

Program/Abstract #494**The evolution of the vertebrate cerebellum**Thomas Butts^a, Richard Wingate^b^aKing's College London, London, UK^bMRC Centre for Developmental Biology, London, UK

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.

doi:[10.1016/j.ydbio.2011.05.556](https://doi.org/10.1016/j.ydbio.2011.05.556)**Program/Abstract #495****A unique secreted peptide regulates early embryogenesis in vertebrates**Serene Chng^a, Jing Tian^b, Bruno Reversade^b^aInstitute of Medical Biology Human Embryology, Singapore, Singapore^bInstitute of Medical Biology, A*STAR, Singapore, Singapore

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.

doi:[10.1016/j.ydbio.2011.05.557](https://doi.org/10.1016/j.ydbio.2011.05.557)**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).

doi:[10.1016/j.ydbio.2011.05.558](https://doi.org/10.1016/j.ydbio.2011.05.558)**Program/Abstract #497****Isolation and characterization of a zebrafish Perilipin**Ryan Thummel^a, Vickie Kimler^b, James Granneman^b^aWayne State University Department of Anatomy and Cell Biology, Detroit, MI, USA^bWayne State University School of Medicine, Detroit, MI, USA

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.

doi:[10.1016/j.ydbio.2011.05.559](https://doi.org/10.1016/j.ydbio.2011.05.559)**Program/Abstract #498****Lung development in lungless salamanders!**Zachary R. Lewis^a, Ryan Kerney^b, James Hanken^a^aHarvard University Dept of Organismic & Evolutionary Biology, Cambridge, MA, USA^bDalhousie University, Halifax, NS, Canada

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-