Current directions and future perspectives from the third *Nematostella* research conference

Ann M. Tarrant[a,*], Thomas D. Gilmore[b], Adam M. Reitzel[c], Oren Levy[d], Ulrich Technau[e], Mark Q. Martindale[f]

[a] *Biology Department, Woods Hole Oceanographic Institution, 45 Water Street, Woods Hole, MA 02543, USA*

[b] *Department of Biology, Boston University, 5 Cummington Mall, Boston, MA 02215, USA*

[c] *Department of Biological Sciences, The University of North Carolina at Charlotte, Woodward Hall 245, Charlotte, NC 28223, USA*

[d] *The Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan 52900, Israel*

[e] *Department of Molecular Evolution and Development, University of Vienna, Althanstr. 14, A-1090 Wien, Austria*

[f] *Whitney Laboratory for Marine Bioscience, University of Florida, 9505 Ocean Shore Boulevard, St. Augustine, FL 32136, USA*

*Corresponding author. Tel.: +1-508-289-3398; fax: +1-508-457-2134.

E-mail address: atarrant@whoi.edu (A.M. Tarrant).

24 pages text

2 tables

Abstract

The third *Nematostella vectensis* Research Conference took place in December 2013 in Eilat, Israel, as a satellite to the 8th International Conference on Coelenterate Biology. The starlet sea anemone, *Nematostella vectensis*, has emerged as a powerful cnidarian model, in large part due to the extensive genomic and transcriptomic resources and molecular approaches that are becoming available for *Nematostella*, which were the focus of several presentations. In addition, research was presented highlighting the broader utility of this species for studies of development, circadian rhythms, signal transduction, and gene–environment interactions.

Keywords: *Nematostella vectensis*; Cnidaria; Genomics; Transcriptomics; Developmental biology

1. Introduction

The starlet sea anemone, *Nematostella vectensis*, is a small, burrowing anemone that can be readily collected from shallow estuarine habitats within its native range on the Atlantic coast of North America, as well as from introduced populations along the Pacific Coast of North America and off southern England (Reitzel et al., 2008). *Nematostella* can be easily propagated both sexually (broadcast spawning) and asexually (transverse fission) under laboratory conditions (Genikhovich and Technau, 2009; Stefanik et al., 2013). Thus, *Nematostella* has become one of the leading model organisms within the phylum Cnidaria, which includes sea anemones, corals, jellyfish, and hydra.

An international group of approximately forty researchers convened for a *Nematostella* Research Conference, which was held as a two-day satellite meeting to the 8th International Conference on Coelenterate Biology (ICCB) in Eilat, Israel on December 5–6, 2013. This meeting followed and built upon two previous meetings that were held in the northeastern United States: Woods Hole in 2011 (Reitzel et al., 2012) and Boston in 2012 (Gilmore et al., 2013). The present report highlights findings presented at the 3rd *Nematostella* Research Conference and emerging research trends using the *Nematostella* model. For completeness, we have also included information from relevant presentations in sessions of the main ICCB meeting.

2. Gene models and genome features

The first analyses of the assembled *Nematostella* genome (Putnam et al., 2007) revealed striking similarities to the human genome, provided an improved understanding of the evolution and diversification of animal lineages and their genomes, and facilitated numerous functional studies of cnidarian development and physiology. Nevertheless, many of those original gene models are

incomplete (e.g., lacking the 3'- and/or 5'-untranslated sequence), and virtually nothing has been documented regarding the structure of gene promoters and other regulatory elements. Ulrich Technau (University of Vienna, Austria) provided an overview of research conducted within his group to improve *Nematostella* gene models. Through extensive transcriptional profiling, they have discovered additional genes not annotated in the first assembly, expanded the number of exons per gene, and identified noncoding RNAs. Furthermore, they have combined this improved picture of gene structures with ChIP-Seq (chromatin immunoprecipitation followed by high-throughput sequencing of associated DNA) to map promoters (e.g., with Pol2 ChIP) and enhancers (ChIP conducted with antibodies targeted toward coactivators and histone modification markers). Overall, the genomic landscape of *Nematostella* gene regulatory elements is similar to that of *Drosophila* and mice, with many regulatory elements within 2 kilobases of the transcriptional start site and within the first intron (Schwaiger et al., 2014). The combination of marks of histone modifications and binding of the transcriptional coactivator p300 facilitated predictions of thousands of enhancers, many of which are stage-specific. Approximately 70% of the predicted enhancers that Technau's group tested were able to drive specific expression in transgenic anemones. These improved genomic datasets, along with the developed ChIP techniques, will be invaluable for research aimed at studying the expression and regulation of individual genes as well as for the construction of gene networks in *Nematostella*, which can be compared with regulatory networks in other animals. The gene models are publicly available as a citable dataset (Fredman et al., 2013).

In addition to the detailed gene predictions described above, several groups are using high-throughput sequencing to conduct transcriptomic profiling studies in *Nematostella* as well as in related species (Table 1). Two recently published studies have characterized transcriptomic

changes during *Nematostella* development (Helm et al., 2013; Tulin et al., 2013). In addition, several studies were presented on transcriptomic profiles in *Nematostella* associated with particular biological processes, tissues, or structures. Complementing targeted studies within *Nematostella*, genomic and transcriptomic studies of related species can provide insight into the diversification and adaptation within the edwardsiid lineage. For example, a reference transcriptome is now available for the confamilial species *Edwardsiella lineata* (Stefanik et al., 2014), which has a parasitic life stage (Reitzel et al., 2009). Further sequencing of transcriptomes paired with proposed sequencing of genomes will enable phylogenetic footprinting studies, which could be used to predict gene regulatory elements.

