Program

TUESDAY MAY 13

9.00 Welcome, Introduction, Background and Goals

Session 1  Eukaryotic Phylogeny
(Chair - Peter Anderson)

9.15 Pawel Burkhardt (Berkeley) - Choanoflagellates and the origin of synaptic proteins.

9.50 Joseph Ryan (Univ. of Florida) - Phylogenetic Position of the Ctenophores

10.25 Break

10.50 Casey Dunn (Brown Univ.) - The evolution of complex traits isn't so simple

11.25 Ken Halanych (Auburn) - Phylogenomic perspectives on animal phylogeny with attention to basal lineages

12.00 Lunch

Session 2  "Neurobiology" of basal metazoans
(Chair - Mark Martindale)

2.00 Sally Leys (Alberta) - Sponge physiology and genomics shed light on the evolution of early coordinating systems.

2.35 Leonid Moroz (Univ. of Florida) - Independent origins and parallel evolution of nervous systems in ctenophores.

3.10 Robert Meech (Bristol, UK) - Ionic mechanisms of integration in Aglantha digitale and other hydrozoan jellyfish.

3.45 Richard Satterlie (UNC, Willmington) - The Cnidarian "Brain"

4.20 Break

5.00 Poster Session, Reception

Session 3  Poster Session and Reception
**WEDNESDAY MAY 14**

**Session 4  Neurogenesis**

(Chair - Sally Leys)

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<tr>
<td>9.00</td>
<td>Claus Nielsen (Copenhagen)</td>
<td>Larval Nervous Systems: true larval and precocious adult.</td>
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<td>9.35</td>
<td>Melissa Rolls (Penn. State Univ.)</td>
<td>Evolution of Neuronal Polarity</td>
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10.10 Break

**Session 5  Contributed Papers I**

(Chair - Casey Dunn)

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<tr>
<td>10.40</td>
<td>Marta Chiodin (Univ. College London)</td>
<td>Molecular mapping of an acoel nervous system.</td>
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<td>11.00</td>
<td>Fred Keijzer (Univ. of Groningen, Netherlands)</td>
<td>The skin brain thesis</td>
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<td>11.20</td>
<td>Michael Layden (Univ. of Florida)</td>
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<td>11.40</td>
<td>Charles David (Univ. of Munich)</td>
<td>Neuronal connectivity and the control of contractile behavior in Hydra</td>
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12.00 Lunch

**Session 6  Neurobiology of Basal Deuterostomes and Protostomes**

(Chair - Richard Satterlie)

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<td>2.00</td>
<td>Pedro Martinez (Barcelona)</td>
<td>The nervous system of Xenoacelomorpha: a tale of progressive cephalization.</td>
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<td>2.35</td>
<td>Elaine Seaver (Univ. of Florida)</td>
<td>Protostome nervous systems</td>
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<td>3.10</td>
<td>Linda Holland (U.C. San Diego)</td>
<td>Evolution of nervous systems in basal deuterostomes.</td>
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3.45 Break

**Session 7  Organized Discussion I**

(Moderator - Dirk Bucher)

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<td>4.15</td>
<td>&quot;What is a Neuron?&quot;</td>
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<td>&quot;Did nervous systems evolve more than once?&quot;</td>
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<td>&quot;Are extant nervous system mono- or polyphyletic?&quot;</td>
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7.00 Banquet
THURSDAY MAY 15

Session 8  Contributed Papers II  
(Chair - Elaine Seaver)
9.00 Fabian Rentzch (Univ. of Bergen, Norway) - Dedicated neural progenitor cells have a pre-bilaterian origin.
9.20 Linda Boland (Univ. of Richmond) - Using Nature's Mutations to Explore a lipid binding site on IR potassium channels.
9.40 Christophe Dupre (Columbia Univ.) - Activity of the nervous system of Hydra
10.00 Break

Session 9  Origins of the "Neurobiological Tool Kit"
(Chair - Robert Meech)
10.30 Yehu Moran (Israel) - The evolution of calcium and sodium selectivity in neuronal conduction.
11.05 Tim Jegla (Penn State) - Nervous system evolution drove functional diversification of metazoan K channels.
11.40 Sakiko Okumoto (Virginia Tech.) - Diverse roles of plant glutamate-like receptors (GLRs).
12.00 Lunch
1.00 Helgi Schioth (Upssala) - Origin of G Protein-Coupled Receptors
1.35 Stefan Gründer (Aachen) - Origin of peptide-gated ion channels

Session 10  Organized Discussion II  
(Moderator - Dirk Bucher)
2.10 Sample Topics
"Revisiting previous questions"
"Common ancestor or convergence"
4.00 Adjourn
CHOANOFLAGELLATES AND THE ORIGIN OF SYNAPTIC PROTEINS
Pawel Burkhardt
Dept. of Molecular and Cell Biology, University of California, Berkeley

The origin of neurons was a key event in animal evolution, allowing animals to evolve rapid behavioral responses to environmental cues. Reconstructing the origin of the synaptic proteins promises to reveal their ancestral functions and might shed light on the evolution of the first neuron-like cells in animals. By analyzing the genomes of diverse animals and their closest relatives, we have reconstructed in detail the evolutionary history of diverse pre- and post-synaptic proteins and find that many evolved before the divergence of choanoflagellates and animals from filastereans. To investigate ancestral functions of synaptic proteins, we have characterized the structure and function of synaptic proteins in choanoflagellates. We identified a primordial neurosecretory apparatus in choanoflagellates and find that the mechanism by which pre-synaptic proteins required for secretion of neurotransmitters is conserved between choanoflagellates and animals. Moreover, studies on post-synaptic scaffolding proteins reveal unexpected localization patterns in choanoflagellates and new binding partners, both which we find to be conserved in animals. These findings demonstrate that the study of choanoflagellates can reveal ancient and previously undescribed functions of neuronal proteins.

THE AFFINITIES OF THE CTENOPHORAE AND THE ORIGIN OF THE NERVOUS SYSTEM, FERTILE SOURCES OF DISCUSSION FOR THE PAST 150+ YEARS
Joseph Ryan
Whitney Laboratory for Marine Bioscience, University of Florida

The conspicuous body plan of ctenophores (comb jellies) simultaneously makes these animals very easy to identify as ctenophores, and very difficult to classify in relation to other animals. Early phylogenetic studies based on gene sequences produced conflicting results and were unable to conclusively resolve the position of Ctenophora. Our recent work examining multiple lines of genomic evidence has conclusively ruled out a close relationship of ctenophores with Bilateria, Cnidaria, or Placozoa and shows strong evidence supporting ctenophores as the sister lineage to the rest of animals. Ctenophores have neural cell types and analysis of the Mnemiopsis leidyi (Ctenophora) genome has raised interesting questions as to the relationship of the ctenophore nervous system to the nervous systems of other animals. On one hand, the genome of Mnemiopsis, like the sponge Amphimedon queenslandica (Porifera), have many of the genes known to be involved in the nervous systems of cnidarians and bilaterians including genes involved in axon guidance, the post-synapse, and neural cell type specification; these data support a common origin. On the other hand, several lines of evidence point to the uniqueness of the ctenophore nervous system including absence of true ionic glutamate receptors, lack of genes involved in the synthesis of dopamine and other catecholamine biosynthesis components, absence of evidence for serotonergic signaling, and a unique synaptic morphology. These data support independent origins of the ctenophore nervous system, or at least, major changes of neural cell types along both the ctenophore and non-ctenophore lineages. While major progress has been made in regards to the phylogenetic position of ctenophores, we are still some way from being able to relate the nervous systems of ctenophores to the nervous systems of other animals with confidence. We do, however, now have a published genome, an increasing number of experimental techniques, and the power to design experiments that will clarify this relationship.
THE EVOLUTION OF COMPLEX TRAITS ISN'T SO SIMPLE.

