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The *Hydra* genome: insights, puzzles and opportunities for Developmental Biologists

ROBERT E. STEELE*

Department of Biological Chemistry and the Developmental Biology Center, University of California, Irvine, CA, USA

ABSTRACT The sequencing of a *Hydra* genome marked the beginning of a new era in the use of *Hydra* as a developmental model. Analysis of the genome sequence has led to a number of interesting findings, has required revisiting of previous work, and most importantly presents new opportunities for understanding the developmental biology of *Hydra*. This review will describe the history of the *Hydra* genome project, a selection of results from it that are relevant to developmental biologists, and some future research opportunities provided by *Hydra* genomics.

KEY WORDS: *Hydra, genome, evolution, Cnidaria*

Introduction

It has become creed that understanding the biology of an organism in depth requires the sequence of its genome. The genome sequence provides a parts list, informs regarding the evolutionary history of the organism, and is essential for carrying out functional studies that involve manipulating genes. While *Hydra* has been used as an experimental organism for nearly 270 years (Trembley, 1744), our ability to investigate its biology has increased dramatically since the sequence of its genome became available (Chapman *et al.*, 2010). *Hydra* is a cnidarian in the family Hydrozoa (Fig. 1). Unlike most hydrozoans, *Hydra* lives in freshwater and lacks the larval and medusa stages typical of hydrozoan life cycles. Two recent phylogenetic analyses (Kawaida *et al.*, 2010; Martínez *et al.*, 2010) have shown that the genus *Hydra* consists of four clades (Fig. 2), that have diverged from a common ancestor over the last 50-60 million years.

Hydra's appeal as a model for studying developmental processes lies in the simplicity of its composition and structure (Campbell and Bode, 1983), its remarkable powers of regeneration (Holstein *et al.*, 2003; Bosch, 2007), and its accessibility to a variety of experimental manipulations (Lenhoff, 1983). The adult *Hydra* polyp consists of two concentrically arranged epithelial layers surrounding a gastric cavity (Campbell and Bode, 1983). The animal has a single axis; at the oral end is the mouth opening, surrounded by a ring of tentacles and at the aboral end is an adhesive disk called the foot or basal disk. In addition to the two epithelial cell layers, which constitute distinct developmental lineages in the adult polyp, *Hydra* has a third cell lineage, the interstitial cell (i-cell) lineage. This lineage contains multipotent stem cells that give rise to nerve cells, nematocytes, gland cells, and gametes (David and Murphy, 1977; Campbell and Bode, 1983; Bode, 1996).

Hydra has served as a research model for pattern formation, cell differentiation, and stem cell biology. Studies of pattern formation in *Hydra* have focused on how the single axis of the adult polyp is maintained during growth, asexual budding, and regeneration (Bode, 2011; Bode, 2012; Shimizu, 2012). Research on cell differentiation in *Hydra* has primarily been concerned with how the various differentiation products of the i-cell lineage – nerves, nematocytes, gametes and gland cells - are generated and maintained (Bode, 1996; David, 2012; Nishimaya-Fujisawa, 2012). The multipotent stem cells that give rise to the differentiated cells of the i-cell lineage (David and Murphy, 1977; Bosch and David, 1987) have been the focus of studies to understand how stem cells function and their evolutionary history in metazoans (Bosch, 2009).

Hydra *developmental biology before genomics*

While studies at the cell and tissue levels had led to many important insights into developmental processes in *Hydra*, it became clear in the mid-1980s that the application of molecular methods, particularly cloning of genes, would be essential for further progress. Reports of the cloning of the first protein-coding genes from *Hydra*, a cytoplasmic actin gene (Fisher and Bode, 1989) and a *src* family protein-tyrosine kinase gene (Bosch *et al.*, 1989), were published in 1989. Subsequently other genes, particularly *Hydra* homologues of genes with known developmental roles in other metazoans (e.g. homeobox genes), were cloned from *Hydra* by screening of cDNA libraries with oligonucleotides, heterologous cloned genes, or differential cDNA probes, and by PCR (Kurz *et al.*, 1991; Chan *et al.*, 1992; Holstein *et al.*, 1992; Schlaepfer *et al.*, 1992; Schummer *et*

***Address correspondence to:** Robert E. Steele. Department of Biological Chemistry Room D240, Medical Sciences I University of California, Irvine Irvine, CA 92697-1700, USA. Tel: +1-949-824-7341. Fax: +1-949-824-2688. e-mail: resteele@uci.edu

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al., 1992; Naito *et al.*, 1993; Shenk *et al.*, 1993). Having cloned genes in hand made it possible to begin investigating the roles of genes in developmental processes in *Hydra*. With the development of a robust whole-mount *in situ* hybridization method for use with *Hydra* (Grens *et al.*, 1995; Grens *et al.*, 1996), it was possible to examine gene expression patterns in spatial and temporal detail in normal and experimentally manipulated animals. These manipulations included regeneration, changing of cell composition (Bode, 1983), and chemical treatments to perturb development (Müller, 1989; Hassel and Berking, 1990; Broun *et al.*, 2005).

