Meetings

Meeting report on "Animal Evolution: New Perspectives From Early Emerging Metazoans", Tutzing, September 14–17, 2015

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The international workshop entitled "Animal Evolution: New Perspectives From Early Emerging Metazoans" was recently held at the Evangelische Akademie in Tutzing, Germany (14–17 September 2015). It was organized by Thomas C. G. Bosch (Kiel), Thomas W. Holstein (Heidelberg), and Ulrich Technau (Vienna) and sponsored by the Deutsche Forschungsgemeinschaft (DFG). Approximately 125 researchers gathered from 18 countries to share their work on non-bilaterian animals, a key group for understanding animal evolution and the fundamental molecular mechanisms common to all animals.

Advances in genome sequencing and assembly

The first part of the meeting brought exciting news about more and better genomic data sets. New genome sequencing results from four species were presented and are summarized in Fig. 1D. Christian Voolstra (KAUST) discussed *Aiptasia*, an experimentally tractable symbiotic sea anemone that may give us novel insights into coral biology [1]. Richard Copley (Villefranche sur mer) discussed the *Clytia hemisphaerica* genome project and newly acquired life stage-specific transcriptomic data (Fig. 1D). Christine Schnitzler (NIH) presented genome assemblies for two species of *Hydractinia* (Fig. 1D). The genome of *H. echinata* is 250 Mb larger than that of *H. symbiolongicarpis*, which is likely due to increased repetitive sequences. The highly repetitive DNA content of the *Hydra* genome has, until recently, stymied efforts to improve the fractured genome assembly published in 2010 [2]. However, Rob Steele (UC Irvine) revealed that the assembly of the *Hydra* genome has been drastically improved by employing a methodology pioneered by the company Dovetail Genomics (arXiv:1502.05331 [q-bio.GN]). The scaffold N50 of the *Hydra* genome now stands at an impressive 1 Mb (Fig. 1D), which allows for synteny studies and improves gene predictions.

New findings in cnidarian stem cells and development

Four sessions of the meeting were devoted to development, stem cells, and regeneration. A very detailed morphological characterization of the early development of *Aglantha*, a member of an enigmatic group within the hydrozoans, was given by Yulia Kraus (Moscow). She found a rather invariant and spiralean-like series of cleavage divisions uncommon for cnidarian embryos, but highlighting the unexplored diversity of developmental programs among basal animals (Fig. 1A).

Grigory Genikhovich (Vienna) hypothesized that cnidarian and vertebrate organizers share a common descent based on the similarity and complexity of their molecular induction modules. He showed that axis induction in the *Nematostella* blastoporal organizer requires the activity of Tcf, and he identified Wnt1 and Wnt3 as potential axis inducers. Thomas Holstein (Heidelberg) showed that inhibition of histone deacetylases (HDACs) in *Hydra* dramatically enhanced polyp growth and led to multiple *wnt* expressing head organizers throughout the body column. Hiroshi Watanabe (Heidelberg) found that organizer formation requires induction of the Activin pathway through β-catenin. Alfredo Ambrosone (Pozzuoli) presented novel methods for manipulating Wnt signaling using laser-destructible microcapsules loaded with small molecule activators.

Aside from Wnt, several presentations were given on the roles of other conserved signaling pathways. Aissam Ikmi (Stowers) showed that FGF is sufficient for tentacle evagination and proposed an ancient FGF-based molecular branching module used for epithelial branching in vertebrates. Monika Hassel (Marburg) characterized a set of potential *Hydra* FGF ligands and cytoplasmic pathway proteins and provided evidence that an FGFf ligand may act as a directional cue in *Hydra* tissue displacement. James Gahan (Galway) provided clear evidence that Notch signaling acts in *Hydractinia* nematocyte differentiation and tentacle regeneration, but surprisingly not in neuronal differentiation. Mona Reineck (Munich) discussed the apoptosis-stimulating role of the *Hydra* TNF receptor pathway. Finally, Helen McNeill (Toronto) demonstrated that the atypical cadherin Fat protein is enriched along the oral-aboral

