Student Bio’s

2014 MBL Embryology Course

Woods Hole, MA
Forward genetic study of neural tube development

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My dissertation work has centered on a mutant with a neural tube defect in the Ascidian, *Ciona savignyi*. The first half of my dissertation work has focused on creating a new computational mutation-mapping program for *Ciona* and identifying the genetic mutation causing the neural tube defect in the *Ciona savignyi bugeye* mutant. I have used whole genome sequencing and computational analysis to compare gross genetic variation and linkage between mutant and wild type genomes. This new method proved to be effective at pinpointing linked regions unique to the mutant genomes and my analysis has explored how the extraordinary polymorphism in *Ciona* actually makes it particularly ideal for mutation mapping and forward genetics. The mutated *bugeye* gene identified through this mapping method is a T-type calcium channel gene.

The second part of my research has involved characterizing the T-type calcium channel gene in the context of normal development and the specific process of neurulation. I have been using a variety of molecular techniques in *Ciona* to pinpoint the gene’s expression domain, necessity for neural tube closure and downstream effects on cell junction proteins. Furthermore, I have been working on addressing whether the role of this T-type calcium channel in regulating neural tube closure is conserved in vertebrates. Using *Xenopus*, I have knockdowned the orthologous T-type calcium channel gene and begun to characterize a neural tube defect phenotype.
Dynein arm assembly and transport in mammals

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Cilia are hair-like organelles that are present in the tracheal, kidney epithelial and other cells.

There are two types of mammalian cilia: motile and immotile cilia. Motile cilia are involved in moving extracellular fluids, like in the respiratory tract, such a mechanism contributes to mucociliary defense. Defects in the structure and function of cilia result in several disorders, known as ciliopathies. Among these congenital diseases, Primary ciliary dyskinesia (PCD) was previously known as immotile cilia syndrome, defects result in neonatal respiratory distress, male infertility, and situs inversus.

To better understand the cilia functions as well as cause of cilia related diseases, we deleted the PCDRP (Primary Ciliary Dyskinesia Related Protein) gene in the mouse. This gene was identified as causing cystic kidney disease in a mutagenesis screen in zebrafish.

Pcdrp null mice displayed aberrant Left-Right (LR) axis and exhibited immotile cilia in the node, trachea, and brain. Pcdrp null cilia morphology was not so different from WT ones, but inner structure of the cilia showed defects, such as cilia in the node and trachea lacked some outer and inner dynein arms. Dynein arms are the molecular motors essential for the ciliary beating. They are assembled in the cytoplasm and carried into the cilia by intraflagellar transport (IFT). Lacking dynein arms in Pcdrp null cilia suggested that Pcdrp has important roles for this dynein arm assembly procedure in the cilium.

Further analysis of Pcdrp functions will reveal more detailed mechanisms of dynein arm assembly.
The role of *Phox2* in neural circuit development and evolution

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I grew up in Buenos Aires, Argentine, where I studied Biology at Buenos Aires University.

I did my Licenciatura thesis (Master’s equivalent) in Molecular Physiology, studying the crosstalk between the Glucocorticoid Receptor (GR) and the two main signaling pathways controlling the mice mammary epithelium cyclical periods of cellular proliferation/differentiation and involution: the signal transducer and activator of transcription factors STAT5A and STAT3.

Afterwards I did my PhD in Molecular biology, in which I focused on understanding the role of intragenic Progesterone Receptor (PR) binding sites on gene transcription. For that, I applied a variety of molecular techniques such as ChIP, co-immunoprecipitations, Pol II elongation measurements, protein knock-downs by siRNAs, enhancer activity measurements, quantitative and radioactive PCRs and Western blots. This work showed a novel mechanism by which intragenic bound PR regulates gene expression of target genes by facilitating RNA PolII elongation.

Even though my background is in Molecular biology, my dream was always to study development with an evolutionary point of view. That is why two years ago I contacted Dr. Arendt to become a member of his lab (at the European Molecular Biology Laboratory in Heidelberg, Germany) where I am currently doing my first postdoctoral training and taking my first steps in the evo-devo field.

In Arendt lab I am studying the role of the paired-like homeodomain transcription factor Phox2 in neural circuit development using the annelid Platynereis dumerillii (Pdu) as animal model. I am interested in characterizing the group of neurons that express Phox2 in Pdu, for which the orthologous genes in vertebrates (phox2a and phox2b) represent the most striking example of circuitmatching transcription factors. In vertebrates the phox2 paralogs are expressed during development in almost all the visceral-reflex circuits partaking neurons (sensory, inter and motor neurons). Even though the visceral neurons differ dramatically in function, neurotransmitter identity, position and embryological origin (neural tube, epibranchial placodes and neural crest), almost all of them express the phox2 genes.

