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MEETING REPORT

A report from the second *Nematostella vectensis* research conference

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Abstract This report summarizes information discussed at the second *Nematostella vectensis* research conference, which took place on August 27, 2012 in Boston, MA, USA. The startlet sea anemone *Nematostella* is emerging as one of leading model organisms among cnidarians, in part because of the extensive genome and transcriptome resources that are becoming available for *Nematostella*, which were the focus of several presentations. In addition, research was presented on the use of *Nematostella* in developmental, regeneration, signal transduction, host–symbiont, and gene–environment interaction studies.

Introduction

The starlet sea anemone *Nematostella vectensis* is a small, burrowing anemone that lives in shallow estuarine habitats on the East and West Coasts of North America as well as southern England. In the past several years, *Nematostella* has become one of the leading model organisms for the phylum Cnidaria (anemones, coral, jellyfish, and hydra) for a variety of reasons, notably its simplicity of being propagated in the lab, its short generation time, its suitability for studies of embryonic development, and the availability of abundant genomic and transcriptomic data (Darling et al. 2005). To foster research collaborations and share knowledge, an international group of approximately 30 researchers convened at Boston University (Boston, MA, USA) on August 27, 2012 for the second *Nematostella* research conference. This report highlights some of the findings presented at this meeting and emerging research trends using *Nematostella* as a model.

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Genomics, transcriptomics, and bioinformatics

The sequenced genome of *Nematostella* revealed striking similarity to that of humans (Putnam et al. 2007). *Nematostella* was found to share more gene orthologs (Putnam et al. 2007), more intron locations (Putnam et al. 2007; Sullivan et al. 2006), and more disease genes (Sullivan and Finnerty 2007) with humans than other invertebrate models more closely related to humans, most notably *Caenorhabditis elegans* and *Drosophila*. However, the original *Nematostella* genome sequence provided only 6.5-fold coverage and the accompanying transcriptomic data were limited in scope and derived solely from embryos and larvae. Furthermore, the data were derived from anemones from only a single geographic locale, a now extinct population from Rhodes River, MD, USA. Deeper sequencing of the *Nematostella* transcriptome, better annotation of the genome, and comparative data from multiple populations will greatly accelerate progress in understanding the evolution and contemporary function of the *Nematostella* genome.

As such, a major thrust of the conference was the generation of improved transcriptomic data. Three new transcriptomic datasets were described for *Nematostella*. David Fredman from the laboratory of Ulrich Technau (University of Vienna) and Antje Fischer from the laboratory of Joel Smith (Woods Hole Marine Biological Laboratory) presented "next generation" RNA sequencing data from *Nematostella* embryogenesis, while John Finnerty (Boston University) described his lab's efforts to obtain transcriptomic data from regenerating animals.

Fredman and co-workers have greatly improved the genome information by extensive sequencing and mapping,

combined with chromatin immunoprecipitation data. The mapping of their improved transcriptome to the existing genome assembly yielded more complete and robust gene models, including the discovery of many genes not previously identified (Putnam et al. 2007). Moreover, they mapped transcription start sites and established developmental stage-specific expression profiles. Upon publication, Fredman and co-workers plan to make their data available via NCBI and an instance of the UCSC Genome Browser (genome.ucsc.edu).

Antje Fischer presented an innovative approach to unraveling the developmental gene regulatory network of *Nematostella*. As a first step, they are using high-throughput sequencing to quantitatively identify changes in gene expression on a fine temporal scale during development. They will construct a synexpression database from this information by determining statistical patterns of dependence of gene expression, including identification of genes likely to play central regulatory roles. The synexpression database will provide a foundation for functional characterization of individual genes and tests of hypotheses regarding their regulatory relationships. The obtained data—including the quantitative analyses, the developmental time course and the synexpression relationships—will be available in the online database SeaBase. Furthermore, SeaBase will provide similar developmental RNA-Seq data from other species, such as the slipper snail *Crepidula fornicata*, a dataset presented by Kimberley Johansson (Joel Smith lab). Updates on the availability of SeaBase will be provided through Twitter (@MBLSeaBase), and will be posted to the Cnidaria Newsgroup (maillists.uci.edu/mailman/listinfo/cnidarian for information about registering).

Derek Stefanik (Finnerty lab) is characterizing the transcriptional profiles of "head" and "foot" regeneration using a clonal line from New Jersey chosen for its rapid regeneration rate relative to other *Nematostella* populations. Adult anemones were bisected through the body column, and RNA was extracted from tissue adjacent to the wound site at 0, 6, 12, 24, and 48 h postbisection in those individuals regenerating either oral structures (mouth, tentacles and pharynx) or aboral structures (a bulbous physa or "foot"). The pooled data were used to create a new reference transcriptome that will serve to anchor a differential expression analysis of regeneration in *Nematostella*. The new reference transcriptome will be publicly available at an updated version of stellabase.org (Sullivan et al. 2006).