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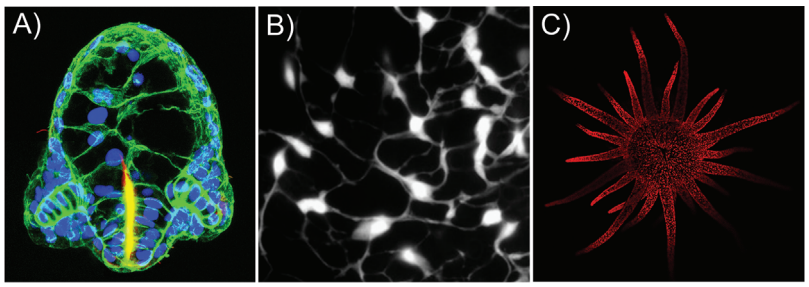

D)

Species (Genomic Data URL)	Assembled Genome Size	% Repetitive	Scaffold N50	Gene Estimate	Presenter
Aiptasia (http://aiptasia.reefgenomics.org)	260 Mb	26	440 Kbp	~26,000	Christian Voolstra (KAUST)
Clytia hemisphaerica	426 Mb	NR	208 Kbp	26, 203	Richard Copley (Villefranche sur mer)
Hydractinia echinata (http://research.nhgri.nih.gov/hydractinia/)	420 Mb	46	64 Kbp	NR	Christine Schnitzler (NIH, Bethesda)
Hydractinia symbiolongicarpis (http://research.nhgri.nih.gov/hydractinia/)	397 Mb	46	260 Kbp	NR	Christine Schnitzler (NIH, Bethesda)
Hydra magnipapillata (https://webfiles.uci.edu/resteele/public)	870 Mb	57	1 Mbp	19,966	Robert Steele (UC Irvine)

Figure 1. Cnidarian models highlighting different focus areas of the meeting. **A:** Late larval stage ("rocket larva") of *Aglantha digitale* stained with phalloidin (green), anti-tubulin (red), and DAPI (blue). This larva emerges from an invariant cleavage program highly uncommon among other cnidarians and then transforms into a prototypic hydrozoan medusa (image courtesy of Yulia Kraus, Moscow State University). **B:** Part of the peduncle nerve net of a transgenic *Hydra* polyp stably expressing GFP driven by an actin promoter in the interstitial lineage (image courtesy of Charles David, University of Munich, and Rob Steele, UC Irvine). **C:** Head of the sea anemone *Aiptasia*, an emerging laboratory model in coral reef research to analyze host-symbiont interaction. Red dots indicate densely packed photosynthetic *Symbiodinium* dinoflagellates based on chlorophyll fluorescence (image courtesy of Sebastian Baumgarten, KAUST Saudi Arabia). **D:** Summary of current genome analysis in various cnidarian models. NR = not reported. Please note that sequencing data for *Hydractinia* are not yet deposited at the URL, but will be within a few months.

plane within each ectodermal epithelial cell of *Hydra*, demonstrating likely conservation of planar cell polarity establishment mechanisms within all eumetazoans.

Many non-bilaterian animals exhibit remarkable plasticity and longevity, which makes these phyla useful for understanding somatic stem cell longevity and aging. Eric Röttinger (Nice) found that *Nematostella* telomeres have the typical structure of human telomeres, including the shelterin complex. Loss of shelterin function affects chromosome integrity. Roger Revilla-i-Domingo (Vienna) is working to uncover the ancient gene regulatory network of stem cells in the sponge *Suberites domuncula* and to this end is developing a stem cell assay to test the self-renewal and differentiation potential of single sponge stem cells. Lucas Leclere (Villefranche-sur-mer) presented the surprising potential of *Clytia* medusae to regenerate various organs and body parts based on the proliferation and differentiation of multipotent stem cells. Celina Juliano (Davis) discussed the PIWI-piRNA pathway, a small RNA regulatory pathway that is essential in the somatic stem cells of *Hydra*. Small RNAs were also discussed by Ulrich Technau (Vienna) who showed that in *Nematostella* mRNA cleavage is the primary mechanism of miRNA-directed gene repression, with clear effects on many biological processes including development [3].