Are the Pdu_phox2+ cells homologous to the vertebrate visceral neurons? Are they interconnected? If yes, are these circuits homologous to the vertebrate visceral-reflex ones? For answering these questions I started characterizing Pdu_phox2+ at molecular level mainly by double whole mount in situ hybridization. For further characterization of these cells I am working on getting a transgenic line expressing EGFP under Pdu_phox2 promoter which I plan to use for (i) Analyse their axonal projections and target cells; (ii) Find out the phox2+ cell-linage; (iii) Perform specific cell ablations and behavioural studies in response to chemosensory stimuli and (iv) Perform single cell RNA-Seq. I have also been setting up the CRISPR technique for analyzing phox2-/- phenotype in this organism and I am generating an anti-Pdu_phox2 antibody for carrying out ChIP-Seq, which will allow us to go further in characterizing the phox2 gene regulatory networks.
Caves harbor a rich diversity of animals including sponges, flatworms, mollusks, annelids, arthropods and vertebrates. This diversity is surprising in face of the constant darkness and severe nutrient depletion of cave habitats. Ancestors of all these animals were originally surface dwellers and during their colonization of subterranean environments evolved a series of adaptations that enabled them to survive in harsh underground conditions. Some of these adaptations, such as the loss of body pigmentation and visual senses, the overdevelopment of non-visual sensory systems, and the increased resistance to starvation, are present in all groups of animals that have successfully adapted to cave life. Cave animals therefore provide a unique opportunity for studying the mechanisms driving convergent evolution of novel phenotypes.

I am working on the loss of pigmentation. Questions I am trying to answer are: i) what molecular mechanisms underlie de-pigmentation of cave dwellers from distant phyla and ii) is de-pigmentation in cave animals a result of neutral evolutionary effects due to absence of purifying selection, which eliminates albinos in surface habitats or alternatively, is it adaptive and driven by selection.
Role of JmjD2B histone demethylase in otic placode development

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I am an MSc in Biotechnology and Molecular Biology, graduated from the University of La Plata, Argentina. As an undergraduate student, I have worked with Dr. Alan Talevi focusing on Molecular Topology, using bioinformatics tools to find novel anticonvulsants and anticancer drugs. These studies were also part of my graduate thesis.

Currently, I am at the third year of my PhD in Molecular Biology and Biotechnology (University of San Martin, Argentina), working in the Developmental Biology Laboratory at the IIB-INTECH under Dr. Pablo Strobl-Mazzulla supervision. The focus of my research is the study of the epigenetic influence on inner ear development using chick embryos as a vertebrate model. Particularly, we are studying the role of JmjD2B histone demethylase.

The inner ear is one of the most sophisticated sensory organs in vertebrates, relaying both acoustic and motion/balance information to the brain. The inner early formation involves an intricately regulated series of events from otic placode induction, to invagination and over differentiation.

In our lab, we have found that an epigenetic modifier JmjD2B, a histone demethylase, plays an important role in chick inner ear development. In situ hybridization (ISH) analysis reveals expression of JmjD2B in the otic placode during its induction and later at the rim of the invaginating otic vesicle. Consistent with this, immunostaining reveals clear variations in the spatiotemporal expression of JmjD2B substrates, the epigenetic marks H3K9me3 and H3K36me3, in ectodermal, invaginating and post-invaginating otic cells.

By unilateral electroporation of JmjD2B translation-blocking morpholino into st8 chick embryos we were able to detect a great reduction of the expression of several inner ear markers, including Pax2, Dlx3, Soho1 and Sox10 by stage 13.

Moreover, JmjD2B knock-down caused a clear defect on the placodal invagination, possibly as a consequence of the observed lack of polarity in the expression of adhesion molecules such as E-cad. Furthermore, by in vivo chromatin immunoprecipitation (ChIP) on otic placode and otocyst we have found that JmjD2B is able to interact with regulatory regions of Dlx3 locus, but not with Pax2 and Soho1. Taking together, we described for first time the epigenetic contribution of histone demethylation to control key genes implicated in inner ear invagination in vertebrate embryos.
Cdk12 and Cdk13 kinases signaling functions in neuron development and axon growth

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At present, my research primarily focuses on (1) investigating the cyclin dependent kinases (Cdk) Cdk12 and Cdk13 functions in mouse neuronal development, and (2) dissecting the mechanisms how Cdk12 or Cdk13 regulating axonal elongation processes. Cdk12 and Cdk13 are Cdc2-related proteins that share 92% identity in their kinase domains. Cdk12 and Cdk13 mRNAs and their proteins are present in developing mouse embryos, especially in the developing nervous system. I explored the roles of Cdk12 and Cdk13 in neuronal differentiation in the P19 neuronal differentiation model and cultured cortical neurons. Upon using these two models, I observed that Cdk12 and Cdk13 regulate axonal elongation through a common signaling pathway. In order to decipher the role of Cdk12 during development of the nervous system in vivo, I investigated Cdk12 by conditional knockout mice (cKO) generated by a Cre-loxP recombinant system to produce CNS-specific Cdk12 cKO mice. Histological and immunohistochemistry analysis of the Cdk12 cKO mice in E14.5 revealed impaired development of the brain. Taken together, how deleting Cdk12/Cdk13 affects embryonic brain development is under investigation.
Development and evolution of the gnathostome axial column