Nematostella shows a surprising complexity of gene repertoire and gene regulation on the DNA level, likely dating back to the ancestral eumetazoan. Gene expression in *Nematostella* is not only regulated at the genomic level, but also post-transcriptionally, for instance by microRNAs (miRNAs); however, the mechanisms of post-transcriptional regulation by miRNAs in *Nematostella* appear to be different from those used by most bilaterians. Yehu Moran (Technau laboratory, University of Vienna, Austria) reported that the repertoire of known *Nematostella* miRNAs has been expanded to 87 by deep sequencing of nine different developmental stages. Unlike bilaterians, *Nematostella* and other cnidarian miRNAs exhibit an almost perfect complementarity to their target mRNAs, which leads to specific slicing of the mRNA by Argonaute proteins. This siRNA-like mechanism is more reminiscent of plants than of bilaterians and likely represents the ancestral mode of action of miRNAs (Moran et al., 2014).

3. The role of transcription factors in cellular differentiation

Several groups are now characterizing individual transcription factors and their associated biological and molecular functions in *Nematostella*.

Fabian Rentzsch (Sars International Centre for Marine Molecular Biology, Bergen, Norway) presented a plenary talk on molecular and cellular aspects of neurogenesis in *Nematostella*. His laboratory has used transgenic lines, transplantation experiments and functional analysis in these studies (e.g., Nakanishi et al., 2012). Using an *NvSoxB2* transgenic reporter line, Rentzsch showed that sensory cells, ganglion cells and cnidocytes derive from a common pool of neural progenitor cells. *SoxB2* knockdown results in strongly reduced numbers of all three cell types, as shown by marker gene expression and effects on an *NvElav1* reporter line. These findings highlight the role of *NvSoxB2* as an essential positive regulator of neurogenesis, which appears to be a conserved function shared with vertebrates.

Uri Gat (Hebrew University, Israel) presented a talk on the evolution of *Runx* transcription factors and Hippo pathway genes and their roles in development and regeneration. Among early-diverging animal lineages, cnidarians and sponges contain *Runx* and often also its dimerization partner *CBFβ* (Sullivan et al., 2008). *Runx* and *CBFβ* share overlapping regions of expression, with the strongest expression observed in the distal ends of the tentacles (Sullivan et al., 2008). Based on in situ hybridization and immunostaining, Gat reported that *Runx* is expressed in neurons and neural progenitors during development in *Nematostella*. *Runx* is also an early expressed gene following induction of oral regeneration.

The Gat laboratory is also characterizing the Hippo/YAP pathway, which helps to regulate tissue proliferation and apoptosis during animal development. Orthologs of most known mammalian components of the Hippo/YAP pathway are present in *Nematostella* (Hilman and Gat, 2011). Within this pathway, the phosphorylation state of the transcriptional co-activator *YAP* provides a

pivotal regulatory point. The sequence and domain structure of *YAP* are highly conserved between cnidarians and mammals, in contrast to several bilaterian groups in which *YAP* has diverged. Gat's lab has been characterizing the developmental expression of genes in the Hippo/YAP pathway in order to gain insight into function. Preliminary results reveal high expression in early stages of development as well as distinct activity of pathway members upon regeneration.

The laboratory of Thomas Gilmore (Boston University, USA) has been characterizing the NF-κB signaling pathway in *Nematostella* (Wolenski et al., 2013b). Gilmore presented evidence that there is a single NF-κB protein in *Nematostella,* in contrast to flies and mammals that have several (Sullivan et al., 2007). Moreover, using protein-binding microarrays, he showed that the DNA site-specificity and predicted target gene profile of *Nematostella* NF-κB have extensive overlap with human NF-κB and that two natural variants of Nv-NF-κB differ significantly in their recognition of DNA target sites (Sullivan et al., 2009; Wolenski et al., 2013a). These results provide the first empirical evidence in *Nematostella* linking specific nucleotide polymorphisms with differences in protein function. These data also provide a foundation for correlating the extensive genetic diversity present in natural populations of *Nematostella* with functional variation at the phenotypic level.

Ann Tarrant's laboratory (Woods Hole Oceanographic Institution, USA) has been characterizing nuclear receptor expression and function. Among the 17 nuclear receptors present in *Nematostella, HNF4* (NR2A, hepatocyte nuclear factor 4) is expressed at the highest levels from embryonic to adult stages (Reitzel and Tarrant, 2009). Tarrant's laboratory has also identified a homolog of *PGC-1* (PPARgamma co-activator 1), a gene that serves as a co-activator for many vertebrate nuclear receptors including HNF4 (Puigserver and Spiegelman, 2003). Interestingly,

the leucine-rich motifs that enable PGC-1 binding to vertebrate nuclear receptors are not present in the *Nematostella* homolog. Reporter assays conducted in Tarrant's laboratory indicate that the transcriptional activity of NvHNF4 differs substantially from that of the vertebrate homologs. Studies are in progress to determine whether NvPGC1 can bind to and activate NvHNF4. In preliminary studies, expression of both *NvHNF4* and *NvPGC1* can be induced upon starvation, indicating that the role of these genes in maintaining energetic homeostasis may be conserved in cnidarians.