Casey Dunn  
Dept. of Ecology and Evolutionary Biology, Brown University

There is now broad consensus for many deep relationships of central interest in the animal tree of life. It is now clear that many "complex" characters that were thought to exhibit minimal evolutionary change in fact have extensive converge, with many independent gains and losses. There is growing focus on a diminishing number of important questions that still do not have strong, consistent support. With this growing consensus for many relationships, these remaining questions have become much clearer and there are well-defined alternative hypotheses that can now be evaluated. Some of these open questions may never be resolved, but current advances in analysis methods, taxon sampling, and data acquisition suggest promising approaches for tackling them.

LOPHOTROCOZOA & BASAL METAZOA

Ken Halanych, Dept. of Biological Sciences, Auburn University

Since the dawn of the molecular age and with improve phylogenetic tools, our understanding of animal phylogeny and evolution has improved immensely. Recent phylogenomic analyses have been reaching consensus for a number of outstanding issues across the animal tree of life. Here I will review some of the recent findings on metazoan phylogeny and discuss some of the major outstanding questions. Attention will be given to regions of the tree that are particularly important for understanding key transitions in functional morphology of animals.

SESSION 2: "NEUROBIOLOGY" OF BASAL METAZOANS

SPONGE PHYSIOLOGY AND GENOMICS SHED LIGHT ON THE EVOLUTION OF EARLY COORDINATION SYSTEMS.

Sally Leys  
Dept. of Biology, University of Alberta, Canada

Genomic and transcriptomic analyses show sponges possess a large repertoire of genes associated with neuronal processes in other animals, but what is the evidence these are used in a coordination or sensory context in sponges? The very different phylogenetic hypotheses today suggest wildly different scenarios for the evolution of tissues and coordination systems in early animals. Either the sponge genomic tool-set reflects a simple, pre-neural system used to protect the sponge filter, or the sponge tool-set reflects remnants of a more complex signaling system and sponges have lost cell types, tissues and regionalization to suit their current suspension feeding habit. Comparative transcriptome data can be informative but need to be assessed in the context of current knowledge of sponge tissue structure and physiology. The evolution of tissues and coordination should also be considered in light of what is expected to have been the paleoecological framework in which the first animals evolved.
INDEPENDENT ORIGINS AND PARALLEL EVOLUTION OF NEURAL SYSTEMS IN CTENOPHORES

Leonid L. Moroz
The Whitney Laboratory of Marine Bioscience and Dept of Neuroscience, University of Florida, FL 32080, USA

The origins of neural systems remain unresolved. In contrast to other basal metazoans, ctenophores, or comb jellies, have both complex nervous and mesoderm-derived muscular systems. These holoplanktonic predators also have sophisticated ciliated locomotion, behaviour and distinct development. Here, I will summarize the analysis of ctenophore’s neuro-muscular organization performed by our laboratory and our collaborators. First, by sequencing of the genome of Pleurobrachia bachei, Pacific sea gooseberry, together with 14 other ctenophore transcriptomes, we show that they are remarkably distinct from other animal genomes in their content of neurogenic, immune and developmental genes. Our integrative analyses place Ctenophora as the earliest branching lineage within Metazoa. This hypothesis is supported by comparative analysis of multiple gene families, including the apparent absence of HOX genes, canonical microRNA machinery, and reduced immune complement in ctenophores. Although two distinct nervous systems are well-recognized in ctenophores, many bilaterian neuron-specific genes and genes of “classical” neurotransmitter pathways either are absent or, if present, are not expressed in neurons. Second, we discover several dozen of candidates for novel signal molecules in ctenophores, and showed their recruitments in both neural and developmental functions. Our developmental, metabolomic, proteomics, physiological and pharmacological data are consistent with the hypothesis that ctenophore neural systems, and possibly muscle specification, evolved independently from those in other animals. Finally, we started to extend similar integrative (genomic-proteomic-physiological) analyses to other animal lineages suggesting that there were at least 9 independent events of nervous system centralization from a brainless common bilaterian ancestor without a CNS. Combined, these data provide the alternative scenario to the most widely accepted ‘single-origin’ hypotheses of both neurons and central nervous systems in animals.

Supported by NSF, NIH and NASA grants.

IONIC MECHANISMS OF INTEGRATION IN AGLANTHA DIGITALE AND OTHER HYDROZOAN JELLYFISH.

Robert Meech, University of Bristol, United Kingdom

It is evident that many of the ion channel variants involved in propagating signals in the earliest multicellular life-forms were already present in the protozoa. In fact propagating impulses were the end product of at least three different evolutionary time-lines. Each time-line co-opted much the same set of ion channels but inserted them into a different cell matrix. The matrices were: the loose three-dimensional trabecular reticulum of the glass sponge, the two-dimensional epithelial sheets of the hydrozoan jellyfish and the two and three dimensional neuronal networks found in the ctenophores and cnidaria. The apparent ease with which natural selection assembled the building blocks for such systems, makes it likely that ctenophore and cnidarian neurons evolved independently.

It seems that the first neurons simply conveyed information; the process of integrating the information took place at the effector. If the effector was a muscle epithelium it made an integrative surface. Key stages that lead to a system based on neural integration were the evolution of i) all-or-nothing regenerative activity; ii) a way of translating all-or-nothing signals into graded responses; iii) the means of generating rhythmic activity. Rhythmic muscle activity appeared first in the cnidaria and marked a giant step in the role of neurons because responsiveness became graded not simply by force but also by frequency. The significance of frequency is that it can be adjusted down as well as up; there is the potential for inhibition. Some groups, notably the crustacea, are associated with peripheral inhibition but inhibitory control is centralized in jellyfish.

The presentation will focus on the role of ion channels in central and peripheral mechanisms of integration in Aglantha during slow and fast swimming and when feeding.
THE CNIDARIAN BRAIN
Richard Satterlie
University of North Carolina Wilmington

Despite being widely considered to be “nerve net animals,” the medusoid generations of cnidarians possess various levels of concentration of neurons and sensory cells into integrating units that warrant consideration as centralized nervous systems. As a consequence of radial symmetry, this centralization is distributed differently than in bilateral organisms. Yet the equivalent of ganglia, commissures and connectives, and condensed nerves are seen. A comparative look at representatives of the three groups with medusoid members supports this organizational view, and highlights the existence of identifiable neurons and neuronal groups in cnidarians.

SESSION 4: NEUROGENESIS

LARVAL NERVOUS SYSTEMS: TRUE LARVAL AND PRECOCIOUS ADULT.
Claus Nielsen
The Natural History Museum of Denmark, University of Copenhagen. Denmark

Ciliated larvae of cnidarians and bilaterians show a truly larval organ, the apical organ, which is sensory, probably involved in metamorphosis, and disappears before or at metamorphosis. The ciliated protostome larvae show ganglia/nerve cords which are retained as the adult central nervous system (CNS). Two structures can be recognized, viz. a pair of cerebral ganglia, which form the major part of the adult brain, and a circumblastoporal nerve cord, which becomes differentiated into a perioral loop, paired or secondarily fused ventral nerve cords, and a small perianal loop. The anterior loop becomes integrated in the brain. This has been well documented through cell-lineage studies in a number of spiralians and homologies with similar structures in the ecdysozoans are strongly indicated. The deuterostomes are more difficult to interpret. The nervous systems of echinoderms and enteropneusts are very difficult to homologize with those of the protostomes. The ontogeny of the chordate CNS can perhaps be interpreted as a variation of the ontogeny of the circumblastoporal nerve cord of the protostomes, and this is strongly supported by patterns of gene expression. Presence of homologues of the protostomian cerebral ganglia has not been indicated through morphological studies, but patterns of gene expression indicate presence of homologous areas.