Expression patterns in and of themselves often provided insight into likely developmental roles for genes in *Hydra*. Particularly good examples include the *HyAlx* gene and the *Wnt3* gene. The *HyAlx* gene is expressed in a ring of ectodermal epithelial cells at the base of the tentacles (Smith *et al.*, 2000). This expression pattern immediately suggested a role for the *HyAlx* gene in the formation of tentacles, a role that was confirmed experimentally (Smith *et al.*, 2000). The *Wnt3* gene is expressed in a small number of epithelial cells at the tip of the hypostome (Hobmayer *et al.*, 2000). Because it was known that the tip of the hypostome had organizer activity (Browne, 1909), the expression pattern of *Wnt3* suggested that Wnt signaling was a component of the axial organizer. Subsequent studies (Broun *et al.*, 2005) demonstrated that the canonical Wnt signaling pathway is a key component of the *Hydra* head organizer.

While expression studies of cloned genes accelerated progress in our understanding of the genes involved in developmental processes in *Hydra*, methods for specifically altering the expression of a given gene were clearly needed. With the discovery of RNA interference (RNAi), it was obvious that this method could be an important tool for carrying out functional studies of genes in *Hydra*. The first attempt to perturb gene expression in *Hydra* by RNAi involved electroporation of dsRNA corresponding to the *ks1* gene, a gene expressed in the tentacles and the hypostome (Lohmann *et al.*, 1999). As expected for a gene putatively involved in head formation, polyps exposed to the *ks1* dsRNA showed marked delays in head regeneration. RNAi carried out by electroporation has been used to study the functions of additional *Hydra* genes (Lohmann and Bosch, 2000; Smith *et al.*, 2000; Cardenas and Salgado, 2003; Takahashi *et al.*, 2005; Amimoto *et al.*, 2006; Khalturin *et al.*, 2008), and a method has now been developed for carrying out RNAi in *Hydra* by feeding the animals bacteria producing dsRNA for the target gene (Chera *et al.*, 2006; Miljkovic-Licina *et al.*, 2007; Chera *et al.*, 2009; Chera *et al.*, 2011). Despite these efforts, RNAi is not yet a robust, widely used technology for functional studies in *Hydra*. It is hoped that continued efforts will lead to gene knockdown methods that can be easily and reproducibly applied to any *Hydra* gene of interest.

By the end of the 1990s it became clear that the "one gene at a time" approach to identifying and studying genes involved in development in *Hydra* was limiting the rate at which knowledge could be acquired. A genomics solution was clearly needed.

Genomics come to Hydra *developmental biology*

Genomics research with *Hydra* began in 2001 with two EST projects, one funded by the United States National Science Foundation and carried out as a collaborative effort between UC Irvine and the Genome Sequencing Center at Washington University and the other carried out at the National Institute of Genetics in Mishima, Japan. Together these projects generated ~170,000 ESTs from *Hydra*. The U.S. project also sequenced ESTs from two other hydrozoans: ~3500 ESTs from *Podocoryna carnea* and ~9000 ESTs from *Hydractinia echinata* (Soza-Ried *et al.*, 2010).

Sequencing of a *Hydra* genome was recommended by the Comparative Genome Evolution Working Group at the National Human Genome Research Institute (NHGRI) in 2004. Approval for sequencing of 18 organisms, including *Hydra magnipapillata*, was announced in an NHGRI press release on August 4, 2004. Strain 105 of *Hydra magnipapillata* was chosen to be sequenced because of its widespread use for *Hydra* research and the fact that it was used for construction of most of the cDNA libraries for the two *Hydra* EST projects. In addition the 105 strain of *Hydra magnipapillata* was used for the *Hydra* Peptide Project (Takahashi *et al.*, 1997; Fujisawa, 2008) and a number of mutant strains of *H. magnipapillata* had been isolated by Tsutomu Sugiyama's group in Mishima (Sugiyama and Fujisawa, 1977; Sugiyama, 1983; Shimizu, 2012). The 105 strain of *H. magnipapillata* was recloned from a single polyp in Hans Bode's lab at UC Irvine to provide a source of DNA. The genome was sequenced by the Sanger whole genome shotgun method at the J. Craig Venter Institute; sequencing coverage was ~6x. Fixed voucher specimens of the 105 strain from UC Irvine were deposited in the invertebrate collections at Yale's Peabody Museum. In

