genes evolve to maintain developmentally important patterns of expression.

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Program/Abstract # 95
Evolution of SoxB1 regulation and function: Neural development from marine worm to frog
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SoxB1 transcription factors are encoded by early neural genes expressed in a subset of ectoderm cells that are directed to become proliferating neural progenitors. Expression of proneural proteins then drives the progenitors to exit the cell cycle, decrease the expression of the SoxB1 genes and differentiate into neurons to form the central nervous system (CNS). Interestingly, although the basic organization of the CNS in vertebrates is conserved, the pathways driving induction and differentiation vary, making it difficult to identify an ancestral molecular mechanism. To define these basal mechanisms, we will study the regulation and function of the highly conserved SoxB1 transcription factors in organisms with a simple nervous system — the hemichordate, Saccoglossus kowalevskii and the urochordate, Ciona intestinalis — and compare this to what we and others have found in the vertebrate, Xenopus laevis. We have begun to investigate their role in neural development by using gain and loss of function experiments of SoxB1 proteins from Xenopus, Ciona and Saccoglossus in Xenopus and Ciona embryos. Also we are comparing the neural induction mechanisms by studying the regulation of the SoxB1 genes in Ciona and Xenopus. With this approach we can determine which mechanism is ancestral and if the functions of the proteins involved in neural development are conserved.

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Program/Abstract # 96
Function and regulation of Xenopus laevis Sox21
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Primary neurogenesis can be broken down into two key steps: (1) induction — cells are induced to express SoxB1 transcription factors and (2) differentiation — the neural progenitors are instructed to decrease SoxB1 expression, cease proliferation and differentiate. It has been proposed that neural progenitors are instructed to differentiate in part due to the decrease in SoxB1 expression imposed by the function of Sox21, a member of the SoxB2 group of transcription factors. To further understand the role of Sox21 during this step of neural development, we have investigated Xenopus laevis Sox21 expression, regulation and function. The current model for Sox21 function would predict that it be expressed in all domains of the central nervous system, we find that sox21 is expressed in discrete domains of the CNS including the forebrain and midbrain—hindbrain barrier, two sites in which neural differentiation is delayed and under the influence of the secreted molecule fibroblast growth factor-8. Furthermore, sox21 is expressed in only a dorsal domain of the neural tube, suggesting that Sox21 may either direct distinct populations of progenitors to differentiate or that it has another function during neurogenesis. Using gain of function experiments, we find that Sox21 like the SoxB1 proteins acts to expand the pool of progenitors but unlike the SoxB1 transcriptional activators, functions by acting as a transcriptional repressor. To analyze the regulation of Sox21 expression, we have isolated evolutionary conserved regions necessary to restrict and drive expression in the forebrain and MHB, and are in the process of identifying the signals driving expression and the targets of Sox21.

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Program/Abstract # 97
Development of the central catecholamine systems in a frog without a tadpole
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Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine synthesis. This study examined the development of the catecholaminergic system in the brain of a directly developing frog using immunohistochemistry and high performance liquid chromatography. Eleutherodactylus coqui is a frog that has evolved a life history that has eliminated the free swimming tadpole common to most anurans. E. coqui embryos hatch as small froglets that have directly developed into the adult phenotype. This type of life history mode and development displays both derived and ancestral (metamorphic) characters. Embryonic development in E. coqui is classified into 15 stages (TS 1–15; 1=oviposition and 15=hatching). TH immunoreactivity was initially detected at TS 5–6 in the nucleus of the posterior tubercle. Catecholamines and their respected metabolites were detected electrochemically at TS 5. At mid-embryonic stages, immunopositive perikarya were observed in the locus coeruleus, cells adjacent to the paraventricular organ, and suprachiasmatic nucleus. The majority of TH cell and fiber development occurs in the later stages of embryonic development. Compared to metamorphic frogs, catecholaminergic development initiates slightly later in E. coqui and the majority of perikarya and fiber development occurs in the later stages of embryonic development, which appears to correlate with development that transpire during metamorphosis in anuran tadpoles.

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Program/Abstract # 98
Small molecule-mediated “phenotypic engineering” reveals a role for retinoic acid in anuran gut evolution
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The evolution of diverse digestive organs in vertebrates with distinct feeding strategies is poorly understood. In contrast to typical omnivorous anuran larvae (Xenopus laevis), the obligate carnivorous tadpole of the South American frog, Lepidobatrachus laevis, has a large stomach, elongated gastroduodenal (GD) loop, and rudimentary
pancreas. We used a “phenotypic engineering” approach to determine whether exposing Xenopus embryos to chemical inhibitors of specific signaling pathways could induce Lepidobatrachus–like gut features. Compounds that inhibit retinoic acid (RA) signaling caused the Xenopus foregut to adopt features similar to Lepidobatrachus, including an elongated GD loop and reduced pancreas. Reciprocally, Lepidobatrachus embryos treated with ectopic RA developed a more characteristic anuran foregut, with a shortened GD loop and more apparent pancreas tissue. Interestingly, the expression domain of a gene involved in shaping the left–right asymmetry of the GD loop, Pitx2, is located more posteriorly in Lepidobatrachus. Lepidobatrachus Pitx2 expression is shifted anteriorly upon RA treatment, while Xenopus treated with RA signaling inhibitors exhibits a posteriorized Pitx2 domain. These results suggest that alterations in RA and/or Pitx2 domains underlie the evolution of novel digestive anatomy, and illustrate the utility of small molecule-mediated phenotypic engineering for uncovering morphogenetic mechanisms in non-model species.