EVOLUTION OF NEURONAL POLARITY
Melissa Rolls
Dept. of Biochemistry and Molecular Biology, Pennsylvania State University

When the cell theory was initially proposed in 1839, the nervous system was considered a special case, and perhaps not made up of cells like the rest of the body. Fifty years later neurons were finally accepted as cells, but what are the key features of these cells that unify them and differentiate them from other cell types? One of the first ideas was that directional information transfer required a cell with two different ends, axons and dendrites. One hypothesis then, is that neuronal polarity is an essential part of neuronal identity and should be a shared feature of all neurons. Alternatively, it has been suggested that only vertebrate neurons are truly polarized with distinct axons and dendrites arising from the cell body. To distinguish between these hypotheses, one first needs to identify the underlying features that distinguish axons and dendrites. I will argue that the essential difference between axons and dendrites is distinct microtubule polarity in the two compartments. With this key feature in mind, it is possible to determine which animals have neurons with axons and dendrites. Based on microtubule polarity, axons and dendrites exist in both Drosophila and C. elegans. Surprisingly, a specialized cytoskeleton that organizes a membrane diffusion barrier at the axon initial segment is also present in Drosophila neurons and thus this feature may also be widespread in bilaterians. We propose that the ancestral bilaterian had polarized neurons with true axons and dendrites distinguished by distinct microtubule organization and an axon initial segment. We are now examining the evolutionary origins of axons and dendrites using live imaging of genetically encoded polarity markers in cnidarians.
THE NERVOUS SYSTEM OF XENOACELOMORPHA: A TALE OF PROGRESSIVE CEPHALIZATION

Pedro Martinez
Dept. of Genetics, University of Barcelona, Spain

Xenoacelomorpha is, most probably, a monophyletic group that includes three clades: Acoela, Nemertodermatida and Xenoturbellida. The group still has contentious phylogenetic affinities, though most authors place it as the sister group of the remaining bilaterians some would include it as a fourth phylum within the Deuterostomia. Traditionally, the characteristic of having relatively "simple" body plans has been critical in ascribing these animals to basal groups and, thus, they have played an important role in most hypotheses regarding the origin of Bilateria. Most characteristics used in their classification are morphological, though molecular cloning has provided various types of sequences that have been used in more recent phylogenetic analysis.

Over the last few years, our group, along with others, has undertaken a systematic study of the microscopic anatomy of these worms; we mostly focus our attention on those structures (tissues) that are especially relevant to our understanding of the origin of bilaterians; for instance, the mesoderm and nervous system. Our studies have been aided by the use of molecular/developmental tools, the most important of which has been the sequencing of the complete genomes and transcriptomes of different members of the three clades. The data obtained has been used to reanalyze phylogenetic relationships but, most importantly, has allowed us, for the first time, to follow the evolutionary history of gene families and to study their expression patterns during development, both in space and time.

A major aim of our group is to understand the origin of "cephalized" nervous systems. How complex brains are assembled has been a matter of intense debate for at least a hundred years. We are now tackling this problem using Xenoacelomorpha models. These represent an ideal system for this work, since the members of the three clades have nervous systems with different degrees of cephalization; from the relatively simple sub-epithelial net of Xenoturbella to the compact brain of acoels. How this process of "progressive" cephalization is reflected in the genomes or transcriptomes of these three groups of animals will be the focus of my talk. Genome evolution, the simplification of gene families and the specific use of several neural transcription factors will represent the core of what I will discuss at the meeting.

PROTOTOMES NERVOUS SYSTEMS

Elaine Seaver
Whitney Laboratory for Marine Bioscience, University of Florida

The anatomical organization and development of nervous systems in protostomes are widely diverse. However, there are numerous conserved organizational features including the presence of nerves, bilateral organization, an anterior concentration of sensory cells, a high density of neurons in the head that is often condensed into a brain, and longitudinal nerves running along the anterior-posterior axis. A defining organizational feature of many protostome nervous systems is the presence of the orthogonal arrangement of nerves. From this basic plan, there have been repeated independent condensations in different clades. The relationship between the orthogonal nervous system and the segmented body plan will be discussed. Historically, our knowledge of the cellular development of the protostome nervous system has been disproportionately influenced by studies in model insects. Recent data representing more broad taxon sampling will be presented.

EVOLUTION OF NERVOUS SYSTEMS IN BASAL DEUTEROSTOMES

Linda Z. Holland
Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093-0202 USA
The deuterostomes include the Ambulacraria (echinoderms and hemichordates) and the chordates (cephalochordates, tunicates and vertebrates). Xenoturbellids, sometimes together with acoel flatworms, occupy an unresolved position—either as sister group of Ambulacraria, as basal deuterostomes or as basal to the bilaterians. A major question of nervous system evolution is whether the basal deuterostome had an epidermal nerve net or a central nervous system. *Xenoturbella* has an epidermal nerve net. However, as *Xenoturbella* it is thought to have lost many deuterostome features, it cannot be determined whether a nerve net is an ancestral feature of Xenoturbellids or a secondary simplification. Echinoderms have an epidermal nervous system, a circumoral nerve ring and radial nerves. Hemichordates have a basiepidermal nerve net and dorsal and ventral nerve cords but appear to lack a brain, while all chordates have a dorsal nerve cord with an anterior brain or sensory vesicle.

Several schemes have been proposed for the evolution of deuterostome nervous systems. 1) the ancestral deuterostome had both an apical nervous system and a longitudinal one. The two remained separate in Ambulacraria while the latter evolved into the radial nerves in echinoderms and the nerve cord(s) in hemichordates. In chordates, the two nervous systems fused, the apical system becoming the brain. 2) the ancestral deuterostome had only a nerve net; nerve cords in echinoderms, hemichordates and chordates evolved independently. 3) the ancestral deuterostome had a longitudinal nerve cord with an anterior brain plus an ectoderm with numerous sensory neurons. The nerve cord was lost in echinoderms; echinoderm nerve cords, which unlike chordate nerve cords, do not express *Hox* genes, evolved independently. In hemichordates, either the dorsal or ventral nerve cord is homologous to chordate nerve cords; the brain was lost. Concomitantly, the subepidermal nerve net was elaborated. Opinions concerning the relative merits of these schemes, particularly schemes 2 and 3 are highly divided. Missing from the discussion are detailed data on gene expression and on neuronal subtypes in the developing nerve cords of hemichordates. If gene expression and types of neurons are very different from those in chordates, scheme 2 would be supported. If, on the other hand, if gene expression and types of neurons in developing hemichordate and chordate nerve cords are similar, the balance could tip in favor of scheme 3.

**SESSION 9: ORIGIN OF THE "NEUROBIOLOGICAL" TOOL KIT.**

**THE EVOLUTION OF CALCIUM AND SODIUM SELECTIVITY IN NEURONAL CONDUCTION**

**Yehu Moran**
Department of Ecology, Evolution and Behavior, Hebrew University of Jerusalem, Israel

Ion selectivity of metazoan voltage-gated sodium channels (Navs) is considered critical for neuronal signaling and has long been attributed to a ring of four conserved amino acids that constitute the ion selectivity filter at the channel pore. Yet, heterologous expression and electrophysiological characterization of Na, homologs from the sea anemone *Nematostella vectensis*, a member of the early-branching metazoan phylum *Cnidaria*, revealed besides channels with preference for calcium ions ("Nav-like channels"), a sodium-selective channel bearing a non-canonical selectivity filter. Mutagenesis followed by physiological assays suggests that pore elements additional to the selectivity filter determine in this channel the preference for sodium ions. Phylogenetic analysis assigns the *Nematostella* sodium-selective channel to a channel group unique to Cnidaria, which diverged more than 500 million years ago from a calcium-conducting sodium channel homolog in the last common ancestor of all extant cnidarians. The identification of cnidian Na+-selective ion channels distinct by filter structure and phylogeny from the channels of bilaterian animals indicates that selectivity for sodium in neuronal signaling emerged independently in these two animal lineages. This convergence strongly suggests that sodium selectivity has a significant functional advantage. However, the calcium-conducting Na,-like channels are still retained in many phyla including cnidarians, mollusks, annelids, insects and urochordates in parallel to Navs, raising the possibility that calcium-based action potentials are advantageous in yet unknown contexts.
NERVOUS SYSTEM EVOLUTION DROVE FUNCTIONAL DIVERSIFICATION OF METAZOAN K⁺ CHANNELS.