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I am a third year doctoral student in the department of Organismal Biology and Anatomy at the University of Chicago. My research combines developmental biology, paleontology, and comparative anatomy to explore the evolution of vertebrate anatomical systems. For my dissertation project I am studying the primitive conditions and convergent evolution in the vertebral column of jawed vertebrates. The vertebral column is a defining feature of vertebrates, but its evolutionary history and the mechanisms governing its development are greatly understudied. Fishes, in particular, display an impressive array of morphology, composition, and developmental diversity in this system, but no modern synthesis of the evolution of these structures exists. I am interested in determining how variation in vertebral development across fishes has resulted in the diversity of vertebral morphology seen today. To do this I use microCT scans of iodine-treated fish embryos to document the timing and study the embryonic morphology of the axial column in three dimensions. I am also using the little skate, *Leucoraja erinacea*, as a chondrichthyan model system to identify the embryological origin of neural arches and vertebral centra through fate mapping and cell ablation experiments. By comparing vertebral development in cartilaginous fishes with studies from several different bony fishes, like zebrafish, salmon, and medaka, I can reconstruct ancestral conditions for vertebrates and examine patterns of convergent or parallel evolution. I am excited to spend my time in the Embryology course gaining experience working with a range of different organisms and exploring new developmental techniques.
The embryonic diapause of African killifish
Nothobranchius furzeri

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My current project focuses on embryonic diapause in vertebrates, using the African killifish Nothobranchius furzeri (N. furzeri) as my model organism. Diapause is a unique embryonic state where all developmental processes are suspended. The suspended embryonic development will resume after the ‘right cues’ are received. This allows organisms to perfectly time their offspring births with right seasons or right environmental conditions. Diapause is a very common strategy and well known in plants and insects. However, it is also practiced in vertebrates even mammals (for example, panda), in a less understood mechanism. This is possibly due to the difficulty to observe, follow, and cue embryonic diapause inside mothers. As a vertebrate, fish, which has embryo developed in eggs, would be a good model system to study embryonic diapause.

N. furzeri could enter diapause right after the completion of somitogenesis. I am fascinated by the fact that the robust embryo development can be stopped precisely at one specific point and suspended for months or even years. With an average lifespan of 6 months, N. furzeri is also the shortest lived known vertebrates that can be maintained in labs. Some N. furzeri embryos directly develop through without diapause; but the rest would enter diapause. Compared to the former, the latter spend a few additional months in diapause. This raises another interesting question – whether embryos still age in diapause? My preliminary data suggest that the period of time N. furzeri staying in diapause does not affect the post-diapause adult lifespans. Thus the embryos in diapause might not experience aging at all. Or alternatively, rejuvenation is coupled with diapause exit to have the ‘clock’ reset. For my projects, I would need to know better about the embryology part of my project, such as the main signaling pathways or key regulators at specific developmental stages which might cross-talk with diapause and aging pathways.
The establishment of left-right asymmetry in zebrafish

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I’ve just recently finished my PhD at the University of Cambridge, in the lab of Fiona Wardle, where I’ve been looking at the establishment of left-right asymmetry in zebrafish. I’m currently getting all my work ready for publication, and I’m hoping to move on and start a postdoc somewhere new, sometime soon.

My undergrad degree was in genetics, and I’ve really enjoyed working on zebrafish development during my PhD and gradually becoming a developmental biologist. I’d like to move into evo-devo next, and this summer I’m looking forward to learning how to work with lots of different animals! I’m especially interested in the evolution of gene regulation during development, particularly in early patterning.

Outside the lab, my interests include science communication (I spend my weekends working in a museum), taking photos, doing yoga, etc.
Organ shape and size control in the developing zebrafish posterior lateral line

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The zebrafish (*Danio rerio*) lateral line system consists of mechanoreceptive organs - neuromasts, closely related to the auditory system of higher vertebrates. Lateral line is used to sense water motion, thereby enables fish to detect pray, avoid predators and swim in the same direction in the coordinated manner with other fish. The posterior lateral line (pLL) is established when a cluster of cells, known as the primordium detaches from the lateral line placode just behind the developing ear and migrates caudally to the tip of the tail by periodically depositing neuromasts. The lateral line while being a relatively simple system found in aquatic vertebrates can serve as an excellent in vivo model to study fundamental developmental mechanisms like cell migration, cell proliferation, cell fate determination and organogenesis. The mechanisms governing the primordium migration, segmentation and neuromast deposition are relatively well investigated. Yet, much less is known on how neuromasts arrange their cells into polarized rosettes and develop into functional organs of particular shape and size. I want to understand the mechanism of neuromast formation, specifically, to figure out if there is a cell or a group of cells in the middle of the neuromast, which organizes its construction, or whether the neuromast formation is regulated by the “rosette gene”, which is expressed in all cells of the future neuromast. Most importantly, results from my studies will help to better understand tissue polarization and organ size control mechanisms, crucial for the developmental biology and cancer fields.
Scaling chromosome compaction to cell size

