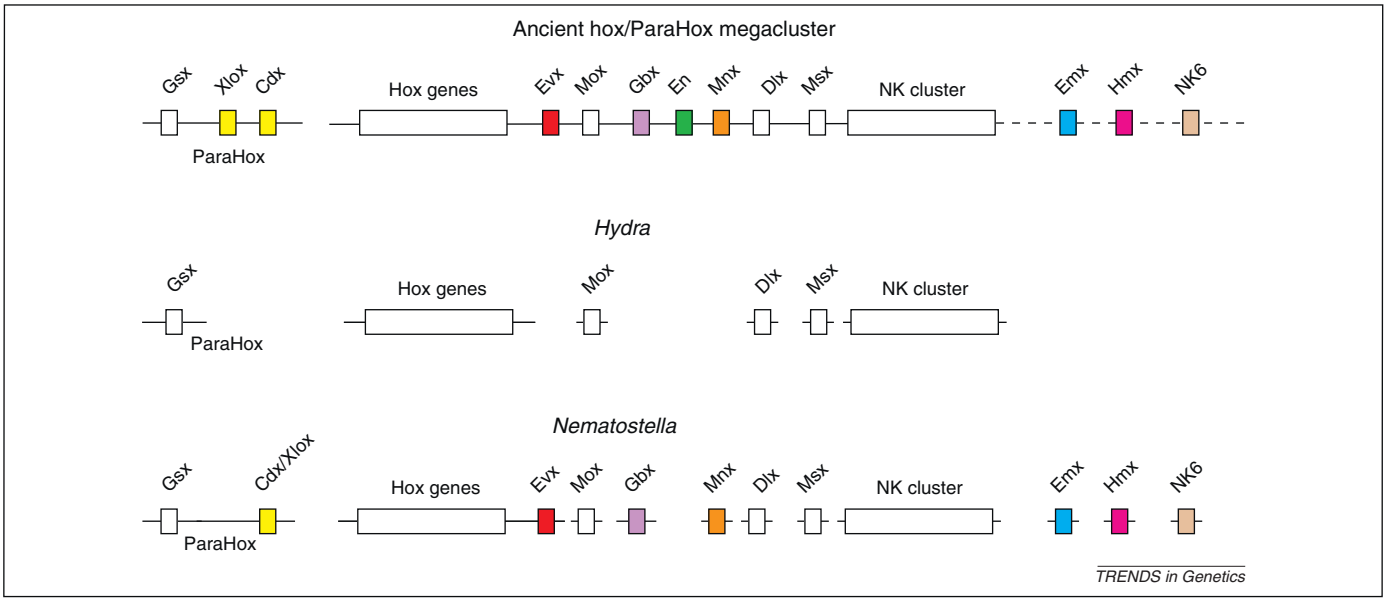

Figure 2. Fate of Hox megacluster genes (top) in *Hydra* (middle) and *Nematostella* (bottom). The figure shows the genes present in *Hydra* and *Nematostella* that derived from the Hox megacluster present in the ancestor of cnidarians and bilaterians [45]. The megacluster contained the Hox and ParaHox genes, and genes of the NK cluster. Uncolored genes are present in both *Hydra* and *Nematostella*. Colored genes are missing from *Hydra*. Some of the details of the current gene linkage patterns have been ignored for simplicity (e.g. the *Evx* gene in *Nematostella* is located between two Hox genes, and the *Mnx* gene is linked to the Hox/*Evx* cluster). Gene nomenclature and arrangements in the megacluster are from [45]; gene presence/absence data are from [46].

sequences. From an analysis of ESTs from *Nematostella* and the coral *Acropora* [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 HGT 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 homologs have not yet 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 to be transmitted to subsequent generations. This is particularly problematic in metazoans, because 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 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, researchers are beginning to explore how these mechanisms are used by cnidarians. Alternative splicing occurs in >95% of all transcribed genes in humans, yielding a significant expansion of the proteome [59]. From EST datasets 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, *trans*-spliced 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. More than 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 miRNAs have been detected so far [2], but this is probably 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 homologs [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 fish [72], but it has not been reported outside of vertebrates. No clear homolog 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 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 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 arising within hydrozoans after the diversification of cnidarian classes. It will be of interest to understand this independent evolutionary experiment in producing stem cells, which could reveal the molecular logic underlying 'stemness'.

Concluding remarks

With the sequencing of the *Hydra* and *Nematostella* genomes, one can see the end results of two ~500-million-year-long experiments in cnidarian genome evolution. It is now clear that cnidarian genomes are more plastic than one 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.

However, researchers are still missing the historical record written in the pages of other cnidarian genomes, including those from three of the five cnidarian classes. More cnidarian genome sequences are required and, to this end, 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, several other cnidarian genome sequences are likely to be revealed, as well as deep sequencing of cnidarian transcriptomes.

In addition to providing a wealth of data for bioinformatics mining, the *Hydra* and *Nematostella* genome sequences provide 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 facilitated by: (i) robust methods that are now available for generating stably transgenic *Hydra* [84] and *Nematostella* [85]; and (ii) 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 them 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 are found in 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.

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