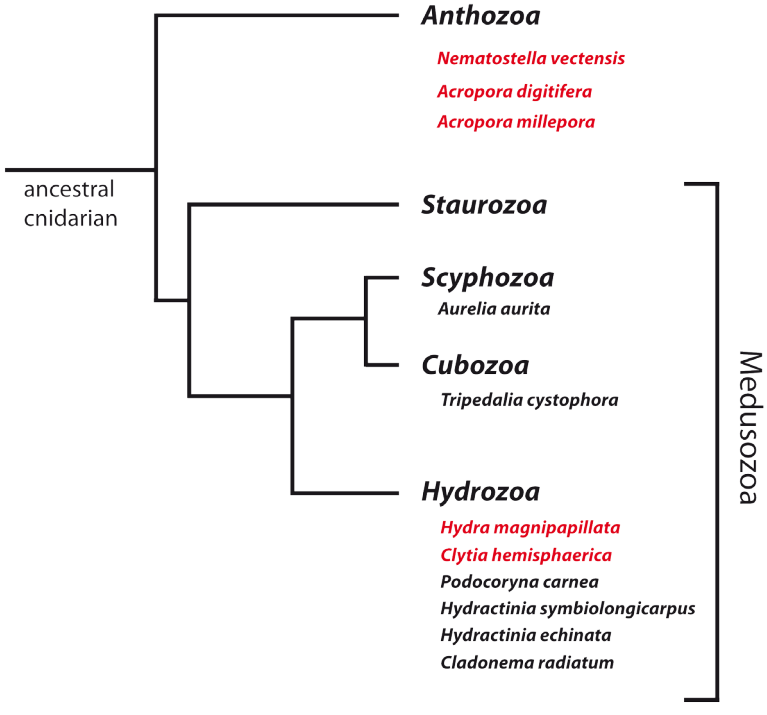

Fig. 1. Cnidarian phylogenetic tree. *The branching order for the five cnidarian classes is shown (Marques and Collins, 2004; Collins* et al.*, 2006). Species that are model systems for studies at the molecular and genomics level in each class are shown and those whose genomes have been sequenced are in red.* Podocoryna carnea *is commonly, but incorrectly, referred to as* Podocoryne carnea *(Calder, 1988). Citations for the genome projects are as follows:* Hydra magnipapillata *(Chapman* et al.*, 2010);* Nematostella vectensis *(Putnam* et al.*, 2007);* Clytia hemisphaerica *(Houliston* et al.*, 2010);* Acropora digitifera *(Shinzato* et al.*, 2011);* Acropora millepora *(www.coralbase.org).*

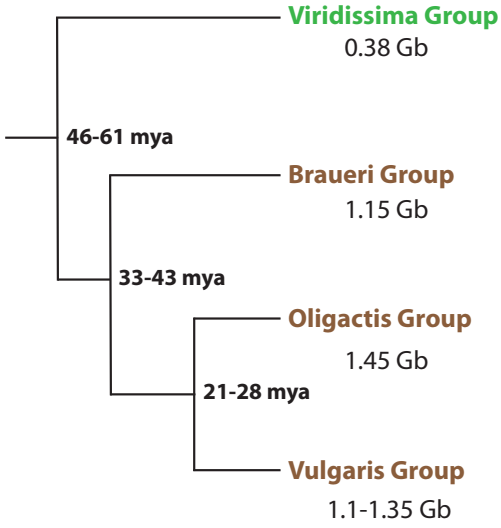

Fig. 2. Phylogenetic tree of the genus *Hydra*. *The tree is adapted from Martínez* et al., *(Martínez* et al., *2010). Estimated times in millions of years ago (mya) for the divergences of the various clades are indicated. The Viridissima group consists of the green* Hydra *species* H. viridissima, *which contains algal endosymbionts. The Braueri, Oligactis, and Vulgaris groups contain brown* Hydra *species, so-called because they lack algal endosymbionts. Under the group names are the genome sizes (in gigabase pairs) measured for species within the group (Zacharias* et al., *2004; Hemmrich* et al., *2007). Only single species have been measured from the Viridissima, Braueri, and Oligactis groups. Three species have been measured from the Vulgaris group.*

addition, the Venter Institute deposited the remaining genomic DNA from the project in the Peabody Museum's Cryo Collection (http://peabody.yale.edu/collections/cryo-facility/cryo- facility).