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During early development in many organisms, cells reduce in size due to consecutive cell divisions without increase in embryo volume and must adapt organelle sizes accordingly. Despite dramatic changes in cell size, the genome size is constant in all diploid cells of an organism. Thus, it is expected that during anaphase, condensed mitotic chromosomes must be half the length of the mitotic spindle to be properly segregated to each daughter cells, a length that varies according to cell size. Using high-resolution time-lapse microscopy of C. elegans embryos expressing H2B-GFP and γ tubulin-GFP, we have measured chromosome length in 3D in living, developing embryos of different developmental stages, ranging from the 1 cell to the 16 cells stage embryos. We have derived a rule, expressed in the form of a linear regression, for chromosome length regulation over cell size and confirmed that prometaphase condensed chromosomes are smaller in length as cell reduces in size. We have found that artificially changing cell size reduced chromosome length compared to control of corresponding developmental stage although maintaining the same constant of cell size regulation of chromosome length. Inversely, using a worm strain with double the amount of DNA resulted in smaller chromosomes, and an overall lower constant of chromosome length regulation compared to Control embryos. Those results suggest the ratio of DNA over cytoplasm is a determining factor regulating chromosome length. We hypothesized this ratio could also be affected by nuclear size. It was shown previously that nuclear size is regulated through nuclear import. Depleting the nuclear import factor RCC1 dramatically reduced chromosome length with a fixed amount compared to control embryos although maintaining the same constant of chromosome length regulation. This result suggests RCC1 prevents the incorporation of a specific state of chromatin compaction. Therefore, deriving a linear regression of chromosome length over cell size or nuclear size allowed us to determine that chromosome size was regulated in 2 different ways: one following proportional reduction in length according to cell size and the second with a fixed magnitude. We suggest a model where the first mechanism is controlled through adaptive regulation and the second through inheritance of an epigenetic mark.
The genome of the inarticulate brachiopod *Lingula anatina*

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I am studying an inarticulate brachiopod called *Lingula anatina*, which is often referred as “living fossil”, since their shell morphology changes very little during evolution. There are many unique features that make *Lingula* a good model to study evolutionary developmental biology. First, unlike the bivalve molluscs which have left-right shells made by calcium carbonate, *Lingula* has dorsal-ventral shells composed by calcium phosphate, suggesting that it has different axial patterning mechanism. More intriguingly, among all animals, only some of brachiopods such as *Lingula* and vertebrates use organic phosphate to make their hard tissues. It will be interesting to see whether they share ancestral biomineralization machinery or they are evolved independently. Second, although it belongs to protostome, its early embryonic development exhibits deuterostomic features such like radial cleavage and enterocoely coelom formation. Third, the evolutionary origin of brachiopods is unknown and their phylogenetic position within Lophotrochozoa is highly debating.

To resolve these questions, I sequenced the genome as well as embryonic and tissue transcriptomes of *Lingula* collected in Amami, Japan. To answer whether the evolutionary rate of *Lingula* is slow, I compared one-to-one orthologs among metazoans and estimated its phylogenetic relationship compared to other lophotrochozoans. I also applied comparative genomic analyses to study micro-synteny and intron evolution to give evidence of its evolutionary origin. By comparing the mantle specific and early developmental genes, I would like to reveal some interesting stories about its deuterostomic development and shell formation mechanisms. In addition, I performed artificial spawning during last summer and manipulated BMP signaling during early embryonic stage to study its dorsal-ventral patterning. By combining genomic, transcriptomic, and embryological studies, I hope that I can provide new insights about the evolution origin and unique body plan of *Lingula*. 
The spatial-temporal dynamics of WNT signaling in mammalian gastrulation

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Classical embryology and recent advances in molecular biology and stem cell biology have gone a long way to uncovering the key molecular elements for cell signalling during mammalian gastrulation. However, little is known quantitatively about exactly when, where, and to what degree and effect these signals interact with each other and determine cell fate and patterning. I am specifically interested in WNT signaling and its role in determining the anterior–posterior axis. Using mouse and human stem cells, I make use of microfluidics, micropatterns, and fluorescent reporters to take quantitative measurements of the time course of fate commitment and patterning upon exposure to various signaling ligands. It is my hope to then fit this data to a predictive mathematical model.
Modeling tissue polarization and break of symmetry in development and disease

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I started my academic education as a pure physicist, but very early on I became interested on how patterns emerge in nature and on how I could explain/simulate such processes using theoretical and computational models.