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Program/Abstract # 99
Conservation in a frog of the retinoic acid requirement for forelimb initiation
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Retinoic acid (RA) is required for initiation of zebrafish pectoral fins, chicken wings, and mouse arms, suggesting that this character is basal for Osteichthyes. A gap in this list is amphibians, primarily because limbs originate late in embryos of Xenopus and other frogs, so this development is not investigated. In the direct developing frog, Eleutherodactylus coqui, limbs form shortly after neural tube formation, as in amniotes, allowing us to ask whether the RA requirement is conserved in frogs. When neurulae were treated with citral, the embryos had hindlimbs but lacked forelimbs. Citral inhibits retinaldehyde dehydrogenase (Raldh), an enzyme required for generating RA from vitamin A. We cloned two E. coqui genes coding for Raldh, EcRaldh1 and EcRaldh2. EcRaldh1 was expressed well after limb initiation in dorsal retina, otic capsule, and pronephros. EcRaldh2 was expressed on the blastoporal lip of gastrulae and lateral and posterior to the head of neurulae. The latter expression, as well as expression in the first four somites, was present before limb buds appear, so it likely accounted for the RA production, required for forelimbs. EcRaldh2 was later expressed in the retina, lens, ventral spinal cord, and at the base of both forelimbs and hindlimbs. Direct development in E. coqui is derived from frogs with tadpoles and delayed limb development, so we conclude that the RA requirement for forelimb initiation is conserved in frogs.

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Program/Abstract # 100
In limb development BMP and FGF signaling interact through Sproutys
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During limb development, Fibroblast Growth Factors (FGFs), produced by the Apical Ectodermal Ridge (AER) at the distal tip of the limb bud, signal to the underlying mesenchyme are required for proximal–distal limb outgrowth. A variety of studies indicate that patterning of the limb also requires Bone Morphogenetic Protein (BMP) signaling. In this study we provide evidence that BMP signals modulate the FGF signal by regulating the expression of the FGF antagonist genes, Sprouty 2 and 4. We have conditionally inactivated the Bmp receptor gene, Bmpr1a, specifically in the limb bud mesenchyme, thus causing a proximal–distal truncation that worsens with additional loss of Bmpr1b. This truncation is also reminiscent of a defect caused by a loss of AER-FGF signaling. Consistent with this idea, expression of the AER-FGF targets, Pea3, Ern and Fgf10, is reduced in mutant limb bud mesenchyme. However, we found that expression of the FGF antagonists, Spry2 and Spry4, is upregulated. Thus we hypothesize that BMPs regulate AER-FGF signals through the regulation of Spry gene expression. In support of this hypothesis, Bmpr mutants with genetically enhanced AER-FGF gene expression show a significant rescue of both limb bud mesenchymal gene expression and proximal–distal patterning. Our current efforts are focused on the molecular mechanism by which BMPs regulate Spry gene expression.

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Program/Abstract # 101
Building a marsupial neonate: Evolution of the limb development program in opossum
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Marsupial neonates are born at an embryonic state compared to their eutherian mammal counterparts, yet certain features are accelerated to aid in survival. The most conspicuous of these features are the precocial forelimbs, which the newborns use to climb unaided from the opening of the birth canal to the teat. The mechanism by which the forelimbs become so well developed at birth is unknown. Here we show that multiple, early changes to the limb development program contribute to the forelimb heterochrony. Using Tbx5 and Tbx4 as fore- and hindlimb field markers respectively, we have found that the limb fields arise extremely early during development of the opossum, Monodelphis domestica. In addition, the forelimb buds grow out early, a greater proportion of the lateral plate mesoderm is devoted to the forelimb field, and more somites contribute myocytes than have been reported for eutherians. Furthermore, we found that both fore- and hindlimb fields arise at the posterior end of the embryonic axis directly adjacent to the primitive streak. Our results show a surprising evolutionary flexibility in the early limb development program of mammals and suggest that initial establishment of the limb fields is either due to inducing signals from very caudal axial structures or an autonomous property of the lateral plate mesoderm itself.

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