Following the completion of sequencing, two assemblies of the genome were done. A detailed description of the two assemblies is given in the Supplementary Material included with the paper describing the genome sequence (Chapman *et al.*, 2010). Both assemblies are of relatively low quality, i.e. the contigs are short, compared to the assemblies of other animal genomes. This is largely because it was not possible to obtain large insert libraries (e.g. BAC libraries) due to the instability of the A+T-rich *Hydra* DNA in *E. coli* (Pieter de Jong *et al.*, unpublished observations). Fosmid cloning was attempted at the Venter Institute but only about one third of the resulting clones yielded usable sequence. Efforts are under way to use a combination of happy mapping and Illumina sequencing to improve the assembly (Jiang *et al.*, 2009). Long read sequencing methods, e.g. strobe sequencing with the PacBio RS system (Ritz *et al.*, 2010), provide another possible approach for improving the assembly of the *Hydra* genome.

What have we learned from the genome sequence?

The results from a number of analyses of the *Hydra* genome sequence have been reported by Chapman *et al.,* (2010) and recently reviewed by Steele *et al.,* (2011). The focus here will be on those aspects of the genome that are particularly relevant to developmental biology.

What genes are present?

One of the first questions that the genome sequence provides an answer to is "are there *Hydra* orthologues of genes known to play key developmental roles in the well-studied bilaterian developmental models? The answer to this question is a definite yes. *Hydra* has all of the major classes of signaling molecules and transcription factors that are well known from studies in bilaterians. And in some cases, the number of genes in the family is similar to that in bilaterians. For example, the Wnt gene family in *Hydra* is comparable in size to that of bilaterians (Lengfeld *et al.*, 2009), indicating that much of the diversification of this developmentally important family preceded the divergence of cnidarians from other metazoans.

Having a sequenced genome has substantially accelerated progress in delineating the components of developmental pathways in *Hydra*. Rather than having to identify genes encoding the various components in a developmental pathway or developmental process one at a time, all of the genes can be identified quickly in silico. Examples of this are presented in the *Hydra* genome paper (Chapman *et al.*, 2010) in which genes potentially involved in head organizer function and in cell-cell and cell-substrate interactions were cataloged.

While the *Hydra* genome was being sequenced, a suite of genes (*Myc*, *Nanog*, *Klf4*, *Oct4*, and *Sox2*) that confers pluripotency on differentiated mammalian cells was identified by Yamanaka's group (Takahashi and Yamanaka, 2006). Given *Hydra*'s history as a model for the study of stem cells (Bode, 1996; Bosch, 2009), it was of considerable interest to search the genome for homologues of the mammalian pluripotency-inducing genes. This search has not yet reached a clear conclusion. We can say with certainty that *Myc* genes are present in *Hydra (Hartl et al., 2010)*. At least one of the *Hydra Myc* genes is expressed strongly in the stem cells of the interstitial lineage (Hartl *et al.*, 2010; Hobmayer *et al.*, 2012).

It appears that *Nanog* and *Klf4* genes are absent from the *Hydra* genome (Chapman *et al.*, 2010). The situation with *Oct4*, a member of class 5 of the POU family, is currently unclear. An *Oct4* homologue appears to be absent from *Hydra*. However, *Polynem*, a POU family gene in *Hydractinia*, has been shown to have a role in stem cell development (Millane *et al.*, 2011; Plickert *et al.*, 2012). Phylogenetic analysis does not resolve the relationship of *Polynem* to other members of the POU family (Millane *et al.*, 2011). Additional studies of the POU family in cnidarians are clearly warranted to clarify the evolutionary history of members of this family and their role in stem cell biology. Functional studies, for example determining whether putative cnidarian homologues of *Oct4* can functionally substitute for *Oct4* in a mammalian setting, are particularly important in addressing this issue since sequence comparisons alone cannot determine whether genes have shared functions.