Tim Jegla
Dept. of Biology, Pennsylvania State University

Metazoan K⁺ channels display a high degree of molecular and functional diversity encoded by 13 gene families shared between cnidarians and bilaterians. We have examined the functional evolution of voltage-gated K⁺ to better understand how neuronal excitability evolved. Several studies have demonstrated that the Shaker family voltage-gated K⁺ channels are functionally conserved between cnidarians and bilaterians indicating that they play conserved roles in action potential regulation. We now show that EAG and KCNQ family channels also show remarkable functional conservation between vertebrates and cnidarians. For instance, the biophysical characteristics of Erg K⁺ channels that are specialized for repolarization of broad action potentials were present in the cnidarian/bilaterian ancestor but have been lost in two protostome lineages. Genomic analysis indicates that the much of diversification of voltage-gated K⁺ channels occurred after the evolution of the first nervous systems. Ancestral Shaker and Eag channels first appear in ctenophores, but the functionally distinct gene subfamilies shared between cnidarians and bilaterians are absent. Many of the channel types that evolved after the split from ctenophores regulate dendritic excitability in bilaterians. These results support the early divergence of ctenophores and suggest that the first neurons had fewer mechanisms for the regulation of intrinsic excitability.

DIVERSE ROLES OF PLANT GLUTAMATE-LIKE RECEPTORS (GLRs)
Michelle B. Price and Sakiko Okumoto
Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University

The plant glutamate receptors (GLRs) are initially discovered as potential homologs of mammalian ionotropic glutamate receptors (iGluRs). Since their first discovery, genetic and pharmacological approaches implicated plant GLRs in diverse physiological processes such as C/N ratio sensing, cation transport, root formation, pollen germination and plant-pathogen interaction. However, the exact properties of these channels, such as the spectrum of ligands, ion specificities, and subunit compositions are still not well understood.

It is well established that animal iGluRs form homo- or hetero-tetramers in order to form ligand-gated cation channels. In order to understand the subunit composition of plant GLRs, a modified yeast-2-hybrid system approach was taken and applied to 15 of the 20 AtGLRs. Using this approach, we have successfully identified GLR subunits that are capable of interacting with multiple other GLRs. Unlike iGluRs, sequence similarity between the subunit was not correlated with the likelihood of interaction among two given subunits. Interactions between the subunits were further examined using Förster resonance energy transfer (FRET). Using this approach, only homomeric interactions were identified between GLRs 1.1 and 3.4 in HEK293 cells.

Further, analysis of publicly available microarray data shows altered gene expression of a subfraction of GLRs in response to pathogen infection and bacterial elicitors. Indeed, recent studies implicate GLRs in pathogen- and wounding-induced signaling. We will discuss the potential roles of amino acid perception through plant GLRs in plant-pathogen interaction.

ORIGIN OF G PROTEIN-COUPLED RECEPTORS
Helgi Schiöth, and A. Krishnan
Dept. of Neuroscience, Univ. of Uppsala, Sweden

G protein-coupled receptors (GPCRs) are the largest superfamily among membrane bound proteins. The main GPCR families in mammals can be classified into the five main families named Glutamate, Rhodopsin, Adhesion, Frizzled and Secretin according to the GRAFS classification. There exist also other families that undergone several species specific expansions related to roles in sensing the environment, such as the vomeronasal receptors, taste receptors and nematode chemosensory. It has been debated if the different families of GPCRs share a common origin as they show low sequence similarities. We have aimed to delineate the overall GPCR repertoire in number of species and establishing long distance relationships. Mining of fungal genomes suggests that four of the five main
mammalian families of GPCRs, namely *Rhodopsin, Adhesion, Glutamate* and *Frizzled*, are present in fungi. We have provided evidence for that the *Rhodopsin* family emerged from the cAMP receptor family. The *Rhodopsin* family then expanded in metazoans while the cAMP receptor family is found in few invertebrate species and lost in the vertebrates. We estimate that the *Adhesion* and *Frizzled* families evolved before the split of unikonts from a common ancestor of all major eukaryotic lineages. Also, recently we clarified the origins of nematode chemosensory GPCRs providing further insights into the evolutionary events that shaped the GPCR chemosensory system in protostome species. Furthermore, we also aimed to explore the GPCR repertoire in several model organisms, including hemichordate (*Saccoglossus kowalevskii*), and as well as in the earliest diverging phyletic branch of the metazoa, the sponges (*Amphimedon queenslandica*). Although, having a diffuse nervous system, the hemichordate contains conserved orthologues for human *Adhesion* and *Glutamate* family members, with similar N-terminal domain architecture. This is particularly true for several genes involved in CNS development and regulation in vertebrates. Similarly, the sponges that lack the gut and the nervous system harbour a rich GPCR repertoire, including large expansions in the *Rhodopsin* family. Recently, the Ctenophore (*Mnemiopsis leidyi*) has been sequenced and they may form a sister lineage to animals including the most ancient sponges. Interestingly, a preliminary mining of *M. leidyi* suggests that they contain a remarkable rich repertoire of over 600 genes coding for families belonging to the *Rhodopsin* family. This could be one of the first large expansions of GPCR genes at the early origins of metazoa. These findings provide a better platform to perform systematic genome comparative analysis on a rich set of GPCRs in species that are known to lack nervous system.

ORIGIN OF PEPTIDE-GATED ION CHANNELS
Stefan Gründer
Institute of Physiology, Aachen University, Germany

Chemical synapses use small molecule transmitters and neuropeptides for transmission. It is textbook knowledge that only small molecule neurotransmitters directly gate ion channels, mediating fast neurotransmission, whereas neuropeptides activate exclusively G-protein coupled receptors, mediating slow neurotransmission. Cnidaria are animals with a simple body plan; nervous systems probably appeared in animals similar to Cnidarians. We used a model Cnidarian, *Hydra magnipapillata*, to characterize ion channels, the *Hydra* Na$^+$ channels (HyNaCs), that are directly gated by neuropeptides. The *Hydra* genome contains genes encoding 12 HyNaCs. We found that HyNaCs assemble in specific combinations into heterotrimeric ion channels, which, when expressed in *Xenopus* oocytes, are directly activated by neuropeptides of the *Hydra* nervous system, Hydra-RFamides. Using in situ hybridization, we located the HyNaC-expressing cells to either the base of the tentacles or the foot region, adjacent to the peptide-expressing neurons, suggesting that Hydra-RFamides are the natural ligands of HyNaCs. Our results suggest that the simple nervous system of *Hydra* uses neuropeptides for fast neurotransmission, suggesting that neuropeptides were among the first neurotransmitters mediating fast synaptic transmission. Related ion channels are also present in lophotrochozoa (protostomes) but apparently absent from chordates (deuterostomes), suggesting that fast transmission by neuropeptides was lost in the deuterostome lineage.
SESSION 3: POSTER SESSION

P1. EVOLUTION OF MEMORY MOLECULES: FROM MOLLUSK TO MAMMALS

Bostwick, Caleb J1, Yang Q, Fodor A., Moroz T.P. Kohn A.B., Hawkins R.D., Moroz L.L.

1University of Florida Whitney Laboratory

Memory is an ancient and highly conserved biological process. The molecular components of memory storage are conserved from invertebrates to mammals, including human memory. Proteins and RNA integral to invertebrate memory circuits may also be vital to mammalian circuit functions. Recent advances in next-generation sequencing and molecular biology have enabled the study genomic organization of single neurons. We examined molecular portraits of sensory, motor, and interneurons in memory-forming circuits of Aplysia californica. cDNA libraries were constructed for each neuron and subsequently sequenced using Ion Torrent semiconductor sequencing. Differential expression analyses and hierarchical clustering were then performed to reveal which cells had similar expression profiles and which transcripts were differentially expressed among cells. Neurons clustered into distinct groups based on cell type (sensory, motor) whether the sample was a single neuron or a small group of neurons. LFS motor neurons preferentially expressed an allatotropin-like peptide, while LE sensory neurons seemed to express higher levels of TBL1. Single-cell RNA-Seq sampling provided an unbiased transcriptome profile of each component (sensory, motor, interneuron) of the defensive siphon-withdrawal reflex. By studying the complete memory circuits of invertebrates such as Aplysia, we can gain insight into the more complicated circuits of the mammalian brain.

