Program/Abstract #494
The evolution of the vertebrate cerebellum
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The vertebrate cerebellum develops from the rostral hindbrain and in mammals is responsible for the integration of sensory and proprioceptive inputs in the coordination of motor function. While its cellular composition is highly conserved, it represents one of the most morphologically adaptive structures in the central nervous system of vertebrates, and is thus an excellent model for studying the evolution of CNS development. In particular the foliation of the amniote cerebellum reflects a unique pattern of extra-ventricular cell division within a transient, superficial proliferative layer, the external germinal layer (EGL). Transit amplification of EGL precursors is driven by local sonic hedgehog signalling and yields a vast number of a single cell type, the granule cell, which then migrate radially into an internal granule layer. Recent data from more basal vertebrates suggest that the EGL evolved in the sarcopterygian lineage. Where and how it arose remains unclear. To investigate this we have analysed the molecular anatomy of the developing cerebellum in the frog, *Xenopus laevis*, and compared it to that of the chick, a representative amniote. Remarkably, the frog cerebellum displays temporally discrete developmental motifs of both anamniote and amniote development but lacks transit amplification in the EGL. From comparative expression analyses, we present a model whereby changing bHLH gene regulation underlies the evolution of a proliferative window with the EGL.

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Program/Abstract #495
A unique secreted peptide regulates early embryogenesis in vertebrates
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In a locus associated with identical twinning in humans, we discovered a gene encoding for a unique secreted peptide of less than 40 residues. Conserved throughout vertebrates, this gene is expressed during early embryogenesis in signaling centers such as the Spemann Organizer, the Midbrain–Hindbrain boundary and the Chordo–Neural hinge. To address its function we pursued a MO knock-down strategy in frogs and a Zinc-Finger Nucleases (ZFN) knock-out in zebrafish. Zygotic null fish cannot survive past 3 days and display growth and patterning defects reminiscent of Oct4 (spg) mutant embryos.

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Program/Abstract #496
The evolution of mesoderm from pluripotent tissue
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All vertebrate tissue is derived from one of three somatic germ layers, ectoderm, endoderm, or mesoderm. Of these, mesoderm was last to evolve, as indicated by the absence of mesoderm in ancient vertebrate ancestors, such as sea anemones. Understanding how mesoderm evolved is a central question of evolutionary and developmental biology, but it is also important for practical reasons since it instructs how genetic regulatory networks (GRNs) that govern mesoderm specification can be manipulated for regenerative medicine. Our lab pioneered the use of axolotl embryos as a model system to investigate vertebrate development because axolotls are representative of the amphibians from which mammals evolved. In the last year we reported that the mechanisms governing mesoderm specification and pluripotency are conserved from axolotls to mammals, and we showed that the master regulator of mammalian pluripotency, Nanog, is conserved in axolotls, even though it is not present in Xenopus or zebrafish (Swiers et al., 2010; Dixon et al., 2010).

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Program/Abstract #497
Isolation and characterization of a zebrafish Perlipin
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Perilipins (Plin) are evolutionarily conserved proteins involved in neutral lipid metabolism. In mammals, five Plin family members are targeted to intracellular lipid droplets (LD) and regulate various aspects of triglyceride metabolism. The function of Plin proteins in other organism is not well studied, and there are no data regarding Plin function in zebrafish. Query of the zebrafish genome with the conserved PAT domain of mouse Plin1 identified three putative paralogs. Conservation between mammalian and fish genes was limited to the N-terminal PAT domain, which directs protein targeting to neutral lipid droplets, with virtually no conservation in C-terminal regions, which confer specialized functions. We focused our attention on zgc:162150 (plin1), which encodes a 490 amino acid protein with protein kinase A (PKA) consensus sites in the putative C-terminal regulatory domain. RT-PCR showed that plin1 was maternally expressed and was observed in multiple stages of embryonic and larval development. In adults, plin1 mRNA was found in skin and scales, but not in adipose tissue. Immunohistochemistry demonstrated strong Plin1 expression in adult xanthophores, where it was targeted to carotenoid bodies (CB). Expression was not seen in adipose tissue. Mass spectrometry confirmed the targeting of Plin1 to purified carotenoid droplets (CD) and phosphorylation of C-terminal PKA sites. CB are intracellular organelles that mediate pigment dispersion in response to PKA activation. We suggest that the conserved PAT domain targets Plin1 to CD and the divergent C-terminus confers control of pigment dispersion. We are currently testing the role of Plin1 in CB organization and function using gain and loss of function approaches.