At the time the genome paper was published, the evolutionary relationship between the two *Hydra* genes in the *SoxB* group (the *SoxB* group includes *Sox2*) and *Sox2* genes in vertebrates was not clear. Since then, a detailed study of the phylogeny and expression patterns of the *Sox* genes in the hydrozoan *Clytia hemisphaerica* has appeared (Jager *et al.*, 2011). This study showed that members of the *SoxB* group in *Clytia* do not have an orthologous relationship to the *SoxB* group members in vertebrates. Nonetheless, some of the *SoxB* group genes in *Clytia* are expressed in stem cells (Jager *et al.*, 2011). An interpretation of these results is that the ancestral *SoxB* group gene had an ancient, conserved role in stem cell function that pre-dates the allocation of this role to individual

SoxB group paralogues in hydrozoans and vertebrates. As with *Oct4*, functional studies will be required to test this hypothesis.

What genes are absent?

Perhaps as interesting as finding out what genes involved in development are present in the *Hydra* genome is finding out what genes are absent. Comparisons of the hox/parahox superfamily of homeobox genes in *Hydra* with other hydrozoans and the anthozoan *Nematostella* have shown that this superfamily has suffered a number of losses in *Hydra* (Chourrout *et al.*, 2006; Quiquand *et al.*, 2009; Chapman *et al.*, 2010; Steele *et al.*, 2011). These losses have generated in *Hydra* what is essentially a "natural knockout" line for the missing genes, demonstrating that it is possible to build a metazoan without these particular genes. Most interesting of these losses is the absence of *Emx* and *Evx* orthologues. Orthologues of these two genes have been identified in other cnidarians including the hydrozoans *Sarsia* sp. (Bridge and Steele, unpublished; GenBank accession number AF326771), *Hydractinia symbiolongicarpus* (Mokady *et al.*, 1998), *Clytia hemisphaerica* (Chiori *et al.*, 2009), *Podocoryne carnea* (D. Bridge and D. Martínez, unpublished), and *Cladonema pacificum* (R. Greenspan *et al.*, unpublished). Comparisons of the life cycles of *Hydra* and these other hydrozoans leads to the conclusion that absence of the *Emx* and *Evx* genes in *Hydra* correlates with the absence of the planula larva stage (Chapman *et al.*, 2010). This is an interesting finding that suggests the possibility that loss of these genes was causative in the loss of the planula stage of the *Hydra* life cycle or that these two genes became dispensable when the planula stage was lost. It would obviously be of interest to know whether either of these genes is missing in other members of the clade Aplanulata (i.e. hydrozoans that lack a planula larva) (Collins *et al.*, 2006).

Surprisingly absent from the *Hydra* genome assembly are genes encoding two previously characterized proteins that have been reported to play roles in regeneration and patterning. An 11-amino acid peptide termed head activator (HA) was isolated from *Hydra* in the late 1970s based on its ability to accelerate head regeneration (Schaller, 1973; Schaller and Bodenmüller, 1981). HA was studied intensively, but a sequence that could encode it has not been identified in the *Hydra* genome. In addition to being reported from cnidarians, HA was also reported to be present in mammals (Schaller, 1975). This suggests that it should be present in the genomes of many metazoans. Thus we are left with a mystery. Given that it contains only 11 amino acids, HA could be synthesized by a route that does not involve translation. If HA is encoded by a gene and its predicted 33 nucleotide coding sequence is interrupted by one or more introns, it would be difficult to identify the gene. It is also possible that the HA gene is contained in a portion of the *Hydra* genome that is not included in the assembled version.

Another puzzling case of a missing gene is that of the peptide HEADY (Lohmann and Bosch, 2000). A fragment of HEADY cDNA was identified by a differential display PCR screen for genes whose expression changes during regeneration. The putative full-length 369 nucleotide HEADY cDNA is predicted to encode a 23-amino acid precursor protein, which is predicted to yield the mature 12 amino acid HEADY peptide following proteolytic processing. The HEADY cDNA sequence is not found in the *Hydra* genome assembly. As with the case of HA, the absence of the HEADY gene from the *Hydra* genome assembly could be due to incompleteness of the assembly. Since the original publication on HEADY in 2000, no additional information on this gene has appeared.