Finally, talks highlighting nematocyte biology were included in this session. Yehu Moran (Jerusalem) analyzed venom production in *Nematostella* by combining molecular approaches and protein mass spectrometry. Unexpectedly, venom production appears to start in early embryonic stages, thus uncovering a complex scenario of venom composition and usage at various life cycle stages. Suat Özbek (Heidelberg) has succeeded in studying the binding dynamics of cysteine-rich protein domains characteristic for *Hydra* minicollagens at a structural level. Based on this information, he has developed versatile and patented polymerization units useful for diverse applications in protein chemistry.

News from the nerve nets of cnidarians

Early diverging metazoans offer important opportunities for furthering our understanding of neurobiology. Over the course of three sessions a wide variety of presentations covered many topics in neuroscience, including developmental, evolutionary, mechanistic, and behavioral studies. Michael Layden (Lehigh University) discussed *Nematostella* neurogenesis and Delta-Notch signaling; while Notch represses neural fate, Delta activates neural fate through the activation and repression of specific targets. Gemma Richards (Bergen) found that *SoxB2* and *NvAth-like* (high identity to neurogenin/neuroD) are required for neurogenesis, but their expression is independent of each other in *Nematostella*. SoxB2 and Delta-Notch signaling act in separate, but parallel pathways to specify nerve cells. Hakima Flici (Galway) found that in *Hydractinia* neurogenesis SoxB1 activates the expression of *HDAC2*, and subsequently SoxB1 and HDAC2 proteins interact, a phenomenon conserved in mammals.

Several talks investigated the evolutionary origins of the nervous system. Pawel Burkhardt (MBA, UK) is investigating the evolutionary origins of the SNARE proteins using choanoflagellates, which are sister to all metazoans. SNARE complexes predate the nervous system and therefore were likely co-opted for synaptic transmission [4]. Gáspár Jékely (Tübingen) discussed

the evolution of neuropeptides, which are difficult to compare between species because of their diversity and lack of conservation across large phylogenetic distances. Stefan Gründer (Aachen) discussed the Hydra HyNaC cation channels which surprisingly are activated by neuropeptides. *Hydra* does not have acetylcholine-activated neuromuscular junctions [2]; these results suggest that instead *Hydra* has peptide-activated neuromuscular junctions. Sumiko Minobe (Fukuoka) discussed her research on a novel gene, *hmp4846*, which encodes a neuropeptide that is expressed in nerve cells at the tentacle base and induces rhythmic movement of the tentacles.

Considerable effort is being focused on understanding how neural circuits translate to behavior. Rafael Yuste (Columbia University) spoke about the BRAIN initiative, which aims to establish a functional connectome for the entire human brain [5]. The Yuste group has turned to *Hydra* in order to develop the necessary technologies to attain this goal. To this end, Christophe Dupre (Columbia University) is monitoring the activity of the *Hydra* nerve net in real time using the calcium indicator GCaMP6 and has already identified three distinct neural circuits in *Hydra*. Ekaterina Taralova (Columbia University) is building a computer program to track *Hydra* behavior and neural circuit activation over time. The ultimate goal of the Yuste group is to build connectivity, activity, and behavior matrices for *Hydra* and then link these together in order to understand how a nervous system creates behavior. Similarly, the Jékely research group has completed an impressive large scale project to map nerve connectivity in the *Platynereis* larva down to the level of synaptic vesicles using electron microscopy.