As a physics undergrad I joined a multidisciplinary lab at Universidade Federal do Rio Grande do Sul, Brazil to study foam and cellular structures and worked on 3D model of cell movement and shape driven by cell-matrix adhesion. During my master degree I developed a 2D model to describe collective motion of cells in culture that predicted (later confirmed experimentally) that the presence of even small amounts of coordinated motion between cells greatly enhance basic morphogenetic processes such as cell sorting.

During my PhD at Indiana University, under the direction of Dr. James Glazier, I built new computational models of basic developmental events - such as PCP, cell-intercalation and polarization - and used them to create cell-based, multi-scale models of diseases and developmental processes. Under close collaboration with developmental biologists and clinicians, we studied and proposed new mathematical/theoretical models for how chicken limb buds grow, how somites arise from the interaction of a clock with a wavefront, and why human renal tubules develop cysts when injected with cadherin-8.

Currently I am working on a new mechanical model for vertebrate somitogenesis, where segmentation and somite formation is a self-organized process regulated by a mesenchymal-to-epithelial transition without the need of an oscillatory gene network.
Skeletal patterning in the sea urchin embryo

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In the Bradham lab at Boston University, I study the sea urchin larval skeleton as a model for three-dimensional tissue patterning during embryogenesis. During gastrulation, the skeletogenic primary mesenchyme cells (PMCs) use thin filopodia to receive patterning information from the overlying ectoderm, and this conversation directs PMC migration and positioning. Shortly thereafter, the PMCs begin to secrete the larval endoskeleton, a bilaterally symmetric pair of calcium carbonate crystals, which elongate and branch to give structure to the sea urchin larva. The PMC positioning during gastrulation ultimately dictates the pattern of this skeleton. Until recently, the molecular mechanisms by which the PMCs and the ectoderm communicate have remained largely unknown.

To identify skeletal patterning genes, we have performed a three-way comparative RNA-seq-based screen. Nickel chloride treatment and p38 MAPK inhibition with SB203580 (SB) elicit reciprocal effects on ectodermal specification and development; however, both treatments result in similar skeletal patterning perturbations. We therefore hypothesize that skeletal patterning genes are mutually downregulated by both treatments. By comparing control, nickel-, and SB-treated transcriptomes, we have identified a set of 57 candidate skeletal patterning genes. Of these candidate genes, we have tested 10 by performing loss-of-function (LOF) analysis by morpholino microinjection. Each of these 10 genes tested are specifically required for normal PMC positioning and normal skeletal patterning, which demonstrates the success of our screen. Several projects have arisen from this data set, including investigations into the roles of ion channels and sulfated proteoglycans, BMP signaling, lipoxygenase activity, and adhesion molecule expression on skeletal patterning during development.

Additionally, we have developed a project in which we demonstrate the role of late TGF-β-like signaling through the type I receptor Alk4/5/7 in skeletal patterning. Pharmacological inhibition of Alk4/5/7 with the specific inhibitor SB431542 (SB43) during gastrulation demonstrates that TGF-β-like signaling is specifically required for PMC migration and patterning of the animal skeleton. Gene expression and LOF analysis suggest that the ligand, Univin, is the relevant Alk4/5/7 activating signal during patterning the anterior skeletal elements.
Functional analysis of the Krüppel-like factor gene family during embryogenesis in the early branching metazoan Mnemiopsis leidy

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The Krüppel-like factor (Klf) gene family is a well characterized group of transcription factors that is defined by a highly conserved triple-C2H2 DNA binding domain composed of three tandem zinc fingers that have approximately the same length. KLF transcription factors function in a wide variety of biological processes including embryonic development, cell differentiation, cell proliferation, human diseases and cancer. Most functional studies have been limited to traditional bilaterian model organisms, and no studies have extensively identified or functionally characterized Klf genes in non-bilaterian organisms. Recently, I identified two Klf genes in the ctenophore Mnemiopsis leidy, MleKlf1/2/4a and MleKlf1/2/4b, in a broad-scale study that identified members of this gene family in a range of organisms spanning the Unikonta. The Klf gene complement found in Mnemiopsis is more similar to that found in unicellular eukaryotes than to other non-bilaterian organisms such as sponges and cnidarians. This pattern of gene under-representation is seen in other gene families and provides support for inferring Ctenophores as extant members of the earliest diverged lineage of animals.