That gap junctions are present in *Hydra* was convincingly demonstrated by electron microscopic studies (Wood and Kuda, 1980) and by dye coupling experiments (Fraser and Bode, 1981). That they have developmental roles in *Hydra* was first suggested by the study of Fraser *et al.,* (1987) in which a polyclonal antiserum against a vertebrate connexin was shown to decrease the efficacy of the head inhibition gradient when the antibodies were introduced into *Hydra*. These results were interpreted as indicating that the inhibitor either moved through gap junctions are acted through a pathway involving gap junctions. Given these results, it came as a surprise when no connexins gene could be found in the *Hydra* genome. There were however, genes encoding innexins (Alexopoulos *et al.*, 2004), the proteins that were known to form gap junctions in other invertebrates. Given the absence of any sequence conservation between innexins and connexins, it seems unlikely that the results of Fraser *et al.,* can be explained by cross-reaction between the vertebrate connexin antibody and *Hydra* innexins. Nonetheless, the hypothesis that gap junctions have a role in patterning as originally tested by the studies of Fraser *et al.,* (1987) is still valid and clearly worth revisiting, with innexins being the test subject this time around.

What genes are novel?

While genes that are conserved among animals are of obvious importance for understanding *Hydra* development, there was reason to expect that *Hydra* had evolved novel genes that play important roles in development. This possibility was the motivation for the *Hydra* peptide project (Takahashi *et al.*, 1997; Fujisawa, 2008), which had the goal of identifying novel peptides involved in developmental processes in *Hydra* (Fujisawa and Hayakawa, 2012). This project has led to the identification of the Hym-301 peptide, which is involved in head formation, Hym-355 and the PW peptides, which play stimulatory and inhibitory roles, respectively in nerve cell differentiation, and Hym-346 and Hym-323 peptides, which have roles in foot formation (Grens *et al.*, 1999; Takahashi *et al.*, 2000; Takahashi *et al.*, 2005; Takahashi and Fujisawa, 2009; Takahashi *et al.*, 2009). Although Hayakawa *et al.,* (2007) have shown that a computational approach can be used to identify potential precursors to peptides in *Hydra* in ESTs or in gene models from the *Hydra* genome, functional screens of the sort developed by the *Hydra* Peptide Project clearly still have a role to play in defining the molecules that control developmental processes in *Hydra*, with the genome sequence playing an enabling role.

Further studies of Hym-301 (Khalturin *et al.*, 2008) led Khalturin *et al.,* (2009) to develop the concept of taxonomically restricted genes (TRGs). TRGs are genes that are found in a restricted set of taxa and which control taxon-specific features. The Hym-301 gene fits the criterion for a TRG since it is found in *Hydra* but in no other animals, including the anthozoan *Nematostella*. When a transgenic line was created that overexpressed Hym-301, alterations in tentacle development and number were seen (Khalturin *et al.*, 2008), suggesting that relatively subtle changes in the regulation of TRGs such as Hym-301 can alter morphological features of the animal. Extending these studies to other TRGs will provide

insight into how many of the features of *Hydra*'s development and morphology are based on TRGs.

From colonial to solitary – using genomics to understand the evolution of the* Hydra *foot

The evolution of the genetic circuits underlying novel structures in animals is of long-standing interest to developmental biologists. Cnidarians provide particularly fertile material in this regard because of their remarkable diversity of form. It is believed that the adult form of the ancestor of Aplanulata was a solitary polyp (Paulyn Cartwright, personal communication). The clade Aplanulata belongs to the subclass Hydroidolina, and most of the clades in Hydroidolina are colonial. Thus those Aplanulata species with a solitary polyp (such as *Hydra*) likely evolved from colonial forms. This scenario raises the interesting question of how the *Hydra* foot evolved – did it primarily involve well-known developmental regulatory genes or did TRGs play a significant role? From studies of expression patterns of various genes and results from the Hydra Peptide Project, it appears that foot formation involves both conserved genes (Grens *et al.*, 1996; Steele *et al.*, 1996) and TRGs (Grens *et al.*, 1999; Bridge *et al.*, 2000). In at least one case for the foot we even have regulatory linkage between a conserved gene (and NK homeobox gene) and a TRG (Hym-346) (Thomsen *et al.*, 2004).

Genome sequences will be key to understanding the evolutionary origins of the *Hydra* foot. By comparisons between the genome of *Hydra* and the genomes of closely related species that are colonial or solitary one can determine whether genes known to play roles in foot formation in *Hydra* are present in colonial forms too, present only in solitary forms, or present only in *Hydra*. *Clytia hemisphaerica*, like *Hydra*, is a member of Hydroidolina but it is colonial. The *Clytia* genome is being assembled and should be available soon (Houliston *et al.*, 2010). RNA-seq datasets from normal and regenerating feet in *Hydra*, coupled with *in situ* hybridization will increase the number of genes for comparative purposes.