The statistical characterization of transcriptional co-expression patterns provides a tool for inferring gene regulatory networks. This approach has been extensively applied in characterizing embryonic development of several model species including fruit fly (Jaeger et al., 2004; Aerts et al., 2010), sea urchin (Smith et al., 2007), and zebrafish (Chan et al., 2009; Greenhill et al., 2011). Given the relatively simple body plan and cell division pattern during *Nematostella* embryogenesis, the availability of a relatively large amount of gene expression data, and the potential to manipulate gene expression levels through morpholino knockdown and overexpression, *Nematostella* provides a tractable species for computational modeling of development (Helm et al., 2013; Tulin et al., 2013). Jaap Kaandorp's laboratory (University of Amsterdam, Netherlands) has developed methods to quantitatively analyze images from in situ hybridization studies conducted in *Nematostella* (Botman and Kaandorp, 2012). Kaandorp described how his laboratory has incorporated published gene expression data into a spatial database, creating a three-dimensional "virtual embryo," and used the dataset to build a mathematical model of the developmental regulatory network and to infer regulatory network parameters. Ultimately, the regulatory network model could be coupled to a biomechanical model of cell movement and division (Tamulonis et al., 2011).

3. Structures and organismal processes

3.1. Oogenesis

Previous transcriptional profiling studies in tunicates and diverse vertebrates have shown that most genes expressed in oocytes are conserved among chordate species (Evsikov et al., 2006; Vallee et al., 2008). However, it is not known to what extent oocyte composition is conserved between chordates and animals from early-diverging lineages.

Tamar Lotan (University of Haifa, Israel) presented a proteomic characterization of mature ovulated oocytes of *Nematostella* (Lotan et al., 2014). Her group has identified 1,837 proteins in the *Nematostella* oocyte; among these proteins, vitellogenin is the most abundant, comprising about two-thirds of the total protein. Homologs of maternal and germ cell-specific proteins were identified, and several functionally enriched metabolic pathways were shared with mouse oocytes, indicating extensive conservation of oocyte composition between cnidarians and mammals.

3.2. Axial patterning

Wnt signaling directs formation of the primary body axis in both cnidarians and bilaterians, specifying formation of the oral pole in *Nematostella* and the posterior pole in bilaterians (Petersen and Reddien, 2009).

Bert Hobmayer (University of Innsbruck, Austria) characterized the *sFRP1/2/5* genes, which encode secreted proteins (secreted frizzled-related proteins, SFRPs) that bind Wnt ligands to modulate Wnt signaling in both *Nematostella* and *Hydra*. He found that expression of *sFRP1/2/5* genes is restricted to the aboral pole during early embryogenesis in *Nematostella* and that

morpholino-mediated knockdown results in termination of aboral development around the gastrula stage, affecting positional values along the entire primary larval body axis.

Fabian Rentzsch (Sars International Centre for Marine Molecular Biology, Bergen, Norway) and colleagues demonstrated that *NvSix3/6* regulates aboral development in *Nematostella* (Sinigaglia et al., 2013). Through knockdown and overexpression experiments he showed that *NvSix3/6* maintains aboral expression of the Wnt receptor *NvFrizzled5/8*, and *NvFrizzled5/8* in turn prevents expansion of *NvSix3/6* expression towards the oral pole. This feedback loop determines the boundary between oral and aboral territories of the gastrula embryo.

3.3. Mesentery development

Mesenteries are tissue sheets that extend radially from the gastric cavity to the inner surface of the body wall and harbor muscles, nematocytes, gland cells, germ cells and neurons. They provide structural support to the polyp and increase the surface area available for respiration and other metabolic exchanges.

Lucas Leclère (Rentzsch laboratory, Sars International Centre for Marine Molecular Biology, Bergen, Norway) demonstrated that bilateral symmetry is established morphologically in *Nematostella* immediately after gastrulation by the formation of the first two mesenteries, and he has characterized the role of bone morphogenic proteins (BMPs) and repulsive guidance molecule (RGM) in patterning mesentery formation.

Andy Aman (Technau laboratory, University of Vienna, Austria) presented a detailed examination of mesentery development in *Nematostella.* He found that mesenteries are initially formed through a surprisingly complex series of cellular rearrangements. The primary mesenteries first appear shortly after gastrulation and establish a visible bilateral symmetry.

Subsequent development of six secondary mesenteries occurs simultaneously, rather than with a defined progression. In addition, Aman presented a new method of long-term mapping of cell fate in which cells from a blastocyst expressing a fluorescent transgene are fused into a wild-type blastocyst. The fate of cells derived from the fused blastocyst can then be visualized by fluorescence in the developing animal. Using this method, he showed that all mesenteries have a dual germ layer origin in which the distal portion of the mesentery is derived from the ectodermal pharynx and the remainder is derived from the body wall endoderm.

