Meetings

Establishing a model organism: A report from the first annual *Nematostella* meeting

Introduction

The sea anemone *Nematostella vectensis*, a member of the phylum Cnidaria, has rapidly developed into a cornerstone taxon in comparative studies of genome evolution and animal development. Originally described in 1935 by T.A. Stephenson, *Nematostella* was largely studied in the context of estuarine ecology and comparative taxonomy for the next 60 years. Beginning in the 1990s, descriptive work by Hand and Uhlinger [1], as well as gene expression and comparative phylogenetics research by Finnerty and Martindale [2], laid the foundation for the emergence of *Nematostella* as an informative laboratory model for cnidarian evolution and development [3, 4]. In 2007, *Nematostella* became the first non-bilaterian animal with a sequenced genome [5]. The availability of the genome quickly established *Nematostella* as a key invertebrate animal for both developmental biology and comparative genomics. Indeed, one of the immediate and major surprises was the realization that, in many respects, the *Nematostella* genome is more similar to the human genome than it is to two more commonly studied invertebrate models, *Drosophila melanogaster* and *Caenorhabditis elegans* [6]. Since then, research on *Nematostella* has rapidly grown in prominence, extended into other areas of biology, and is continually being adopted by labs throughout the world.

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Moreover, this growing community has developed a suite of molecular techniques for *Nematostella* that mirror those developed for traditional model systems (Table 1). The overall growth of this species as a laboratory model is reflected in the dramatic increase in publications that mention or feature *Nematostella* (Fig. 1). In response to this growing interest, we organized the first *Nematostella* meeting, which was held at the Woods Hole Oceanographic Institution on June 27, 2011, in Woods Hole, Massachusetts, USA. The meeting focused on developing strategies for improving and expanding community resources, on expertise related to animal culturing and experimental techniques, as well as highlighting the diversity of *Nematostella* research topics.

Developments in tests of gene expression and protein function

Studies involving *Nematostella* have been integral to our understanding of how embryogenesis and animal evolution are interconnected. In particular, spatial and temporal characterizations of gene expression have been useful in identifying likely roles for genes involved in axial patterning [7], germ layer development [8], and cell specification [9], to name a few. However, far fewer studies have been conducted involving techniques that directly test gene function (see [10, 11]). Mark Martindale (Kewalo Marine Laboratory, University of Hawaii) presented an overview of his lab's efforts to address this limitation. He provided a brief overview of a project with *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis coupled with restriction site associated DNA (RAD) mapping, deep sequencing, and forward genetics to identify mutations causing abnormal phenotypes in *Nematostella*. The Martindale lab has also developed a microarray for comparing gene expression in response to chemical (e.g. lithium chloride and alsterpaullone for inhibition of glycogen synthase kinase 3, GSK-3), misexpression, and gene knockdown manipulations of development.

In the discussion following Mark's presentation, several participants brought up examples of widely-used techniques that work well in other models, but perform poorly or in an unexpected manner in *Nematostella*. For example, while morpholino-based methods to suppress expression of *Nematostella* genes have proven effective in some studies, several groups reported difficulties doing the same with RNA interference, despite this technique's broad utility in another cnidarian, *Hydra*. Based on this discussion, we have listed some comments from meeting participants in reference to particular techniques (see Notes column in Table 1). We hope that by sharing the experiences of the participants at this meeting we can help researchers to avoid duplication of effort, select the most appropriate techniques, and identify areas where additional method development is needed.

Table 1. Summary of experimental methods that have been successfully applied to *Nematostella* research

Method	Approach	Representative publication(s)	Meeting notes[a]
In situ hybridization		[8, 18, 19]	
Immunohistochemistry		[11, 14]	Visualization of broad spatial expression in whole mount and subcellular localization in thin sections (TDG)
Gene knockdown	Morpholino	[10, 11]	Morpholinos are typically injected, but animals can also be soaked for incorporation (MQM)
	RNAi	[20]	Participants at meeting suggested RNAi has been less reliable or ineffective (MQM)
Heterologous protein expression	Cell-free	[21]	
	Vertebrate cell lines	[14]	
EMSA for DNA binding		[14]	
Protein crystallography		[22]	
Transgenics		[23]	
Spawning and culturing		[24, 25]	Varied culturing conditions (salinity, temperature) may favor better cultures for some populations (AMR)

We have also included a notes column with observations from participants from the meeting.
[a] Initials indicate source for observation from meeting. MQM: Mark Q. Martindale, AMR: Adam M. Reitzel, TDG: Thomas D. Gilmore.