Transgenics – manipulating the genes the genome sequence provides

Following the publication of RNAi methods for *Hydra* but before the completion of the genome sequence, a landmark in *Hydra* research was established by the publication in 2006 of a method for making stably transgenic *Hydra* (Steele, 2006; Wittlieb *et al.*, 2006). With this method, a new set of options became available for functional tests of the genes that the genome sequence provided for study. Making a transgenic *Hydra* involves injection of plasmid DNA into a blastomere of a 1-8 cell embryo. Use of this method in a number of labs over the past five years has revealed that it is remarkably robust, technically simple, and easy to implement in any lab that has experience culturing *Hydra*, which is itself quite easy. It is amusing that GFP is now being used as a research tool in a hydrozoan, given that it was originally isolated from a hydrozoan (*Aequorea victoria*). Fortunately, *Hydra* has no endogenous fluorescence, the fluorescent protein genes that it might be expected to contain given that it is a hydrozoan, have apparently been secondarily lost, as no GFP-related genes are present in the *Hydra* genome (Chapman *et al.*, 2010). This absence of endogenous fluorescence makes it particularly straightforward to image any aspect of a transgenic animal.

Transgenic methods for *Hydra* not only allow one to manipulate genes, but they also make it possible to investigate the cis-acting elements that regulate gene expression during development in *Hydra*. The results of the first such promoter "bashing" study in *Hydra*, an analysis of the promoter of the Wnt3 gene, was recently published. By classical promoter deletion studies and use of GFP as a reporter, Nakamura *et al.,* (2011) were able to define both positive and negative cis-elements in the Wnt3 promoter. Despite the large size of the *Hydra* genome (1 Gb), the promoters that have been characterized so far by construction of transgenic lines are surprisingly small (on the order of 1-2 kb). This finding bodes well for further efforts to define the logic of gene regulation in *Hydra*.

Opportunities provided by the *Hydra* genome sequence

The availability of a genome sequence has already provided us with much insight into developmental processes in *Hydra*. But even more importantly, the sequence points toward exciting opportunities for learning more. Some of these possibilities are discussed below.

The* Hydra *transcriptome – still much to learn

There are currently ~170,000 *Hydra* ESTs in GenBank, generated by Sanger sequencing. By today's standards this is a tiny number. The *Hydra* transcriptome clearly needs revisiting using next generation sequencing technologies (i.e. RNA-seq). Data from RNA-seq would improve gene annotation for the existing genome sequence, and more importantly, show the extent of alternative splicing in *Hydra*. We currently know very little about alternative splicing in *Hydra*, but what we do know (Hwang *et al.*, 2010) suggests that it would be of interest to know more.

Epigenetics - How is the* Hydra *genome marked?

The past few years have seen an explosion of interest and effort in the area of epigenetics. A polycomb gene from *Hydra* has been cloned and characterized (Khalturin *et al.*, 2007), but otherwise *Hydra* has missed out in this area. With a sequenced genome, a clearly defined stem cell system, and very plastic patterning, *Hydra* is ripe for studies to determine how its genome operates at the epigenetic level. Mapping of the *Hydra* methylome and the application of ChIP-seq to *Hydra* using antibodies that detect histone modifications are advances that are eagerly awaited.

Do non-coding RNAs regulate developmental processes in* Hydra*?

Non-coding RNAs play important roles in regulating developmental processes in bilaterian animals (Pauli *et al.*, 2011). What role they play in *Hydra* development is completely unexplored territory. A small number of microRNAs were identified as part of the *Hydra* genome project (Chapman *et al.*, 2010), but this is clearly a very incomplete accounting. And no attempt has been made to identify genes for long non-coding RNAs in the *Hydra* genome. This is clearly an area where attention is needed.

Using zinc-finger and TALE nucleases to manipulate the* Hydra *genome

With a genome sequence available, we now have ~20,000 *Hydra* genes that are targets for disruption to understand their function. RNAi is not yet up to the task, and gene disruption by