In addition to sequence data from a new population of *Nematostella*, Stefanik presented RNA-Seq data from a closely related outgroup species, the lined sea anemone *Edwardsiella lineata*. *E. lineata* belongs to the same family as *Nematostella* (Edwarsiidae), but it exhibits an unusual life cycle where it can either parasitize the comb jelly *Mnemiopsis leidyi* or exist as a free-living anemone (Reitzel et al. 2007). As a parasite, *E. lineata* assumes a vermiform body. When excised from the host, the parasite rapidly morphs into a typical anemone-type larva. In the absence of a second host, this larva will develop into an adult anemone, but in the presence of a new host, it can re-assume the vermiform body plan. Stefanik has collected RNA-Seq data from the adult, parasite, and larva as well as individuals transitioning from parasite-to-larva and larva-to-adult stages. These data from a closely related outgroup will be useful for placing the evolution of the *Nematostella* genome in a phylogenetic context.

Two public databases and a new computational tool from the Finnerty lab were described. Finnerty detailed a major overhaul to StellaBase (The *Nematostella vectensis* Genomics Database), and Brian Granger (Finnerty lab) described Edwardbase (The *Edwardsiella lineata* Genomics Database; EdwardsiellaBase.org). Both databases share the same underlying architecture based on PostgreSQL and Perl, and both will house reference transcriptomes that incorporate user-submitted sequencing data. These databases will eventually permit users to conduct differential expression queries using cnidarian-specific search terms from a geographically and genetically diverse collection of *Nematostella*. Tristan Lubinski (Finnerty lab) described a computational pipeline he is developing to map transcriptomic data to the assembled genome and to predict transcription factor binding sites in the vicinity of particular genes using position weight matrices. Simultaneous application of this pipeline to the increasing number of cnidarian species with sequenced transcriptomes and genomes will allow identification of genes that are likely targets of a given transcription factor in multiple species, which should accelerate the identification of targets of conserved transcription factors in cnidarian genomes.

Biological processes

Regeneration

Increasing efforts are being made to develop *Nematostella* into a model for studying regeneration (Burton and Finnerty 2009; Trevino et al. 2011). In this vein, Dr. Patricia Bossert, working in the lab of Gerald Thomsen (SUNY Stony Brook), presented a Reference System for Staging Regeneration (RSSR) in *Nematostella*. The RSSR establishes a formal staging scheme for regeneration using discrete anatomical landmarks. This system should facilitate the study of pathways involved in regeneration by allowing investigators to monitor the regeneration process using a common metric and to describe deviations from normal using a standard vocabulary. Using Bossert's RSSR, Matthew Dunn (Thomsen lab) reported that treatment of bisected anemones with dorsomorphin, a type I BMP receptor inhibitor, results in slowed and abnormal regeneration, implicating the BMP pathway in this

important process. Similarly, Amos Schaffer (Uri Gat lab, Hebrew University) presented evidence that the transcription factor Runx may be involved in early regeneration stages, possibly under the control of the Wnt pathway. In addition, Schaffer presented evidence that Runx may also be involved in gastrulation and neurogenesis.

MicroRNAs

By computational methods, previous research identified ~40 microRNAs (miRs) in *Nematostella* (Grimson et al. 2008), as well as the miR processing Dicer enzymes (de Jong et al. 2009). By direct RNA sequencing, Yehu Moran (Technau lab) has identified dozens of additional miRs in *Nematostella*. He presented data on the mechanism of action of these miRs and showed that the miRs are expressed in spatiotemporally distinct patterns during embryogenesis and larval development. These data shed new light on the role and mode of action of miRs in this basal metazoan.

NF-κB

In diverse organisms from insects to humans, one of the primary conserved biological processes controlled by transcription factor NF-κB is the innate immune response; however, NF-κB also has a number of additional functions in bilaterian development. Francis Wolenski (Gilmore lab, Boston University) has been characterizing the NF-κB signaling pathway in *Nematostella* (Wolenski et al. 2011b). Wolenski presented evidence that most of the NF-κB signaling pathway components are encoded by single genes, unlike more complex animals, which generally have multiple duplicated genes. For example, *Nematostella* has a single NF-κB transcription factor and a single IκB inhibitor, whereas there are five and six such proteins, respectively, in humans. Moreover, by using morpholinos to knock down NF-κB activity in early embryos, he showed that NF-κB is necessary for the development of cnidocytes, a phylum-specific cell type that performs variety of sensory and prey capture/defense effector functions (Wolenski et al. 2013).