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Program/Abstract #498
Lung development in lungless salamanders!
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Lungs have played a key role in the extraordinary adaptive diversification of terrestrial vertebrates. Yet, independent instances of lung loss (lack of lungs as an adult) have occurred within each of the three clades of living amphibians—Caudata, Anura, and Gymnophiona. The morphological and molecular developmental pathways involved in lung loss remain unexplored. However, growing under-

standing of the mechanisms of pulmonary development presents the opportunity to examine this issue in greater detail, beginning with morphological description. We compare lung morphogenesis in a lunged salamander (*Ambystoma mexicanum*) to the lungless plethodontid salamander *Plethodon cinereus*. Both species undergo similar early stages of pulmonary morphogenesis, including the formation of a median fold in the ventral foregut and the evagination of the laryngotracheal tube from the foregut endoderm. The laryngotracheal tube represents the anlage of the trachea and lung buds in *A. mexicanum*. However, in *P. cinereus* this structure quickly regresses without forming a trachea or lung buds. The presence of pulmonary vestiges in *P. cinereus* indicates that lung loss likely involves the disruption of proper lung growth or maintenance, and not the specification of pulmonary rudiments. Formation of a transient laryngotracheal tube in *P. cinereus* suggests conservation of essential inductive interactions between lung rudiments and surrounding tissues. These results have implications for both the evolution of lung loss and the developmental mechanics of lung development. This work was supported by a grant from the NSF to JH (NSF EF-0334846, AmphibiaTree). RK is an American Association of Anatomists (AAA) postdoctoral fellow.

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Program/Abstract #499
Investigating the role of FGF-regulated transcription factors ETV4 and ETV5 in lung development and maturation

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The Fibroblast Growth Factor (FGF) signaling pathway has been shown to play a central role in lung development and maturation. However, the specific molecular program that mediates FGF activity remains poorly understood. ETV4 and ETV5 are Ets domain containing transcription factors that are under the control of FGF signaling activity. Both genes are expressed in the developing lung epithelium, and have been implicated in proper lung formation. To address their precise role in lung development, we generated conditional knock out of both Etv4 and Etv5 in the lung epithelium. These mutant lungs display dilated airways, decreased lung branching, and a thickened mesenchyme. After birth, these early developmental defects manifest into a postnatal phenotype that resembles the lungs of emphysema patients. Characterization of changes in signaling pathways revealed that there is a distinct downregulation of sonic hedgehog (Shh), another key signaling gene essential for proper lung formation. Our results confirm that FGF signaling positively regulates the expression of Shh in the lung, and identifies Etv4 and Etv5 as potential mediators of the crosstalk between FGF and SHH, two major signaling pathways critical for lung development.

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Program/Abstract #500
How the chicken lost its penis: Developmental basis of external genital reduction in birds

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The external genital organs of amniotes show a high degree of morphological variation. Birds are a particularly interesting case; some lineages have evolved elongated, coiled and highly ornamented phalluses whereas others have reduced or completely lost their external genitalia. Crocodilians (the sister group to birds), paleognaths (the most basal birds) and anseriformes (e.g., ducks, geese) have well-developed external genitalia, whereas galliformes (e.g., chickens, quails, turkeys) have lost the intromittent phallus. In order to identify the developmental mechanism underpinning loss of the phallus in galliform birds, we have performed a comparative study of external genital development in members of the galliform and anseriform sister clades. We find that genital tubercle development is initiated in members of both clades, but that outgrowth of the tubercle arrests prematurely in chick and quail embryos. Analysis of cell proliferation and cell death shows that apoptosis is mis-regulated in chick and quail embryos, which leads to arrest and subsequent regression of the genital tubercle. A comparative study of gene expression in chick and duck embryos shows that the mechanisms that regulate genital outgrowth are intact in both groups, but that chickens show ectopic activation of Bmp signaling at the distal tip of the genital tubercle. To test whether Bmp expression underlies reduction of chick genitalia, we antagonized Bmp signaling by implanting beads soaked in Noggin protein. We find that Noggin is sufficient to switch off expression of Bmp target genes and to rescue genital tubercle mesenchyme from cell death. Consequently, outgrowth of the chicken genitalia is enhanced. We propose that reduction of the external genitalia during the evolution of galliform birds resulted from acquisition of a novel Bmp expression domain after the divergence of galliformes and anseriformes.

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Program/Abstract #501
Trunk neural crest cells form an ectomesenchymal dermis in the turtle plastron

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Turtle plastron bones develop by intramembranous ossification, suggesting that they are derived, like the facial bones, from neural crest cells. Well after the initial wave of neural crest migration, cells expressing HNK1 and the early neural crest marker FoxD3, begin accumulating in the thickened dermis of the carapace and migrating to the developing plastron. This second, later wave of HNK1+ cells can also be observed migrating away from cultured neural tubes from St.17 embryos. These late emerging neural crest cells also express PDGFRa, which is typically expressed by cranial neural crest cells. When the lipophilic dye DiI was injected into the lumen of the neural tube, DiI-positive cells were observed in the neural crest "staging area" within a day. After several days, DiI-positive cells reached the ventral mesenchyme. Plastron mesenchyme cells have a gene expression pattern similar to cranial skeletogenic neural crest cells, and appear to have functional similarities to cranial neural crest cells as they differentiate readily in culture to form clusters of collagen I-positive cells. These data support our hypothesis that the plastron of the turtle is formed by a late emerging population of neural crest cells that collect dorsally in the carapace, migrate ventrally to the plastron, and undergo intramembranous osification.

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