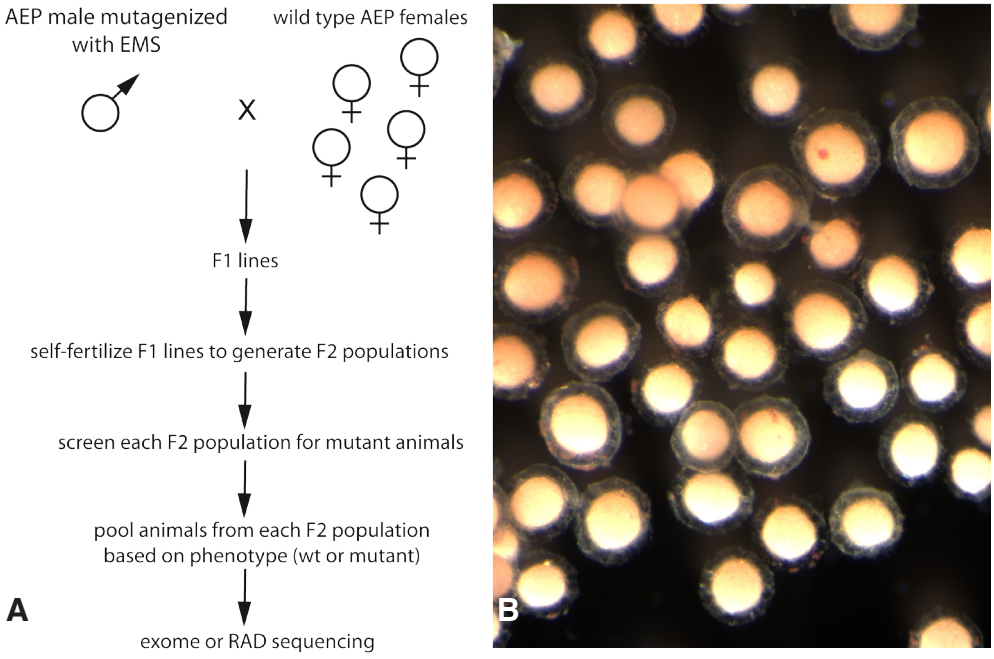

Fig. 3. A genetic screen using *Hydra*. (A) *An approach for screening for mutants in* Hydra. **(B)** *A population of cuticle stage* Hydra *embryos. The mutant screening approach is described in detail in the text.*

site-specific recombination appears impossible. Recently two new methods have become available for creating loss of gene function – zinc-finger nucleases (Urnov *et al.*, 2010) and TALE nucleases (Miller *et al.*, 2011). Both of these methods seem applicable to *Hydra* and it is expected that they will be tried sooner than later.

Is it time to try Hydra *genetics again?*

One glaring omission from the toolkit for exploiting the *Hydra* genome is classical genetics. Sugiyama and Fujisawa attempted to develop *Hydra* as a genetic model in the 1970s (Sugiyama and Fujisawa, 1977). They began by carrying out sexual crosses between animals from ponds in the area of Mishima, Japan. This work led to two clear conclusions – doing classical genetics in *Hydra* is not easy, but very interesting developmental mutants can be identified (Sugiyama and Fujisawa, 1977; Sugiyama, 1983; Shimizu, 2012). Such mutants included ones that differed dramatically in size (Sugiyama, 1983), ones with impaired regeneration capacity (Sugiyama and Fujisawa, 1977), ones that failed to differentiate particular nematocyte lineages (Fujisawa and Sugiyama, 1978), and one with temperature-sensitive interstitial cells (Terada *et al.*, 1988). These mutants have proved very valuable for developmental studies even though we have no idea what genes are mutated.

With the sequencing of a *Hydra* genome, it is worth revisiting the possibility of using genetic approaches to identify genes involved in developmental processes in *Hydra*. With next generation sequencing, it is now possible to map mutant genes by segregation analysis of sequence tags in bulked samples (Miller *et al.*, 2007; Baird *et al.*, 2008) or even by examining the genomes of the members a single human family afflicted with a genetic disease (Bamshad *et al.*, 2011). *Hydra* should be amenable to either of these approaches. Adding chemical mutagenesis to the mix would allow use of the AEP strain of *Hydra*, which produces embryos readily in the lab and is used to make transgenic lines. The genome of the AEP strain has been sequenced and assembled (R. Steele and C. Dana, unpublished). Fig. 3 shows a possible scheme for carrying out a mutagenesis screen with *Hydra*.

Conclusion

The completion of the first *Hydra* genome sequence was a propitious moment for *Hydra* developmental biologists. Labs already working on *Hydra* were quick to take advantage of the sequence for studies of developmental processes. And in an example of "build it and they will come" the availability of a *Hydra* genome sequence has convinced new researchers to use *Hydra* as a developmental model. Sequencing costs continue to drop, bioinformatics methods are becoming more powerful, and tools for manipulating genomes continue to evolve; it's a great time to be a *Hydra* developmental biologist.

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