Surprisingly little is known about the Klf gene family outside of a few well characterized model organisms despite a clear role for these transcription factors in the regulated development of a range of cell types during vertebrate embryogenesis. In an effort to address this gap in our understanding of how this important transcription factor gene family evolved and identify its functions in an early diverged animal lineage, I will investigate Klf activity during embryonic development in Mnemiopsis via gene perturbation techniques (morpholino knockdown and full length mRNA overexpression). Ctenophores, Mnemiopsis specifically, are an emerging model system not only for examining aspects of development and the genetic interactions that control development, but importantly as members of one of the earliest diverged lineages of animals they potentially provide an evolutionary context for improving our understanding of the molecular genetic origins of animal multicellularity. Not only will this work garner new insights into what roles this important gene family has in Mnemiopsis, but it will also provide both a developmental and evolutionary framework for future studies on gene regulatory networks controlling development and how they have evolved during the course of metazoan evolution.
Development of the telencephalon in sharks

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During the last five years, I explored the embryonic development of the anterior-most part of the brain (the telencephalon) in a small shark, the lesser-spotted dogfish, Scyliorhinus canicula. My PhD project was focused on the study of different developmental processes that take place in the telencephalon and peripheral systems associated to it (olfactory and terminal nerve systems). These processes included neuronal differentiation, neuronal migration and axon guidance, morphogenesis or genoarchitecture.

Our work is mainly carried out under an evo-devo approach. S. canicula is emerging as an excellent animal model for developmental and evolutionary studies for several reasons. It belongs to the class of cartilaginous fishes, animals occupying a key phylogenetic position to assess the ancestral condition of the vertebrate brain. Moreover, the dogfish is a good model to perform detailed analysis of developmental processes due to: its protracted embryonic period (from 6 to 8 months) which allows a thorough analysis of the development, evidencing details that can be overlooked in other species of rapid development; its external egg gestation and transparent eggs (easy accessibility to the embryo for the implementation of different experimental approaches); its large size of the embryonic brains and to the availability of embryos at any time of the year. Furthermore, in cartilaginous fishes the telencephalon develops by a morphogenetic process known as evagination (as in all tetrapods) instead of eversion (as happens in teleost fishes like zebrafish), which allows more reliable comparisons with other vertebrates.

Very briefly, I include some results from my thesis work: (1) we characterized a novel cell population of Pax6-positive migrating neurons along the developing olfactory nerve (never described in any other vertebrate) and proposed its implication in axon guidance; (2) we identified the main embryonic telencephalic territories in sharks, by comparative analysis of key developmental transcriptional factors expression; (3) we identified several neuronal tangential migratory routes in the shark telencephalon homologous to those described in tetrapods and (4) we performed the first description of basal ganglia circuitry in cartilaginous fishes. The analysis of shared and derived features in our model is providing us with essential information to fully understand of the evolution of the nervous system.

I have been recently awarded with a Marie Curie postdoctoral fellowship from the European Commission, which will give me the opportunity to work as a postdoc during the next two years in the University of Edinburgh (Scotland). This will represent my first postdoctoral experience and also a significant change in the direction of my research. I will be integrated in the Genes and Developmental Group (directed by Prof David Price) to study the molecular pathways under the control of Pax6 in the developing diencephalon of mammals, paying special attention to its interaction with Shh signaling pathways.
The role of Wnt signaling in olfactory ensheathing cell development

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The neurons of the olfactory system constantly regenerate throughout life. This lends to our interest in this system as a model for axon regeneration and reconnection as well as potential therapy for CNS disease and injury. The glia of the olfactory system, olfactory ensheathing cells (OECs), promote axon-sprouting from olfactory receptor neurons in the olfactory epithelium and guide them to their targets in the olfactory bulb. OECs can also promote spinal cord repair when grafted into rat central nervous system lesions. Our lab has shown that OECs are derived from the neural crest and that their normal differentiation is required for olfactory axon targeting and gonadotropin hormone-releasing (GnRH) neuron migration to the forebrain. Investigating the mechanisms of this process should give insight into the potential mechanisms by which OECs promote spinal cord repair.

I have investigated the expression of both canonical and non-canonical Wnt pathway members during the development of the olfactory system in chick. One intriguing finding is that OECs initially express Wnt5a, encoding a non-canonical Wnt ligand that promotes cell migration and axonal growth. However this expression is down-regulated when most GnRH neurons enter the forebrain (between embryonic day [E]5.5 and 6.5), suggesting a potential role for Wnt5a-secreting OECs in GnRH neuron migration to the forebrain. Down-regulation of Wnt5a coincides with up-regulation of Frzb1, encoding a secreted antagonist of canonical and non-canonical Wnts. Thus, Wnt signalling pathway genes are dynamically expressed during OEC development and OECs may produce and modify Wnt signalling. I am investigating this further in both chick and mouse in order to unravel the roles of Wnt signalling in OEC development and function.
The evolution and development of gastropod shells

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Gastropods (snails, slugs) are a speciose clade characterized by many radiations, morphological novelties, body plan reorganizations, and invasions of land. The gastropod shell is well preserved in the fossil record and has been a classic subject in the study of morphology. Because of the shell’s general adherence to logarithmic coiling, the use of just a few parameters can effectively characterize its morphology, such as the orientation of the aperture (shell opening) and plane of shell coiling. New model systems allow exploration of the underlying processes generating shell diversity at the molecular level.