Table 1 Select websites featuring cnidarian experimental resources

URL	Resources
General invertebrates, including cnidarians	
Cnidbase.org	Comparative cnidarian genomics and transcriptomics
kahikai.org	Comparative gene expression of marine invertebrates (primarily in situ hybridization images)
compagen.zoologie.uni-kiel.de	Comparative genomics/transcriptomics of early-diverging metazoans, esp. *Hydra vulgaris*
MarineGenomics.org	ESTs from many marine species, including six coral species
Metazome.net	Genome information and analysis tools for several model metazoans, including esp. *Nematostella*
SeaBase (Twitter@MBLSeaBase) (posted on the Cnidaria Newsgroup)	Genomic, transcriptomic and gene function data from *Nematostella* and other marine invertebrates (e.g., *Crepidula*)
Anthozoa (corals, anemones, and relatives)	
Stellabase.org	Genomic and transcriptomic data from *Nematostella* searchable by GO terms, protein motifs, and BLAST; PCR primers; genetic stocks; human disease ortholog search; SNP database
Genome.jgi-psf.org/Nemve1/Nemve1.home.html	JGI *Nematostella* genome assembly browser
EdwardsiellaBase.org	Transcriptomic data from *E. lineata* searchable by GO terms, protein motifs, and BLAST; PCR primers; genetic stocks; human disease ortholog search; SNP database
Coralbase.org	*Acropora millepora* genome assembly pre-release
Marinegenomics.oist.jp/genomes/gallery	Entry into *Acropora digitifera* genome browser
www.comp.hkbu.edu.hk/~db/PcarnBase/index.php#&panel1-2	Reference transcriptome for *Platygyra carnosus* searchable by gene ontology terms, protein motifs, and BLAST
Pocilloporabase.org	Reference transcriptome for Pocillopora damacornis searchable by gene ontology terms, protein motifs, and BLAST
Hydrozoa (hydras, hydromedusae, etc.)	
geochembio.com/biology/organisms/hydra/	Taxonomy, life cycle, cell lineages, references
www.metazome.net/cgi-bin/gbrowse/Hmagnipapillata/	*H. magnipapillata* genome browser

Whether NF-κB controls additional biological processes in *Nematostella* or other cnidarians is not known.

Environmental interactions

Symbionts

Two talks focused on the interactions of *Nematostella* with microscopic internal symbionts. Janelle Thompson (MIT) described her ongoing work to develop *Nematostella* as a model for cnidarian-microbial interactions. Her laboratory has identified microbial associates that are shared among distant populations. Some of these microbes are similar to strains hosted by scleractinian corals, suggesting that they have formed symbiotic relationships within the hexacoral lineage, although the nature of the relationships between the microbes and hosts are unknown. Within one of these microbial strains, Thompson's lab has identified genes of potential ecological significance, which exhibit sequence signatures of positive selection. Ashley Power (Gilmore lab) described the isolation, purification, and preliminary molecular characterization of two eukaryotic single-cell organisms that were isolated from embryos and adult *Nematostella*. Limited genome sequencing indicates that one symbiont is in the order *Euglenida*; the identity of the other is unknown, however, it appears to have an early process of rapid endoreduplicative replication which is followed by several rounds of normal mitotic cell division.

Environmental stress responses and phenotypic diversity

Several talks described the utility of *Nematostella* as a model system in environmental stress and toxicology studies. Ann Tarrant (Woods Hole Oceanographic Institution) discussed the diversity of superoxide dismutase and catalase antioxidant enzymes in *Nematostella*. Although superoxide dismutase and catalase activity as well as expression of some of the associated transcripts have been measured in other cnidarians, the availability of the *Nematostella* genome has enabled Tarrant's group to profile the expression patterns for the full suite of transcripts from the superoxide dismutase family (six genes) and the single catalase gene. Her lab has found that the transcription of the genes encoding these enzymes is induced upon exposure to ultraviolet light and to polycyclic aromatic hydrocarbons.

Lauren Friedman (Finnerty lab) identified pronounced differences in peroxide sensitivity among different populations of *Nematostella*, both between geographically distant populations and even between animals in single estuaries, and she is investigating possible linkage between peroxide sensitivity and allelic differences in the gene encoding NF-κB. She described ongoing transcriptional profiling studies comparing peroxide-sensitive and peroxide-resistant clonal lines. Rachel Schweiker (Finnerty lab) is investigating the molecular basis of an established difference in temperature-specific growth rates between *Nematostella* populations from southern and northern latitudes. She confirmed that animals from South Carolina, New Jersey, and Nova Scotia grow at different rates at 21 and 29 °C, and she described her ongoing mRNA sequencing studies designed to test whether the differences in growth rate are attributable to heritable differences in gene expression. Francis Wolenski (Gilmore lab) also provided updates on single amino acid residues that are responsible for the different DNA-binding and transactivation activities of two naturally occurring variants of the NF-κB transcription factor, which may have been selected due to oxidative stress (Sullivan et al. 2009; Wolenski et al. 2011a).

Summary and future perspectives

The research presented spanned a broad range of topics from genomic to environmental. Open discussions included expression of concern regarding needs to house, manipulate, and integrate the vast amount of data being generated by new sequencing technologies. Proposed solutions included posting of new information at Stellabase, along with cross-referencing among the various *Nematostella* and cnidarian websites that are appearing (Table 1). The meeting presented many opportunities for learning about complementary studies, particularly through the use of transcriptional profiling.

A third meeting is currently being planned as a satellite to the 8th International Conference on Coelenterate Biology (ICCB) to be held in Eilat, Israel in December 2013. More information about the ICCB is available at www.iccb2013.com. Updates on the *Nematostella* meeting will be provided on the ICCB website as well as on the dedicated *Nematostella* meeting site (www.nematostellameeting.com).

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