The recent emergence of inexpensive sequencing technologies has presented new opportunities to study the transcriptional changes associated with development in unprecedented detail and to uncover the underlying molecular mechanisms. Casey Dunn (Brown University) presented research utilizing next-generation sequencing for comparative transcriptomic studies. The Dunn lab uses *Nematostella* for establishing techniques that can be applied to other cnidarian species that are less amenable to laboratory study, like large siphonophores [12]. In this context, Casey reported results from detailed comparisons of different sequencing platforms (e.g. Illumina, SOLiD, and Helicos). Additionally, Casey described their protocol for assembling transcriptomes using the OASIS assembly software.

To date, much of the molecular research involving *Nematostella* has focused on gene transcripts. However, it is clear that until we characterize the protein products of these genes, our view of genetic networks will remain partial, and will sometimes be misleading. Experiments describing protein-protein interactions and protein function in the regulation of downstream genes will be crucial for understanding the evolution of metazoan gene function and signaling networks. Tom Gilmore (Boston University) presented one of the most comprehensive protein-based experimental efforts in *Nematostella* to date, a detailed characterization of the *Nematostella* NF-κB signal transduction pathway. His lab has shown that the NF-κB pathway was likely in place prior to the last common ancestor of Metazoa [13]. Using a variety of in vitro and cell-based assays, they have shown *Nematostella* NF-κB can bind to and activate promoters containing canonical κB binding motifs and that activities of NF-κB can be inhibited by two *Nematostella* IκB-like inhibitors [14]. Using a newly developed antibody, they showed that expression of *Nematostella vectensis* NF-κB (NvNF-κB) is restricted to a subset of ectodermal cells in both juveniles and adults.

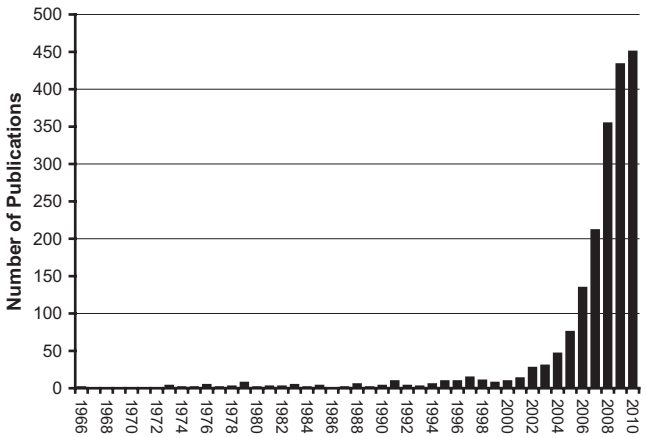

Figure 1. The number of publications per year (1966–2010) containing the search term ''Nematostella'' in the text. The search was conducted with Google Scholar on June 15, 2011. The *Nematostella* genome was published in 2007 and continues to be a strong contributor to the increase in publications.

Expanding comparative genomics in early diverging phyla

Another theme of the meeting was the context for comparative genomics that

has developed around the *Nematostella* genome. Since the sea anemone's genome was published in 2007, two additional cnidarian genomes have been published: *Hydra magnipapillata* (2010) and *Acropora digitifera* (2011). The sequencing and comparative analysis of other genomes will continue to be instrumental in identifying critical molecular events in the evolution of cnidarian and other animal genomes. John Finnerty (Boston University) described a comparative genomics effort to sequence the genomes of several populations of *Nematostella*. The goal of this effort is to characterize intraspecific variation and identify genomic regions under selection. The Finnerty lab has initiated efforts to sequence the genomes of two other species in the family Edwardsiidae (i.e. *Edwardsiella lineata* and *Edwardsia elegans*) as well as other anthozoans. Despite a close relationship with *Nematostella*, *Edwardsiella lineata* has evolved a parasitic life history, thus a comparative genomics study will aim to understand the transition between parasitic and non-parasitic life history stages. In the broader phylogenetic context, Joseph Ryan (National Human Genome Research Institute, National Institutes of Health) provided an overview of the sequencing and annotation of the genome of the ctenophore *Mnemiopsis leidyi*. This presentation focused on lessons learned from a de novo sequencing project based entirely on next-generation sequencing technologies. The ctenophore genome will provide a necessary data source for comparative genomics with other non-bilaterian phyla, such as Porifera and Placozoa, to better assess the branching order at the base of the animal tree and to identify shifts in gene complements in these early diverging phyla.