P2. CEPHALOPOD TRANSCRIPTOMES UNRAVEL DETAILS ABOUT CENTRALIZED BRAIN EVOLUTION IN MULTIPLE METAZOAAN LINEAGES


1The Whitney Laboratory for Marine Bioscience and 2Dept of Neuroscience, University of Florida, FL, USA 3Hebrew University of Jerusalem, Israel 4University of Texas Health Science Center, Houston, TX, USA 5University of Naples Federico II, Department of Biology, Italy

Cephalopod molluscs (Nautilus, Loligo, Octopus, Sepia) are powerful models for comparative biology and neuroscience. The complexity of their nervous systems ranges from simple cords (Nautilus) to one of the most intricate brains of the animal kingdom in Octopus. Of all cephalopod innovations, the most extraordinary structure is the vertical lobe, where we find cell circuits modulating the most advanced learning and memory in all invertebrates. The remarkable morphological, behavioral, and physiological novelties in cephalopods are the result of either a conserved “genomic toolkit” supporting the development of a complex brain across taxa, innovative mechanisms unique to cephalopods, or some combination thereof. To examine this, we sequenced neuronal transcriptomes from key model cephalopods and made comparisons to one another and to our sequenced genome and transcriptomes of the gastropod mollusc, Aplysia. This approach allowed us to identify evolutionarily conserved neuronal genes and numerous genomic innovations within molluscs. For example, we have identified in cephalopods approximately half of all known Aplysia neuropeptides, including putative markers for sensory and motor neuron populations. Of all identified cephalopod neuropeptides, we have cloned and localized expression of seventeen in Octopus vulgaris, and thirteen in the squid Loligo pealei. Neuropeptide expression in cephalopod and gastropod neural tissues support a hypothesis that there has been expansion of potentially homologous neural cell populations across lineages. Of particular importance, we have identified in Octopus four neuropeptides in the vertical lobe that may play a role in cell signaling during memory function: Bradykinin, Conopressin, Buccalin, and FLRFamide. This comparative anatomical and genomic approach provides unique opportunities to reconstruct ancestral neuronal lineages, identify conserved cell types across species, and reveal trends in evolution within neural circuits.
P3. EPIGENETIC MACHINERY IN CTENOPHORES
Dabe, Emily C.; Kohn, A.B.; Moroz, L.L.;
Department of Neuroscience, Whitney Laboratory for Marine Bioscience, University of Florida, USA

DNA methylation and histone modifications are epigenetic modifications crucial to cell differentiation, development and neural functions. Contrary to Drosophila and C. elegans that have lost DNA methylation machinery, possibly due to their compact genome sizes and short life cycle, here we show that the phylum Ctenophora has conserved methylation components. Using the data from the recently sequenced genome of Pleurobrachia bachei we cloned DNA 5-cytosine methyltransferase gene (DNMT) and characterized its expression in major developmental stages and adult ctenophores. Distinctive mRNA expression in the digestive system, (stomach, pharynx and mouth), tentacles and unique patterns in between ciliated comb rows in adult Pleurobrachia collectively suggest that DNMT mRNA expression levels are both cell-specific and noticeable in areas of high proliferation. Next, using colorimetric ELISA assay for methylated DNA we directly showed that DNA methylation does occur in the Pleurobrachia genome, although it was significantly lower than in the molluscan (Aplysia) and mammalian (Ratus) nervous tissues. Additionally, Pleurobrachia possesses a diversity of genes encoding histone modifying enzymes, most of which are highly expressed from 8 to 64 cell stages. Comparative transcriptome data suggests that similar mechanisms of epigenetic regulation are conserved across Ctenophora. Combined, our data indicate that Pleurobrachia bachei has functional DNA methylation and histone modification machinery, possibly involved in epigenetic control of cell fate specification.

P4. INTERPLAY BETWEEN NEURAL DEVELOPMENT AND MEMORY OF INJURY IN APLYSSIA
Sanford, Rachael S., Dabe, E. C., Kohn A. B., Moroz, L. L.
Department of Neuroscience, University of Florida, Gainesville, FL, USA

Genome-wide transcriptional changes in development provide important information on mechanisms underlying neural fate specification, neurogenesis and neural patterning and how these processes relate to neural recovery after injury. Here, we characterize transcriptomes of embryonic, larval, and metamorphic stages in the marine mollusc Aplysia californica and examine novel molecular components associated with the development of the nervous system and injury recovery. To do this, we have performed deep RNA-sequencing of all key developmental stages starting from cleavage and gastrulation to post hatching veligers. We then analyzed this data by examining the genes which are involved in injury recovery. We found that many canonical neural genes are expressed in pre-neural stages such as neuropeptides like cerebrin, NMDA receptors and nicotinic receptors. However, increases in canonical neural gene expression correlate with the development of the CNS. The expression of genes involved in injury related neurogenesis such as Bcl-2, an apoptosis gene, associated with the appearance of neurons in development. We found this is also true with axon guidance genes like neuroglian and netrin. We hypothesize that that development and recovery after injury could share similar mechanisms.

P5. AXON GUIDANCE MOLECULES IDENTIFIED IN THE FOUR CLASSES OF SPONGES THROUGH TRANSCRIPTOME SEARCHES
Farrar, Nathan, Leys, S.P.
Department of Biological Sciences, University of Alberta, Edmonton, AB, CANADA

Axon guidance molecules (AGMs) play essential roles in the patterning of neural architecture by creating attractive and repulsive growth zones to extending neural processes. It is therefore surprising to find AGMs expressed in the transcriptomes of the aneural sponges. Using a set of 10 transcriptomes representing species from each of the four classes of sponges we searched for AGMs from each of the major families including netrin/DCC, slit/robo, semaphorin/plexin and ephrin/Ephs. Although we were unable to identify classical AGMs in each transcriptome searched, representatives from each group of AGMs are found among the sponge transcriptomes searched. Netrin, semaphorin and their receptors are the most frequently identified AGMs in the transcriptome set. The netrin receptor UNCS5 is the gene to be identified only in the homoscleromorphs and calcarea. We
hypothesize that AGMs are involved in patterning the developing canal system in sponges and were later coopted into patterning of neural and circulatory tissues in later evolving metazoans.

P6. THE FUNCTION OF TRP CHANNELS AND MECHANORECEPTION IN HYDRA

Beckmann, Anna, Ziegler, B., Hess, M. and Özbek, S.
1Centre for Organismal Studies, Dept. of Molecular Evolution and Genomics, Im Neuenheimer Feld 329, 69120 Heidelberg, Germany
2Innsbruck Medical University, Division of Histology and Embryology, Innrain 80, 6020 Innsbruck, Austria

TRP (Transient receptor potential) channels are a superfamily of cation channels that are activated by diverse environmental stimuli like pressure, light and temperature. In Cnidarians, basal metazoans that evolved the first nervous system, prey capture by stinging cells (nematocytes) is facilitated by a highly sophisticated mechanosensory apparatus, the cnidocil. Nematocyte discharge, which is triggered by mechanical stimuli, is accompanied by a calcium influx that is very likely induced by a TRP channel associated with the cnidocil.