One specific hypothesis I will investigate is that asymmetric dpp expression causes shell coiling by inducing more cell proliferation on one side of the shell-secreting mantle edge (see figure, Shimizu et al. 2013). A trend in gastropod evolution is limpet-like (non-coiled) shell morphologies being derived from coiled ancestors – Shimizu et al. proposed that repeated evolution of dpp symmetry could be the mechanism for many convergently limpet-like forms. This hypothesis of shell coiling has not been evaluated in caenogastropods, so I will examine two caenogastropod model organisms: Crepidula fornicata (a “limpet-like” shell according to Shimizu and colleagues) and Ilyanassa obsoleta (a coiled shell). In developing veliger larvae, I will assay dpp expression and protein localization using in situ hybridizations and antibody staining. Activity of the dpp pathway can be visualized by using antibodies for phosphorylated SMAD protein. These experiments will answer the question of whether dpp expression is where we would expect based on the Shimizu hypothesis. Additionally, these model systems also allow for functional testing of different dpp configurations. I will manipulate elements of the dpp pathway using mRNA injections, morpholinos, and chemical inhibition. As these elements are perturbed, I will determine whether the resulting shell morphology is consistent with the dpp model of shell coiling proposed by Shimizu et al.

Mechanistically, I can also assay whether dpp is inducing cell proliferation in unperturbed and experimental larvae by staining with BrdU, which marks actively proliferating cells.

To determine whether dpp localization is a common mechanism used to switch between coiled and limpet-like shell morphologies, I will sample limpet-like and coiled species from other lineages of Gastropoda. I will assess whether there is a differential DPP concentration on opposite sites of the mantle in still-growing adult gastropods, as was performed in Shimizu et al. 2013. Because I can collect adults and don’t need to raise animals in the lab, this approach will allow me to take advantage of non-model gastropod diversity in my sampling.
Using sponges as a model to examine the evolution of the Wnt/β-catenin signaling pathway

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The focus of my research is on the evolution of the Wnt/β-catenin developmental signaling pathway and its role in modern sponges, one of the earliest branching metazoan lineages. In bilaterians, the Wnt/β-catenin pathway plays a critical role during animal development, as it controls cell differentiation, proliferation and apoptosis by regulating the expression of a high number of target genes. In addition to its normal functions, misregulation of this pathway can lead to serious diseases in humans, such as colon cancer. The discovery of conserved homologs of the Wnt/β-catenin pathway in sponges raises questions about whether a functional Wnt/β-catenin pathway is present in sponges and what its role may be in organisms of such simplicity – they lack many features of bilaterians, such as muscles, nerves and a gut.

The specific aims of my study are to:

1. test whether the sponge homologs of Wnt/β-catenin components interact. I will use Co-IP to identify the proteins that complex with b-catenin and TCF (these proteins form the transcriptional complex downstream of the Wnt/β-catenin pathway) in vivo. To facilitate the Co-IP experiments, I am working on raising antibodies against b-catenin and TCF.

2. identify the tissue-specific and subcellular localization patterns of β-catenin and TCF by performing immunostaining.

3. identify which target genes are regulated by the β-catenin/TCF transcriptional complex by performing ChIP-sequencing.

4. develop techniques for studying gene function in vivo in sponges. I am working on delivering dsRNA and DNA expression plasmids (with candidate sponge promoters) via lipofection and cell penetrating peptides. An additional strategy I am working on is to use morpholinos to achieve knockdowns.

The overall goal of the proposed research is to learn more about how metazoan morphology evolves, and about how signaling pathways themselves evolve.
Follicular cells and oocyte interactions during *Xenopus laevis* oogenesis

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Currently, I am in the writing process of my PhD thesis in Biochemistry that was carried out in the National University of Tucumán, Argentina. For the next stage of my career I am seeking a post-doctoral position that will allow me to broaden my horizons professionally and personally. With this aim in mind, I have also undertaken a Masters in Molecular Oncology with the ambition of merging both the ordinary processes of developmental biology with the carcinogenic mechanisms of cancer.

Regarding my professional background, during the last 6 years I have been working on different molecular and cellular aspects of *Xenopus laevis* oogenesis, giving special attention to the signalling systems occurring between follicular cells and oocytes during this process.

In the light of our experimental approach, the functional unit is represented by the ovarian follicle comprising the sexual cell (oocyte) surrounded by a monolayer of somatics cells (follicular cells). The most fascinating aspects of this biological framework are not only the processes that supply the oocyte with all the materials needed to support fecundation and early embryogenesis; but also the molecular dialogue occurring between the follicular cell and oocyte allowing those processes to happen. In this sense, we have established a possible interplay of bmp15 and cx45 throughout *X. laevis* oogenesis, determining their implication during the vitellogenin uptake process. We wanted to go deeper in this line of research and we decided to analyze the necessary molecular signals that are required to trigger vitellogenesis in this amphibian, linking a cAMP non-canonical pathway with the IP3-related calcium release pathway.