3.4. Evolution of muscles

In *Nematostella*, there are four types of muscle cells: the relatively weak ring muscles in the endoderm, which run circumferentially between the mesenteries and in the tentacles; the endodermal parietal muscle at the base of the mesenteries; the retractor muscle in the middle of the mesentery; and the ectodermal retractor muscles of the tentacles. All of these muscles are smooth muscles. However, cnidarian medusae also have striated muscles in the ectodermal subumbrella which led to the proposition that striated muscles from cnidarians and bilaterians are homologous (Seipel and Schmid, 2005).

After tracing the evolutionary origin of 47 muscle proteins known in vertebrates or *Drosophila* in 22 fully sequenced genomes, Ulrich Technau (University of Vienna, Austria) reported that many muscle proteins predate the origin of metazoans, while others appear only later in metazoan lineages. Crucial muscle proteins like Titin and troponins are missing from all diploblastic genomes. The comparative study, along with expression studies in two cnidarians and two sponges, led to the suggestion that striated muscles evolved independently in cnidarians and

bilaterians, on the basis of an ancestral core of proteins which predate the origin of metazoans (Steinmetz et al., 2012).

3.5. Cnidocytes

Cnidocytes (also called nematocytes) are a cnidarian-specific cell type with multiple functions and lineages. Some types of cnidocytes contain organelles called nematocysts, which deliver toxins to enable prey capture and defense (reviewed by Beckmann and Ozbek, 2012). Several recent studies have reported gene expression patterns within cnidocytes and begun to characterize regulation of cnidocyte development (Table 2).

Uri Gat (Hebrew University, Jerusalem, Israel) reported that Runx is primarily expressed in elongated cells (likely cnidocytes) in the tentacle and that Runx protein staining is associated with staining by a neuronal marker. In contrast, NF-κB (Wolenski et al., 2013b) and Notch (Marlow et al., 2012) are primarily expressed in cnidocytes along the body column. Morpholino knockdown and pharmacological experiments have shown that Notch (Marlow et al., 2012), NF-κB (Wolenski et al., 2013b), and SoxB2 (Rentzsch, this meeting) are involved in cnidocyte development. SoxB2 appears to be a positive regulator of neuronal precursor cells (as described previously), while Notch and NF-κB apparently negatively regulate neuronal and cnidocyte development. Notch knockdown increases the numbers of both neuronal cells and immature cnidocytes; however, the cnidocytes in Notch knockdown animals do not develop into capsule-positive mature cnidocytes. Like Notch knockdown, NF-κB knockdown results in reduced numbers of capsule-positive mature cnidocytes, but whether NF-κB knockdown animals have normal or increased numbers of immature cnidocytes or neuronal cells has not been determined. The localization of NF-κB in the nucleus of gastrula cells suggests that it exerts its effect on a

program of cnidocyte gene expression at this stage. Treatment of embryos with DAPT, an inhibitor of Notch activation, blocks the appearance of nuclear NF-κB in the gastrula, suggesting that NF-*κB* activation is downstream of Notch activation. Together these studies have revealed several key regulators of cnidocyte development, but the precise structure of the regulatory network remains to be elucidated.

Leslie Babonis (Martindale laboratory, Whitney Laboratory for Marine Bioscience, University of Florida, St. Augustine, USA) reported on her preliminary characterization of the nematosome, a type of highly motile cell mass that is unique to the genus *Nematostella* (Babonis and Martindale, 2014). The function of nematosomes has puzzled researchers for many years. Babonis showed that nematosomes primarily consist of cnidocytes but also contain vacuolated absorptive cells that phagocytose fluorescent latex beads. She has conducted a transcriptomic study comparing gene expression between mesenterial tissues and nematosomes, which arise by budding from the mesenteries. She has also shown that nematosomes isolated from the host retain the ability to discharge their cnidocytes in response to prey, suggesting possible defensive functions.

4. Environmental interactions

4.1. Environmental stress responses and phenotypic diversity

Nematostella inhabits estuarine pools with a wide range of environmental variability. For example, *Nematostella* are year-around residents in locations with large ranges in temperature (–1.5–40 °C), salinity (2–52‰), oxygen availability, ultraviolet radiation, and concentration of peroxides.

Adam Reitzel (University of North Carolina at Charlotte, USA) presented a plenary talk on the genetic and phenotypic diversity of *Nematostella* throughout its natural range along the Atlantic coast of North America. He discussed the role of spatial scale in the genetic relationships of natural populations. Over large geographic ranges (hundreds of kilometers), *Nematostella* has highly structured populations indicating limited genetic exchange between locations (Reitzel et al., 2013b). However, at smaller scales (within one kilometer, single estuary), *Nematostella* shows signs of metapopulation structure where reproductive plasticity and colonization of new habitats occurs at low but quantifiable frequencies. Reitzel also described research quantifying physiological variation between populations, consistent with temperature adaptation (Reitzel et al., 2013a). Finally, he discussed efforts to identify regions of the genome under selection through analyses of expressed sequence tags (Reitzel et al., 2010) and whole genome polymorphisms (Reitzel et al., 2013b). Together, geographically structured genetic and phenotypic variation in *Nematostella* may provide a useful system for discerning the molecular mechanisms that underlie adaptation in a coastal cnidarian.