We have identified and characterized a TRP channel (HyTRPA1) in Hydra nematocytes by molecular cloning, in situ hybridization and antibody staining. The Hydra TRPA1 channel is unambiguously localized to developing nematocyte nests in the body column and the cnidocil apparatus of mature nematocytes in tentacles. Here, it is associated with the ring of stereocilia around the central cnidocil. The organization of this structure is similar to vertebrate hair cells. Our data contribute new and important insights into the feeding behavior and nematocyst function in Hydra. In addition, the molecular and functional characterization of Hydra TRP channels provides answers about the evolution of nociception in early metazoans.

P7. DE NOVO GENOME SEQUENCING AND DEVELOPMENT OF MOLECULAR GENETIC TOOLS FOR MOON JELLY AURELIA SP.1

1Kavli Institute for Brain and Mind, University of California, San Diego, La Jolla, CA 92093, USA
2Department of Ecology and Evolutionary Biology, University of California, Los Angeles, 2154 Terasaki Life Science Building, Los Angeles, CA 90095, USA
3Computer Science Department Rm 1111, Harold Frank Hall University of California Santa Barbara, CA 93106, USA
4Department of Molecular Evolution and Genomics, Centre for Organismal Studies, Heidelberg University, Heidelberg, Germany
5Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
6Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA 93106, USA
7Department of Biological Chemistry, University of California, Irvine, CA 92697, USA

To better understand the neuronal basis of jellyfish behavior, we are developing molecular genetic tools that allow us to observe and manipulate jellyfish nervous systems. Our tools consist of two major parts: genome information and transgenic technologies. We have chosen Aurelia sp.1 (moon jelly) for our experimental animal as it is one of the most-well studied jellyfish species that have an effectively flat and transparent body, which provides unique advantages for studying nervous systems by optical methods. Since no complete genome information has been available from any jellyfish species, we are conducting de novo genome sequencing for Aurelia sp.1. Genomic DNA was prepared from a clonal population of Aurelia polyps and ephyrae, and sequence data were generated from an Illumina paired-end library, an Illumina mate-pair library, and a PacBio long-read library. We have used several different assemblers and error-correction pipelines to obtain a preliminary genome assembly to which over 80% of transcripts (covering >20,000 genes) can be mapped. In parallel with the genome sequencing, we are seeking transgenic and transfection technologies for Aurelia. Using the upstream sequences obtained from the preliminary assemblies, we have created vectors aimed at inducing expression in ubiquitous or neuron specific manners. Currently, attempts for generating germ-
line transformants as well as mosaic animals from re-aggregated polyps are undertaken. Once these genetic resources have been established it would become possible to study anatomy and function of the nervous system from a single-cell to a whole-system level.

P8. NEUROANATOMY STUDY BY IMMUNOFLUORESCENCE ON THE CNIDARIAN CLYTIA HEMISPHERICA
Jager Muriel, Chiori Roxane, Coste Alicia, Quéinnec Eric and Manuel Michaël.
Université Pierre et Marie Curie, Paris, France

The acquisition of neuro-sensory cells has been one of the major events in the evolution of animals (metazoans), enabling the conduction of sensory information and the establishment of coordinated reactions. Within metazoans, a nervous system is absent in sponges but is present in cnidarians, ctenophores and bilaterians. The hydrozoan Clytia hemisphaerica has recently emerged as a new forefront model for evo-devo and development biology studies. We undertook a study by immunohistochemistry of the architecture of the nervous system of C. hemisphaerica using several antibodies (structural antibodies, essentially against tubulin isoforms, and antibodies directed against neuromediators) completed by in situ hybridization using antisense RNA against the precursor of RFamide. We describe the organisation of the nervous system for the three forms that alternate during the C. hemisphaerica life cycle, i.e. planula larva, polyp and medusa. The main findings include substantial changes in nervous system organisation during the three-four days of larval life; marked oral/aboral polarity of the planula nervous system; a surprising degree of oral/aboral regionalisation of neuronal cell types in the feeding polyp (unprecedented in a hydrozoan polyp); and high sophistication of the architecture of the peripheral nervous system of the medusa.

P9. A BEHAVIOURAL PERSPECTIVE ON THE EARLY EVOLUTION OF NERVOUS SYSTEMS: A COMPUTATIONAL MODEL OF EXCITABLE MYOEPITHELIA
1R.A.J. van Elburg, 2O.O. de Wiljes, 3M. Biehl, and 4F.A. Keijzer
1 Institute of Artificial Intelligence, Groningen University
2 Research School of Behavioural and Cognitive Neurosciences, University of Groningen, the Netherlands
3 The Johann Bernoulli Institute for Mathematics and Computer Science, University of Groningen, the Netherlands
4 Dept. of Theoretical Philosophy, University of Groningen, the Netherlands

How and why the first nervous systems evolved remains an open question [1,2,3]. One influential scenario casts excitable myoepithelia, epithelia that combine conductive and contractile properties, as a plausible proto-nervous system [4].

We argue that while modern myoepithelia rely on gap junctions, early myoepithelia had to rely on chemical signalling [5,6] and can themselves be interpreted as a first step towards modern nervous systems [7]. We apply a modelling approach to assess the behaviourally relevant properties of this presumably ancient myoepithelial configuration. Our main questions concern the coordinative possibilities and limitations of such excitable myoepithelia and their potential relevance as a first step to full nervous systems.

We used a neurobiologically realistic simulation environment [8] to create a variety of artificial organisms with different shapes and other characteristics. We also developed a measurement for coordination as patterned activity across the myoepithelium. Our simulations showed that such a structure could induce body-scale patterns of activation, the relevant factors being noise level for spontaneous vesicle release, body dimensions, and body size. The simulations show that in small excitable myoepithelia whole-body coordination emerges from cellular excitability and excitatory chemical transmission alone. For larger animals we find that the physics of chemical transmission intrinsically limit whole-body coordination. Myoepithelia based on chemical transmission can function as modern ones but in contrast to the latter their coordinative properties remain limited to small-sized organisms. We speculate that while proto-neural myoepithelia could have provided a useful solution for basic forms of muscle-based movement, there would have been a strong evolutionary pressure to
improve on this design by (a) the development of non-chemical transmission mechanisms and (b) a
switch to nervous systems proper by including axodendritic processes.


P10. PHYLOGENETIC RELATIONSHIPS WITHIN CTENOPHORES
Paul Simion1, Nicolas Bekkouche1,2, Muriel Jager1, Eric Quéinnec1, Michaël Manuel1
1 Université Pierre et Marie Curie - Paris 6, UMR 7138 UPMC CNRS MNHN IRD, Case 05, 4ème étage, Bâtiment A, 7 quai St Bernard, 75005 Paris, France
2 University of Copenhagen, Department of Biology, Marine Biological Section, Strandpromenaden 5, DK-3000 Helsingør, Denmark

Ctenophores have recently been the focus of many phylogenomic studies, and their disputed
placement (either as sister-group of all other metazoans or as members of Eumetazoa together with
cnidarians and bilaterians) has a dramatic impact on our understanding of the origin of the nervous
system (independently evolved in ctenophores, or acquired once in an ancestor of eumetazoans).
Likewise, phylogenetic relationships within ctenophores remain largely unresolved. Here we present
reconstructions of ctenophore phylogenetic relationships using both traditional phylogenetic markers
(18S rRNA and Internal Transcribed Spacers, ITS1 and ITS2) and transcriptomic data. The use of
different datasets, outgroup choices, molecular evolution models and phylogenetic methods resulted in
a variety of discordant topologies contradicting historical evolutionary hypotheses and allowing an in-
depth discussion of ctenophore phylogenetic relationships. The position of the root within ctenophore
remains problematic. A clarification of internal relationships within the ctenophore phylum is highly
desirable, to allow inferences of their ancestral character states (which in turn should help determining
their phylogenetic position with respect to other non-bilaterian phyla), and to stimulate a diversification
of ctenophore model species notably for evo-devo studies.