In order to define how neural circuits dictate *Hydra* behavior it is important to understand the intricacies of these behaviors. Passano and McCullough [6] proposed that rhythmic potentials produced by the foot control *Hydra* behavior. Charles David (Munich) validated this hypothesis using a series of grafts between nerve-free and wild type *Hydra* and demonstrated that the foot "control center" is dominant over the head (Fig. 1B). Hiroshi Shimizu (KAUST) presented his work suggesting that *Hydra* sense gravity and undergo negative geotaxis. Andrea Murillo (Kiel) demonstrated very convincing evidence that the behavior of *Hydra* is influenced by its microbiome similar to the microbiota-gut-brain axis in mammals [7] and this work emphasizes the importance of considering the holobiome when studying animal biology.

Symbiosis and cnidarians as holobionts

The final part of the meeting was devoted to the impact of symbiosis on cnidarian biology. Because the development and life cycles of non-bilaterian animals are particularly sensitive to environmental factors, they are expected to offer new opportunities in EcoEvoDevo research [8, 9]. Thomas Bosch (Kiel) started the session by emphasizing the necessity to consider animals as developmental and evolutionary units (holobionts) composed of both the host and its associated symbionts and microbes. David Miller (Townsville) has succeeded in characterizing an anti-microbial peptide (AMP) of the Defensin-like family in the coral *Acropora* that is effective against a broad spectrum of gram-positive bacteria. Katja Schröder (Kiel) described two *Hydra* AMPs that act against gram-positive bacteria. Sebastian Fraune (Kiel) analyzed *Nematostella* microbiome dynamics during embryonic development as well as under variable temperature and salinity conditions; in strains collected from different geographical locations, core microbiomes established during embryogenesis are maintained over years of laboratory culture.

The sea anemone *Aiptasia* is a newly emerging laboratory model to study symbiosis between a cnidarian host and the photosynthetic dinoflagellate symbiont *Symbiodinium* with an expanding array of molecular tools (Fig. 1C). Manuel Aranda Lastra (KAUST) found 1,300 differentially methylated genes when comparing symbiotic and aposymbiotic *Aiptasia*; these genes were involved in symbiont recognition, apoptotic cell clearance, and nutrient metabolism. Sebastian Baumgarten (KAUST) found that about ten miRNAs exhibit expression changes as a result of symbiosis and he is now exploring their mRNA targets. Annika Guse (Heidelberg) is developing protocols to improve the use of *Aiptasia* as a laboratory model. Stephanie Barnai-Verdier (Nice) presented her efforts to establish an in vitro model for cnidarian-dinoflagellate symbiosis using primary cell cultures from tentacle cells of the sea anemone *Anemonia viridis*. Mayuko Hamada (OIST) applied transcriptomics and genomics to investigate co-evolution between *Hydra viridissima* and its *Chlorella* symbionts, which depend on *Hydra* for nitrogen metabolism. Finally, a talk by Juris Grasis (San Diego State University) concluded the meeting by extending our discussion into the world of viruses. His data from *Hydra* provide clear evidence that bacteria play a major role in virus recognition and in immune responses upon viral infection.

Outlook for the future

This community has enthusiastically embraced new technologies, which is clearly pushing our research forward at a rapid rate. In particular, the amount of sequencing data collected (both genomic and transcriptomic) is impressive. The next challenge is to further develop reliable methods for functional gene analysis in a wider variety of non-bilaterian models. The *Hydra* community has had several years of success with functional studies using transgenic technology to produce reliable gene knockdown by RNAi. Updates from this meeting from Alex Klimovich (Kiel) have us excited about an inducible system of gene knockdown in *Hydra*. The *Nematostella* community has made notable strides in gene editing using CRISPR-Cas9 technology. Undoubtedly this technology will expand the number of pre-bilaterian systems in which rigorous functional studies can be performed. The most encouraging aspect of this meeting was the large number of young researchers and the presence of experienced researchers who have been newly attracted to the field. With so many enthusiastic minds, young and not so young, we look forward to hearing about many exciting advances when we convene again in 2017.

Acknowledgement

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