Investigators have recently begun to characterize transcriptional responses of *Nematostella* following exposure to external stressors including extreme temperatures (Reitzel et al., 2013a) as well as ultraviolet radiation and environmental contaminants (Tarrant et al., 2014), and several presentations contributed to this growing body of knowledge. Reitzel reported on studies of the diversity and expression of heat shock proteins in *Nematostella* that were conducted with Ann Tarrant. This work showed that while *Nematostella* contains a typical suite of heat shock proteins as compared to other animals, the expression of these genes shows diverse patterns in development and in response to physiological stressors.

Tumor necrosis factor receptors (TNFRs) belong to a superfamily of cell surface receptors, within which distinct receptor types can activate apoptotic or immune pathways in response to stressors (Cabal-Hierro and Lazo, 2012). Nikki Traylor-Knowles (Palumbi laboratory, Stanford University, USA) has surveyed coral and *Nematostella* genomes to characterize TNFR diversity and has studied their expression in response to heat stress (coral expression patterns described by Barshis et al., 2013). She has hypothesized that the diversity of receptors enables stress responses to occur on different time scales and that the TNFR signaling pathway may be a generalized heat response mechanism in cnidarians. Indeed, the coral *Acropora digitifera* has recently been shown to have an extensive TNF/TNFR pathway that can induce apoptosis in much the same way as it does in humans (Quistad et al., 2014).

Ron Elran and Maayan Raam (Lotan laboratory, University of Haifa, Israel) presented a study of the transcriptomic responses of *Nematostella* following exposure to mercury, cadmium, copper and zinc (Elran et al., 2014). Broadly, they found that only a few genes were differentially expressed in response to all four metals and that copper- and mercury-treated anemones exhibited expression patterns that were relatively similar to one another.

4.2. Circadian regulation

Circadian rhythms are temperature-compensated cycles with a periodicity of approximately 24 h that persist in the absence of external stimuli (i.e., are free-running). Circadian rhythms are sensitive to perturbations from the environment and are typically strongly entrained by light. Thus, they provide a means to anticipate cyclic environmental changes and to link behavior and physiology with environmental input. In a plenary presentation, Oren Levy (Bar-Ilan University, Ramat Gan, Israel) described the current state of knowledge regarding circadian signaling in

cnidarians, based on studies conducted both in *Nematostella* and scleractinian corals. The studies conducted in corals are particularly complex because emergent properties in the "holobiont" may result from independent clocks contained within the cnidarian hosts and their dinoflagellate symbionts; it is unknown how these clocks interact. Levy's group has documented physiological, biochemical and transcriptional changes in corals over daily cycles and characterized the dependence of these properties on light (reviewed by Sorek et al., 2014). Recent studies indicate that corals and *Nematostella* contain a similar complement of homologs to bilaterian core clock genes (reviewed by Reitzel et al., 2013c). Surprisingly, through preliminary analysis of transcriptional profiling experiments, Levy found that *Nematostella* may contain a relatively small number of genes exhibiting circadian-like expression patterns. The functions of the circadian clock in *Nematostella* are still not clear, but researchers have characterized circadian cycles in *Nematostella* behavior (Hendricks et al., 2012; Levy, this meeting) and daily cycles in oxygen consumption (Tarrant, this meeting).

5. Nematostella community considerations and future plans

The research presented spanned a broad range of topics related to this cnidarian model. One common theme of open discussions was the need for additional genomic information from related anemones to enable comparative studies, as is now common with *Drosophila* (Drosophila 12 Genomes Consortium, 2007) and nematodes (Coghlan et al., 2006). Recent transcriptome sequencing of *E. lineata* (Stefanik et al., 2014) will allow fruitful comparisons, and a genome assembly is desirable. Several researchers expressed particular interest in obtaining samples of *Nematostella polaris*, which has an arctic distribution but has not been collected for many

decades. In addition, sequencing of different *Nematostella* populations was suggested as a means of enabling continued studies of adaptation and gene function.

As transgenic technology becomes more common with *Nematostella*, Ulrich Technau recommended distribution of stable transgenic animals among community members to avoid unforeseen loss of valuable animals, much as has been done among the *Drosophila* and *C. elegans* research communities. Similarly, the maintenance of animals or embryos by cryogenic means and the development of *Nematostella* cell lines were seen as worthwhile goals. Archiving of plasmids in publicly accessible repositories, such as Addgene (www.addgene.org) was encouraged.

Spatio-temporal expression patterns have been described for a growing number of *Nematostella* genes. Some results from whole mount *in situ* hybridization studies have been archived within the gene expression database in Kahi Kai (www.kahikai.org), a comparative gene expression database for marine invertebrates (Ormestad et al., 2011). Researchers in the *Nematostella* community are invited to submit images to this database. Andy Aman (Technau laboratory, University of Vienna, Austria) recommended the development of a standardized developmental staging system based on morphological structures rather than time post-fertilization. Meeting participants were supportive of this idea, and Aman plans to publish a description of the staging system.

Future *Nematostella* meetings are planned to be held in even-numbered years, with the next meeting to be held in 2016. Announcement of the next meeting will be broadly distributed and posted on www.nematostellameeting.com.