P11. A GROSS OBSERVATION OF SCYPHOPOLYP NEUROMUSCULAR
STRUCTURES
Eason, J., Helm, R., Dunn, C.
Dept. of Ecology and Evolutionary Biology, Brown University, Mansfield, Massachusetts, USA

Aurelia aurita (Cnidaria, Scyphozoa) has a complex lifecycle that includes both polyps and
medusae. Many studies have investigated the musculature and neurobiology of the free swimming
medusa. Little is known about polyp morphology, anatomy and neurobiology. This omission leaves
many questions regarding the morphology and function of the polyp, as well as questions about which
structures arise de novo in the medusa or are remodeled from polyp structures. To help close this gap,
we imaged both the gross musculature and nerve net structures of A. aurita polyps using both
phalloidin and FMRFamide staining. The phalloidin-stained polyps show the location of three cord
muscles. The FMRFamide stained images show patterns of putative neurons around the polyp pedal
disk: longitudinal neurons and neurons that run perpendicular to the longitudinal neurons. Our results
suggest that FMRFamide neurons are located in a similar place as cord muscles. The furrows where
neurons are visible correspond to the placement of the cord muscles. These results suggest that there
are neuromuscular junctions that play an important role in polyp movement such as contraction and
potentially strobilation regulation.
P12. THE PEPTIDE GATED NA⁺ CHANNEL FROM THE FRESHWATER POLYP HYDRA - INSIGHTS INTO ANCIENT ION CHANNEL CHARACTERISTICS.

1 Assmann, M., 1Dürrnagel, S., 2Kuhn, A., 2Holstein, T.W. and 1Gründer, S.
1) Institute of Physiology, RWTH Aachen University, Aachen, Germany
2) Center for Organismal Studies, University of Heidelberg, Germany

So far, the HyNaC gene family from Hydra magnipapillata consists of four subunits, HyNaC2 - HyNaC5, which form the heterotrimeric sodium channel HyNaC2/3/5. HyNaCs belong to the DEG/ENaC ion channel family, a large family of sodium selective ion channels. They are exclusively found in metazoans. HyNaC is expressed at the basis of the tentacles of adult Hydra polyps, presumably in neuromuscular cells, where it is presumably involved in tentacle curling during feeding reaction. Besides FaNaC, HyNaC is the only known ion channel that directly is activated by peptides: HyNaC2/3/5 is activated by the endogenous neuropeptides Hydra-RFamide I and II. Moreover, HyNaC2/3/5 is highly permeable for calcium, a unique feature within the DEG/ENaC ion channel family.

Here we describe cloning of seven novel members of the HyNaC gene family, they were named HyNaC6 - HyNaC12. HyNaC2 - HyNaC11 showed a high degree of sequence homology and were closely related to the acid sensing ion channel (ASIC) and the bile acid sensitive ion channel (BASIC), whereas HyNaC12 showed a large phylogenetic distance to other HyNaCs. Like HyNaC2/3/5, the subunits HyNaC6 - 11 formed heterotrimeric ion channels, while HyNaC12 was inactive. Phylogenetically, HyNaC subunits can be divided into two subgroups: the first subgroup contains HyNaC3, HyNaC4 and HyNaC8 - HyNaC11, whereas the second subgroup contains HyNaC2 and HyNaC5 - HyNaC7. The logic of subunit assembly was always the same: In addition to HyNaC2, one representative of each of the two subgroups was necessary to form a functional heterotrimer. All HyNaCs were unselective cation channels with a high calcium permeability, and all HyNaCs were activated by Hydra RFamides I and II. Interestingly, the apparent ligand affinity of most of the new HyNaCs was up to 40 times higher than of HyNaC2/3/5. In contrast to HyNaC2/3/5, some HyNaC subunits showed expression in the peduncle region, suggesting further physiological functions of HyNaCs in vivo.

SESSION 5: CONTRIBUTED PAPERS I

MOLECULAR MAPPING OF AN ACOEL NERVOUS SYSTEM

1,2 Chiodin, Marta, 12 Telford, M.J. and 2Martinez, P.
1 Dept. of Genetics, Evolution and Environment, University College London, London, UK
2 Dept. of Genetics, University of Barcelona, Barcelona, Spain

The bilaterian central nervous system is patterned in a highly stereotyped manner by a conserved cassette of genes across distantly related taxa. Here we present the expression pattern of several bilaterian neural genes in a xenacoelomorph species: the acocel Symshagittifera roscoffensis.

Xenacoelomorph species present remarkable variations in the organization of their CNS, ranging from an ancestral intra-epidermal nervous system of Xenoturbella, to the condensed sub-epidermal brain and longitudinal nerve cords of the more derived acoels. Nevertheless, the higher complexity of the acoels’ CNS compared to their sister taxa is not reflected in greater complexity at the genome level.

While we were able to recover several of the bilaterian neural patterning genes in the genome of Xenoturbella and two nemertodermatid species, we could not find any clear ortholog of some of
them -e.g. Fez and Gbx- in the assembled genome of *S. roscoffensis*. Likewise, *S. roscoffensis* seems to have lost two key medio-lateral patterning genes, namely Mxr and Gsh orthologs.

The acoel brain consists of an anterior condensation of neurons as revealed by the expression of the pan neural genes *Synaptotagmin* and *Synapsin* and it lacks any morphological indication of regionalization.

When investigating the spatial expression of *S. roscoffensis* anterior neural genes, e.g. *Six3/6* and *Otx*, in juvenile and adult specimens, we found that their expression is compressed in overlapping anterior domains, whereas in most bilaterians their orthologs are expressed in staggered domains along the antero-posterior axis.

Different from most bilaterians, the medio-lateral gene *nk2.1* seems to be equally expressed along the dorso-ventral axis of *S. roscoffensis* juveniles and adults and this condition is apparently reflected in the lack of dorsal or ventral displacement of the acoel nerve cords; a unique condition in the bilaterian taxa investigated so far.

Despite the acoel brain often having been proposed as the bilaterian ‘proto-brain, it now seems likely that it has evolved from a ‘Xenoturbella-like’ epithelial nervous system through gradual condensation steps. Although preliminary, our data suggest that the lack of regionalisation of the acoel CNS may be due to the loss of key neural patterning genes and our results confirm previous observations that a highly stereotyped expression of neural patterning genes, is not a prerequisite for a centralized nervous system.

**THE SKIN BRAIN THESIS**

1*Keijzer, Fred A.*, 2De Wiljes, O.O. and 2,3*Van Elburg, R.A.J.

1Dept. of Theoretical Philosophy, University of Groningen, The Netherlands; 2Artificial Intelligence, University of Groningen, The Netherlands; 3Behavioural Ecology and Self-Organization, University of Groningen, The Netherlands

Our understanding of the origins of the molecular building blocks underlying nervous systems’ functioning rapidly increases, but progress at a systems level has been less strong. We sketch a conceptual approach that derives from embodied approaches to cognition and revitalizes a classic proposal from Chris Pantin: Early nervous systems evolved to coordinate motility derived from contractile tissue [1]. The resulting skin brain thesis proposes a two-step evolutionary sequence. This starts with primitive myoepithelia using chemical signaling to adjacent cells. In a second stage, some of these cells develop axodendritic processes that connect them to non-neighboring cells, providing a diffuse nerve net and more diverse and economic coordination. In both cases, connections are taken as generally diffuse, coordination being the self-organized result of biomolecular and tissue characteristics. While speculative, the proposal has attractive features: First, it links basic conductive cells to full neurons with processes in a gradual way. Second, it targets large-scale coordination of motility, which is a key precondition for more complex nervous systems according to embodied approaches to cognition. Third, rather than being strictly historical, the proposal specifies an empirically tractable organization that invites further specification and elaboration through computational modelling [2]. Fourth, the conceptual and computational elaboration of the basic proposal can be expected to provide new and specific empirical questions and directions for further research on this major evolutionary issue.


**EVOLUTION OF NEUROGENESIS – A DEVELOPMENTAL PERSPECTIVE.**

*Layden, M.J.*, Martindale, M.Q.