If you are interested in knowing more about this project, please click on the following link (https://dl.dropboxusercontent.com/u/26540931/Infographic.png) or scan the QR code here below to see an info-graphic summary.
The role of the neural crest in craniofacial development in basal actinopterygians

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I am interested in large scale evolutionary changes and the development of fishes. For my PhD I will study the cranial neural crest in basal actinopterygians to contribute to a more comprehensive understanding of the role of the neural crest in vertebrates. Many structures that are derived from cranial neural crest cells are evolutionary novelties of the Osteognathostomata, the jaw-bearing vertebrates with a bony skeleton. Within actinopterygians and sarcopterygians, the two osteognathostomatan lineages, major morphological changes occurred in the cranial region and my project will contribute to the understanding of the role the neural crest has in this.

The main organisms I investigate are sturgeons, but gar, bichir and bowfin are planned for a further extension of the study. Sturgeons (Acipenseridae, Acipenseriformes) are primitive actinopterygian fishes that diverged in the Late Cretaceous. For my study I raise several sturgeon species and hybrids in the lab from artificially fertilized eggs which I obtain from local hatcheries. Currently I am describing the development of the bony cranial elements in two species and try to fate map the neural crest with injections of fluorescent dextrane into the neural crest. Morpholino injections and extirpation of neural crest are now getting into focus as we have established the raising of eggs and larvae and enough knowledge on the normal development of sturgeons.

Other ongoing projects with a background in morphology and phylogenetics include the description of the viscerocranium of a deep-sea fish from the family Alepocephalidae and a Bachelor thesis on the ontogeny of the caudal skeleton in a species of pufferfishes (Tetraodontidae) that I am co-advising. A current project that I am pursuing together with another PhD student from the Institute is a muscular developmental study in the longnose gar (Lepisosteus osseus). We focus on the cucullaris muscle that is of debated origin from either lateral plate mesoderm or from the anterior somites. As it is the case for the neural crest project, the cucullaris has been studied in model organisms such as chick and axolotl, but by extending the knowledge on to phylogenetically important taxa we wish to add to a more comparative view.
Investigating functional interactions between the Hippo pathway and polarity proteins

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I am a third year graduate student in the Richard Fehon Lab at the University of Chicago. I am using Drosophila to study the interaction between Hippo signaling pathway and apical-basal polarity proteins.

Epithelial cell polarity and growth control are two important aspects of embryonic development. In addition, disruption of cell polarity and loss of growth control are two major characteristics of cancers derived from epithelial tissues. Understanding the function and regulation of cell polarity and growth control is therefore of great interest for both developmental biology and cancer biology. Epithelial cell polarity is established and maintained through complex interactions among evolutionarily conserved polarity proteins. These polarity proteins localize to distinct cortical domains to execute their respective functions. In developing epithelia, growth is tightly controlled through regulation of cell proliferation and apoptosis. The Hippo tumor suppressor pathway, an evolutionarily conserved signaling pathway, has been identified as a key regulator of these processes. Recent discoveries start to reveal functional interactions between polarity proteins and the Hippo pathway. I am investigating these interactions with genetic, cell biological, and biochemical methods.
Campomelic Dysplasia (CD) is a rare skeletal malformation disease characterized by bowing of long bones, and is often associated with craniofacial and gonad defects. Majority of CD patients contain heterozygous mutations in SOX9 gene, while some present chromosomal lesions at >1Mb from SOX9 coding region. Accordingly, haploinsufficiency of SOX9 and disruptions of SOX9 regulatory elements were postulated to be the underlying causes of CD. However, the exact locations of CD-associated regulatory regions and the molecular mechanism contributing to the characteristic bowing phenotype in CD patients have remained elusive. Here we identified nine putative enhancers of SOX9 that are commonly disrupted in CD patients. A number of these elements activate transgene expression in distinct Sox9-expressing tissues in transgenic mouse embryos. On the other hand, to gain molecular insights into skeletal phenotypes observed in CD patients, we took advantage of our mouse model, Sox9+/Y440X, that phenocopy the human CD syndrome. Advanced primary ossification and hyperplasia of osteoblasts are consistent features in the bowing limbs of Sox9+/Y440X mice. These events could be explained by increased hypertrophic chondrocytes-osteoblasts (HC-OB) transition as shown by lineage tracing of HC. To definitely relate altered HC-OB transition to bowing phenotype characteristic of CD syndrome, we are generating conditional knock-in mice in which the mutant Sox9Y440X protein is specifically expressed in hypertrophic chondrocytes. These studies shall provide novel insights into the molecular etiology of CD syndrome, and more broadly, the regulatory circuit of Sox9 in skeletogenesis.