Acknowledgments

We are indebted to the organizers of the 2013 ICCB, Tamar Lotan, Oren Levy, and particularly Yehuda Benayhu. We thank Joseph Ryan (Whitney Laboratory for Marine Bioscience, University of Florida, St. Augustine, FL, USA) for help with meeting planning and web support. We thank the chairpersons of sessions within the ICCB main meeting for allowing presentations from within their sessions to be included in this report: Genomics, Proteomics and Evolution (M. Martindale, U. Frank and O. Levy), and Cell and Developmental Biology and Neurology, (U. Gat and U. Technau). Research in the authors' laboratories on *Nematostella* is supported by National Science Foundation grants MCB-1057354 to A.M.T. and MCB-0924749 to T.D.G. Travel support for the meeting was provided to T.D.G. by Illumina, Inc. (San Diego, CA, USA), to A.M.R. by the University of North Carolina at Charlotte, and to A.M.T. by the Israel–US Binational Science Foundation (Jerusalem, Israel).

References

Aerts, S., Quan, X. J., Claeys, A., Naval Sanchez, M., Tate, P., Yan, J. and Hassan, B. A., 2010. Robust target gene discovery through transcriptome perturbations and genome-wide enhancer predictions in *Drosophila* uncovers a regulatory basis for sensory specification. PLoS Biol. 8, e1000435.

Babonis, L. S. and Martindale, M. Q., 2014. Old cell, new trick? Cnidocytes as a model for the evolution of novelty. Integr. Comp. Biol., doi: 10.1093/icb/icu027.

Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N. and Palumbi, S. R., 2013. Genomic basis for coral resilience to climate change. Proceedings of the National Academy of Science USA 110, 1387-1392.

Beckmann, A. and Ozbek, S., 2012. The nematocyst: a molecular map of the cnidarian stinging organelle. Int. J. Dev. Biol. 56, 577-82.

Botman, D. and Kaandorp, J. A., 2012. Spatial gene expression quantification: a tool for analysis of *in situ* hybridizations in sea anemone *Nematostella vectensis*. BMC Res. Notes 5, 555.

Cabal-Hierro, L. and Lazo, P. S., 2012. Signal transduction by tumor necrosis factor receptors. Cell. Signal. 24, 1297-305.

Chan, T. M., Longabaugh, W., Bolouri, H., Chen, H. L., Tseng, W. F., Chao, C. H., Jang, T. H., Lin, Y. I., Hung, S. C., Wang, H. D. et al., 2009. Developmental gene regulatory networks in the zebrafish embryo. Biochim. Biophys. Acta 1789, 279-98.

Coghlan, A., Stajich, J. E. and Harris, T. W., 2006. Comparative genomics in *C. elegans, C. briggsae,* and other *Caenorhabditis* species. Methods Mol. Biol. 351, 13-29.

Drosophila 12 Genomes Consortium, 2007. Evolution of genes and genomes on the *Drosophila* phylogeny. Nature 450, 203-218.

Elran, R., Raam, M., Kraus, R., Brekhman, V., Sher, N., Plaschkes, I., Chalifa-Caspi, V. and Lotan, T., 2014. Early and late responses of *Nematostella vectensis* transcriptome to heavy metals. Molecular Ecology, doi: 10.1111/mch.12891 [Epub ahead of print].

Evsikov, A. V., Graber, J. H., Brockman, J. M., Hampl, A., Holbrook, A. E., Singh, P., Eppig, J. J., Solter, D. and Knowles, B. B., 2006. Cracking the egg: molecular dynamics and evolutionary aspects of the transition from the fully grown oocyte to embyro. Genes Dev. 20, 2713-27.

Fredman, D., Schwaiger, M., Rentzsch, F. and Technau, U., 2013. *Nematostella vectensis* transcriptome and gene models v2.0. figshare., http://dx.doi.org/10.6084/m9.figshare.807696.

Genikhovich, G. and Technau, U., 2009. Induction of spawning in the starlet sea anemone *Nematostella vectensis,* in vitro fertilization of gametes, and dejellying of zygotes. Cold Spring Harb. Protoc. 2009, pdb.prot5281.

Gilmore, T. D., Tarrant, A. M. and Finnerty, J. R., 2013. A report from the second *Nematostella vectensis* research conference. Dev. Genes Evol. 223, 207-11.

Greenhill, E. R., Rocco, A., Vibert, L., Nikaido, M. and Kelsh, R. N., 2011. An iterative genetic and dynamical modelling approach identifies novel features of the gene regulatory network underlying melanocyte development. PLoS Genet. 7, e1002265.

Helm, R. R., Siebert, S., Tulin, S., Smith, J. and Dunn, C. W., 2013. Characterization of differential transcript abundance through time during *Nematostella vectensis* development. BMC Genomics 14, 266.

Hendricks, W. D., Byrum, C. A. and Meyer-Bernstein, E. L., 2012. Characterization of circadian behavior in the starlet sea anemone, *Nematostella vectensis*. PLoS ONE 7, e46843.

Hilman, D. and Gat, U., 2011. The evolutionary history of YAP and the Hippo/YAP pathway. Mol. Biol. Evol. 28, 2403-17.

Jaeger, J., Surkova, S., Blagov, M., Janssens, H., Kosman, D., Kozlov, K. N., Manu, Myasnikova, E., Vanario-Alonso, C. E., Samsonova, M. et al., 2004. Dynamic control of positional information in the early *Drosophila* embryo. Nature 430, 368-71.