Studying *Nematostella* in its natural environment

The final major theme of the meeting was interactions between *Nematostella* and the environment. *Nematostella* has a natural distribution along much of the Atlantic coast of North America and has been introduced numerous times to estuaries outside of the natural range [15]. As a denizen of high marsh estuaries, *Nematostella* occupies a highly variable habitat that has frequently been impacted by human encroachment and pollution. Little is known about how *Nematostella* survives and thrives in these environments and if populations throughout the range show any phenotypic variation, potentially consistent with local adaptation.

Janelle Thompson (Massachusetts Institute of Technology) spoke about her lab's work on the microbiome of *Nematostella*. Her lab has compared the microbial community associated with *Nematostella* from wild populations and laboratory strains. To date, most comparisons have used 16S rRNA sequencing for identifying microbial species, but she has also identified broader sequencing approaches. The Thompson lab uses these studies to identify patterns in microbial diversity within these populations and to determine if these associations are stable in laboratory culture. Upon identification of stable associates, Janelle will use experimental approaches to determine effects of microbial associates on *Nematostella* physiology. Early results suggest that there is considerable variation between the microbiomes of the laboratory populations and field-collected populations.

Adam Reitzel (Woods Hole Oceanographic Institution, WHOI) spoke about genetic and phenotypic variation in populations of *Nematostella* collected throughout their known range. Using a suite of neutral markers (e.g. amplified fragment length polymorphisms (AFLPs), and microsatellites), this work has shown extensive genetic variation within and between populations. Additionally, *Nematostella* harbors many non-synonymous polymorphisms in conserved domains that are restricted to particular geographic locations [16]. These markers may provide good candidates for future studies of functional variation between alleles that may underlie molecular mechanisms of local adaptation. He also reported significant phenotypic variation in growth rate and temperature tolerance of individuals collected from populations spanning the thermocline along the Atlantic coast of North America. Finally, Adam highlighted current and future work conducted in collaboration with Ann Tarrant (WHOI) to describe transcriptional responses by *Nematostella* to environmental stress.

Developing research integration and new opportunities

Throughout the meeting, there were frequent discussions of how the *Nematostella* community can create or improve resources to facilitate future research efforts. Some of these efforts are well underway or completed. Mattias Ormestad and Eric Roettinger from the Martindale lab have developed a database of gene expression (KahiKai.org) – a tool for querying expression patterns from *Nematostella* and other marine invertebrates [17]. Synthesizing these data into one location will permit individuals to develop hypotheses about potential gene interactions, given similar temporal-spatial expression, and to select genes to complement future research efforts. The Finnerty lab described ongoing efforts to overhaul current on-line resources (CnidBase, StellaBase, and Nematostella.org) and to integrate these sites with new resources (PdamBase and EdBase) to facilitate comparative genomics within the Cnidaria.

One goal for this first *Nematostella* meeting was to identify a specific area where immediate efforts could have the largest impact on the community. We suggest continuing this goal-oriented discussion as a priority in future meetings. This meeting demonstrated an overwhelming interest in developing an improved genome annotation. To facilitate this, numerous labs will be donating sequence data from transcriptional profiling and targeted cloning to produce improved gene models. In total, these data amount to more than 6×10^{10} bp of sequence, which will result in more complete annotation of transcripts and a catalog of splice variants. On this topic, there was a discussion on how to update current annotations, which have largely remained static since the initial genome release in 2007. Solutions centered on generating an updated transcriptome, implementing a new genome browser,

and creating wiki-based gene pages (similar to those being generated for the *Mnemiopsis* genome project; Ryan, personal communication) that could be edited by the community researchers and easily linked to and from third party resources. The overall goal is to have a single, well-annotated data source for researchers using *Nematostella*.

The final topic of the meeting was a plan for future annual meetings with broader representation. Researchers throughout the world are continually adopting *Nematostella* to explore a broad range of biological fields and it was agreed that future meetings should capitalize on this geographic and interdisciplinary diversity. Attendees suggested holding meetings that alternate between the USA and Europe. The next proposed meeting is scheduled for the summer of 2012 at the Marine Biological Laboratory (Woods Hole, MA), and will be organized by Joel Smith (MBL), Ann Tarrant (WHOI), Adam Reitzel (WHOI), and Joseph Ryan (Sars International Centre for Marine Molecular Biology). For interested individuals, we have created a website that will publish updated information (www.nematostellameeting.com). Future meetings will have workshops that will focus on emerging experimental techniques, *Nematostella*-centric bioinformatic applications, and field collections. Together, efforts to increase geographic representation, broaden the disciplinary scope, and target learning opportunities will continue to develop *Nematostella* as a powerful model in comparative biology.

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