Whitney Laboratory for Marine Bioscience, University of Florida, St. Augustine, FL, USA

Understanding the evolution of animal nervous systems requires comparing neural physiology and investigating the relationship between mechanisms that generate and pattern nervous systems between species. We focused on investigating neurogenesis in the cnidarian sea anemone *N. vectensis*
for three main reasons. First, cnidarians are one of the two basally branching metazoan clades that possess a nervous system, and relatively little is known about neurogenesis in early branching taxa. Second, *N. vectensis* is one of the best genetically tractable model systems to investigate cnidarian nerve net development. Third, cnidarians are the sister taxa to the bilaterians, and our goal is to determine if cnidarian neurogenesis will be informative about the mechanisms that generate the “more complex” nervous systems present in bilaterian taxa. We find the molecular mechanisms governing cnidarian neurogenesis highly conserved with those observed in diverse bilaterian taxa. We have shown that a highly conserved bilaterian neurogenic transcription factor homolog (*NvashA*) is necessary and sufficient to promote expression of neural genes in the anemone. We find that *NvashA* functions within the context of regional patterning genes in *Nematostella* to generate domains expressing molecularly distinct neural cell types. Using transgenic labeling approaches, we further show that the molecularly distinct neural classes give rise to neurons possessing clearly distinct morphologies. These observations reveal an unappreciated morphological and molecular complexity of cnidarian nerve nets. Together our data support the commonly held view that cnidarian nerve nets and the seemingly more complex bilaterian nervous systems are derived from a common ancestor. Our work, and that of others is forming foundation to investigate the evolution of the central nervous systems in bilaterian animals.

**NEURONAL CONNECTIVITY AND THE CONTROL OF CONTRACTILE BEHAVIOR IN HYDRA**

*David, C.N., Heymach, B. and Thuemmler, J.*
Department of Biology, Ludwig-Maximilians-University, Munich, Germany

Hydra is a cnidarian polyp and hence a member of the first phylum to evolve a nervous system. Hydra polyps exhibit spontaneous contractile behavior and this behavior is controlled by a nerve net spread uniformly throughout the body column. Nerve free polyps lack contractile behavior (Campbell et al 1976). Our results show that there are two behavior control centers (BCC), one in the head and one in the foot. Each controls different behaviors and the foot BCC appears to be dominant, since isolated bottom halves of polyps contract similarly to whole polyps, while isolated top halves go “crazy” contracting more frequently and stretching 2-3X longer than normal. When top and bottom halves are grafted together, each half initially behaves independently. Only after 24 hours do grafted individuals recover normal behavior. This recovery parallels repair of the nerve net at the graft junction as demonstrated by immunostaining with an anti-cadherin antibody (Hobmayer and Holstein, unpublished). Results of Takaku et al (2014) have recently shown that nerve cells in the foot expressing the gap junction protein innexin-2 form a network connected by electric synapses and capable of synchronous firing. We suggest that they constitute the BCC in the foot, which controls spontaneous contractile behavior.

**SESSION 8: CONTRIBUTED PAPERS II**

**DEDICATED NEURAL PROGENITOR CELLS HAVE A PRE-BILATERIAN ORIGIN: EVIDENCE FROM TRANSGENIC ANALYSES OF A SOXB GENE IN NEMATOSTELLA VECTENSIS.**

Richards, G.S. and Rentzsch, Fabian
Sars Centre for Marine Molecular Biology, University of Bergen, Bergen, Norway

As an outgroup to bilaterians, and one of the earliest diverging animal clades to possess a nervous system, cnidarians occupy a key phylogenetic position for understanding the evolution of neurogenesis. Our current understanding of the development of cnidarian neurons is mainly restricted to hydrozoans, in which neural cells derive from interstitial stem cells that also possess broader, non-neural, developmental potential. In contrast, bilaterian neurogenesis is characterized by the generation of diverse neural cell types from dedicated neural progenitor cells (NPCs). The evolutionary origin of NPCs is thus unclear, and we address this question by analyzing neurogenesis in an anthozoan cnidarian, *Nematostella vectensis*. Using a transgenic reporter line, we show that *NvSoxB(2)*, an ortholog of bilaterian *SoxB* genes, is expressed in a cell population that gives rise exclusively to the
three primary neural cell types of cnidarians, i.e. sensory and ganglion neurons, and nematocytes. EdU labelling demonstrates that cells express NvSoxB(2) before mitosis, and identifies asynchronous behaviors within NvSoxB(2)+ lineages. Such behavior may reflect an asymmetric acquisition of neural cell fates and/or differing potential for self-renewal in the daughter lineages of NvSoxB(2)+ cells. Morpholino-mediated gene knockdown shows that NvSoxB(2) is essential for the development of the nervous system. Moreover, knockdown of NvSoxB(2) blocks the neurogenic phenotype induced by pharmacological inhibition of Notch signaling. Our results indicate that NvSoxB(2) and Notch signaling control the generation of diverse neural cell types from a population of mitotic cells in Nematostella. We thus propose that neurogenesis via dedicated, SoxB-expressing NPCs predates the split between cnidarians and bilaterians.

**USING NATURE’S MUTATIONS TO EXPLORE A LIPID BINDING SITE ON INWARDLY RECTIFYING POTASSIUM CHANNELS**

Boland, Linda, M., Tang, Q-Y., Larry, T., Hendra, K., Bell, J., Cui, M., Yamamoto, E., Logothetis, D. University of Richmond and Virginia Commonwealth Univ., Richmond, VA.

Ion channel biophysicists often introduce mutations into ion channel genes to study the function of native residues in the protein. We are taking a different approach by using the consequences of nature’s mutations – the source of evolutionary change – as a way to understand ion channel structure and function. Among the various ion channels in the nervous system, inwardly rectifying potassium (Kir) channels are critically important in regulating cellular resting membrane potential and excitability. All vertebrate Kir channels are regulated by the membrane lipid, phosphatidylinositol 4,5-bisphosphate (PIP$_2$). We used Kir channels cloned from the sponge, Amphimedon queenslandica, a valuable model organism, as a way to understand PIP$_2$ regulation of Kir channels by comparative analysis with vertebrate channels. Using patch clamp experiments, sponge Kir currents decreased over time following patch excision. In two electrode voltage clamp, co-expression and activation of a voltage-sensitive phosphatase led to rapid decreases in sponge Kir current and wortmannin pre-treatment to lower endogenous PIP$_2$ levels significantly reduced sponge Kir current amplitudes. While these are characteristics of the PIP$_2$-dependence of all previously studied Kir channels, direct application of the lipid to the cytosolic side of membrane patches could not reactivate the sponge Kir currents. However, mutagenic substitution of two residues in the sponge Kir channel restored high affinity PIP$_2$ reactivation of Kir currents in excised patches. The functional impact of these experimental manipulations which recapitulate nature’s changes in the channel’s structure can be explained using a homology model of the sponge Kir channel. This research will help us resolve the protein/phospholipid interactions required for Kir channel activation and how a phospholipid binding pocket evolved its specificity for PIP$_2$ as observed in vertebrate Kir channels.

**ACTIVITY OF THE NERVOUS SYSTEM OF HYDRA**

Christophe Dupre and Rafael Yuste
Dept. of Biological Sciences, Columbia University

Even though much research is being done on complex nervous systems, our understanding of simple nervous systems is still very poor. Studying the earliest nervous systems could unveil elementary neural network principles that have been conserved throughout the evolution to form more complex systems.

In this regard, it would be very informative to measure the activity of the neurons in an animal that appeared early in evolution. The fresh water invertebrate hydra could be an ideal candidate since its small and transparent body makes it suitable for imaging. Moreover, methods for creating transgenic Hydra have recently become available.

Here we report the generation of a line that expresses GCaMP6s in interstitial cells, a stem cell lineage that includes neurons. This line will help measuring the activity of the neural network of Hydra and thereby study the roles and principles that emerged during the evolution of the first nervous systems.