Layden, M. J., Boekhout, M. and Martindale, M. Q., 2012. Nematostella vectensis achaete-scute homolog NvashA regulates embryonic ectodermal neurogenesis and represents an ancient component of the metazoan neural specification pathway. Development 139, 1013-22.

Lotan, T., Chalifa-Caspi, V., Ziv, T., Brekhman, V., Gordon, M. M., Admon, A. and Lubzens, E., 2014. Evolutionary conservation of the mature oocyte proteome. EuPA Open Proteomics 3, 27-36.

Marlow, H., Roettinger, E., Boekhout, M. and Martindale, M. Q., 2012. Functional roles of Notch signaling in the cnidarian *Nematostella vectensis*. Dev. Biol. 362, 295-308.

Moran, Y., Praher, D., Schlesinger, A., Ayalon, A., Tal, Y. and Technau, U., 2013. Analysis of soluble protein contents from the nematocysts of a model sea anemone sheds light on venom evolution. Mar Biotechnol 15, 329-339.

Moran, Y., Fredman, D., Praher, D., Xi, L. M., Meng Wee, Z., Rentzsch, F., Zamore, P., Technau, U. and Seitz, H., 2014. Cnidarian microRNAs frequently regulate their targets by cleavage. Genome Res. 23, 651-63.

Nakanishi, N., Renfer, E., Technau, U. and Fentzsch, F., 2012. Nervous systems of the sea anemone *Nematostella vectensis* are generated by ectoderm and endoderm and shaped by distinct mechanisms. Development 139, 347-57.

Ormestad, M., Martindale, M. Q. and Röttinger, E., 2011. A comparative gene expression database for invertebrates. EvoDevo 2, 17.

Petersen, C. P. and Reddien, P. W., 2009. Wnt signaling and the polarity of the primary body axis. Cell 139, 1056-68.

Puigserver, P. and Spiegelman, B., 2003. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. Endocr. Rev. 24, 78-90.

Putnam, N., Srivastava, M., Hellsten, U., Dirks, B., Chapman, J., Salamov, A., Terry, A., Shapiro, H., Lindquist, E., Kapitonov, V. et al., 2007. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. Science 317, 86-94.

Quistad, S. D., Stotland, A., Barott, K. L., Smurthwaite, C. A., Hilton, B. J., Grasis, J. A., Wolkowicz, R. and Rohwer, F. L., 2014. Evolution of TNF-induced apoptosis reveals 550 My of functional conservation. Proceedings of the National Academy of Science USA, doi: 10.1073/pnas.1405912111. [Epub ahead of print].

Reitzel, A. M., Darling, J. A., Sullivan, J. C. and Finnerty, J. R., 2008. Global population genetic structure of the starlet anemone *Nematostella vectensis*: multiple introductions and implications for conservation policy. Biol. Invasions 10, 1197-1213.

Reitzel, A. M., Daly, M., Sullivan, J. C. and Finnerty, J. R., 2009. Comparative anatomy and histology of developmental and parasitic stages in the life history of the lined sea anemone *Edwardsiella lineata*. J. Parasitol. 95, 100-12.

Reitzel, A. M. and Tarrant, A. M., 2009. Nuclear receptor complement of the cnidarian *Nematostella vectensis*: phylogenetic relationships and developmental expression patterns. BMC Evolutionary Biology 9, 230.

Reitzel, A. M., Sullivan, J. C. and Finnerty, J. R., 2010. Discovering SNPs in protein coding regions with StellaSNP: Illustrating the characterization and geographic distribution of polymorphisms in the estuarine anemone *Nematostella vectensis*. Estuaries and Coasts 33, 930-43.

Reitzel, A. M., Ryan, J. F. and Tarrant, A. M., 2012. Establishing a model organism: a report form the first annual *Nematostella* meeting. Bioessays 34, 158-61.

Reitzel, A. M., Chu, T., Edquist, S., Genovese, C., Church, C., Tarrant, A. M. and Finnerty, J. R., 2013a. Physiological and developmental responses to temperature by the estuarine sea anemone *Nematostella vectensis:* evidence for local adaptation to high temperatures. Mar. Ecol. Prog. Ser. 484, 115-30.

Reitzel, A. M., Herrera, S., Layden, M. J., Martindale, M. Q. and Shank, T. M., 2013b. Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. Molecular Ecology 22, 2953-70.

Reitzel, A. M., Tarrant, A. M. and Levy, O., 2013c. Circadian clocks in the cnidaria: environmental entrainment, molecular regulation, and organismal output. Integr. Comp. Biol. 53, 118-130.

Schwaiger, M., Schönauer, A., Rendiro, A. F., Pribitzer, C., Schauer, A., Gilles, A. F., Schinko, J. B., Renfer, E., Fredman, D. and Technau, U., 2014. Evolutionary conservation of the eumetazoan gene regulatory landscape. Genome Res. 24, 639-50.

Seipel, K. and Schmid, V., 2005. Evolution of striated muscle: Jellyfish and the origin of triploblasty. Dev Biol 282, 14-26.

Sinigaglia, C., Busengdal, H., Leclère, L., Technau, U. and Rentzsch, F., 2013. The bilaterian head patterning gene six3/6 controls aboral domain development in a cnidarian. PLoS Biol. 11, e1001488.

