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*, *Erm* 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 modulate 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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