Smith, J., Theodoris, C. and Davidson, E. H., 2007. A gene regulatory network subcircuit drives a dynamica pattern of gene expression. Science 318, 794-7.

Sorek, M., Diaz-Almeyda, E. M., Medina, M. and Levy, O., 2014. Circadian clocks in symbiotic corals: The duet between *Symbiodinium* algae and their coral host. Mar. Genomics 14, 47-57.

Stefanik, D. J., Friedman, L. E. and Finnerty, J. R., 2013. Collecting, rearing, spawning and inducing regeneration of the starlet sea anemone, *Nematostella vectensis*. Nat. Protoc. 8, 916-23.

Stefanik, D. J., Lubinski, T. J., Granger, B. R., Byrd, A. L., Reitzel, A. M., Defilippo, L., Lorenc, A. and Finnerty, J. R., 2014. Production of a reference transcriptome and transcriptomic database (EdwardsiellaBase) for the lined sea anemone, *Edwardsiella lineata*, a parasitic cnidarian. BMC Genomics 15, 71.

Steinmetz, P. R., Kraus, J. E., Larroux, C., Hammel, J. U., Amon-Hassenzahl, A., Houliston, E., Wörheide, G., Nickel, M., Degnan, B. M. and Technau, U., 2012. Independent evolution of striated muscles in cnidarians and bilaterians. Nature 489, 231-4.

Sullivan, J. C., Kalaitzidis, D., Gilmore, T. D. and Finnerty, J. R., 2007. Rel homology domain-containing transcription factors in the cnidarian *Nematostella vectensis*. Dev. Genes Evol. 217, 63-72.

Sullivan, J. C., Sher, D., Eisenstein, M., Shigesada, K., Reitzel, A. M., Marlow, H., Levanon, D., Groner, Y., Finnerty, J. R. and Gat, U., 2008. The evolutionary origin of the Runx/CBFbeta transcription factors - Studies of the most basal metazoans. BMC Evolutionary Biology 8, 228.

Sullivan, J. C., Wolenski, F. S., Reitzel, A. M., French, C. E., Traylor-Knowles, N., Gilmore, T. D. and Finnerty, J. R., 2009. Two alleles of NF-κB in the sea anemone *Nematostella vectensis* are widely dispersed in nature and encode proteins with distinct activities. PLoS ONE 4, e7311.

Tamulonis, C., Postma, M., Marlow, H. Q., Magie, C. R., de Jong, J. and Kaandorp, J. A., 2011. A cell-based model of *Nematostella vectensis* gastrulation including bottle cell formation, invagination and zippering. Dev. Biol. 351, 217-28.

Tarrant, A. M., Reitzel, A. M., Kwok, C. K. and Jenny, M. J., 2014. Activation of the cnidarian oxidative stress response by ultraviolet light, polycyclic aromatic hydrocarbons and crude oil. J. Exp. Biol. 217, 1444-53.

Tulin, S., Aguiar, D., Istrail, S. and Smith, J., 2013. A quantitative reference transcriptome for *Nematostella vectensis* early embryonic development: a pipeline for *de novo* assembly in emerging model systems. EvoDevo 4, 16.

Vallee, M., Aiba, K., Piao, Y., Palin, M.-F., Ko, M. S. H. and Sirard, M.-A., 2008. Comparative analysis of oocyte transcript profiles reveals a high degree of conservation among species. Reproduction 135, 439-48.

Wolenski, F. S., Bradham, C. A., Finnerty, J. R. and Gilmore, T. D., 2013a. Two polymorphic residues account for the differences in DNA binding and transcriptional activation

by NF-κB proteins encoded by naturally occurring alleles in *Nematostella vectensis*. J. Mol. Evol. 73, 325-36.

Wolenski, F. S., Bradham, C. A., Finnerty, J. R. and Gilmore, T. D., 2013b. NF-κB is required for cnidocyte development in the sea anemone *Nematostella vectensis*. Dev. Biol. 373, 205-15.

Table 1. Transcriptomic and proteomic studies on sea anemones (*Nematostella vectensis* and *Edwardsiella lineata).*

Species	mRNA/protein source	Reference
Transcriptomes		
N. vectensis	6 stages (post-fertilization through polyp)	(Helm et al., 2013)
N. vectensis	Multiple stages	(Fredman et al., 2013)
N. vectensis	Adult, 24-hour cycle, different light conditions	(Levy, this meeting)
N. vectensis	Nematosome, mesentery	(Babonis, this meeting)
E. lineata	Multiple stages (parasitic, larval, adult, transitions)	(Stefanik et al., 2014)
Proteomes		
N. vectensis	Oocyte	(Lotan, this meeting)
N. vectensis	Discharged proteins released by nematocysts	(Moran et al., 2013)

Table 2. Genes involved in cnidocyte development in *Nematostella.*

Gene	Function	Reference
soxb2	Differentation of neural precursors	(Rentzsch, this meeting)
nfκb	Body column cnidocytes	(Wolenski et al., 2013b)
Notch	Inhibitor of neurons and cnidocytes	(Marlow et al., 2012; Rentzsch, this meeting)
Runx	Tentacle sensory cell development	(Gat, this meeting)
ashA	Positive regulator of ectodermal neurons	(Layden et al., 2012)