The mechanisms regulating morphogenesis, homeostasis, and repair of the vertebrate skeleton remain unclear. Zebrafish are a well-suited model for investigating skeletal biology. They possess a classic vertebrate skeleton including endochondral and membranous elements and they have the three signature skeletal cell types (osteoblasts, osteoclasts, and chondrocytes). Therefore, zebrafish mutants that alter skeletal morphology and physiology may provide insight into the fundamental mechanisms involved in human skeletal development. The rapunzel mutant acts globally, resulting in profound overgrowth of the axial and appendicular skeleton. We mapped rpz to a 46 kb critical interval on chromosome 16 that was found to contain a family of five novel, paralogous genes. I examined the coding sequences of all five paralogs and found that one gene, rapunzel (rpz), contained a missense mutation resulting in a non-conserved amino acid substitution. Using the embryonic phenotype, we show that knockdown of rpz completely rescues the homoygous embryonic phenotype, demonstrating that rpz is a gain of function allele. This evidence provides the first gene identification for a mutation affecting development of both the fin ray and the axial skeleton. We are exploiting the rpz mutant to obtain new information regarding skeletal development and homeostasis. Using bone morphometry, in situ hybridization and tissue culture experiments, we will learn more about how rpz is regulating skeletogenesis. Understanding these fundamental aspects of skeletal biology will offer broad insight into skeletal disorders.

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Program/Abstract # 252
Development of cranial foramina in the chick embryo
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Cranial foramina are holes in the skull allowing the entry/exit of blood vessels and nerves. They remain open once formed to make a good model of bone inhibition. Malformation and closure of cranial foramina lead to raised intracranial pressure. Blindness, deafness and facial paralysis can occur if cranial foramina close. We have established nomenclature and locations of chick embryo cranial foramina. Cellular appearance of cranial foramina and structure of the blood vessels and nerves within them have been investigated, using immunohistochemical techniques. In-situ hybridisation analysis of expression of skeletal markers has indicated that mesenchyme adjacent to the blood vessels and nerves initially embarks upon the skeletogenic differentiation fate but this halts and the mesenchyme forms a “zone of inhibition” around these structures. Role of nerves in cranial foramina development is investigated via ablation of hypoglossal and optic nerves. Neurofilament antibody staining has confirmed the absence of these nerves after ablation and albinian blue/alizarin red stained skeletal preparations demonstrate the effect on resulting cranial foramina. Presence of antichondrogenic/antiosteogenic signals within cranial foramina is being investigated. C type natriuretic peptide (CNP) is known to bind to its receptor GC-B to cause chondrogenesis. If CNP binds to the clearance receptor (NPR-C), inhibition of chondrogenesis occurs. Expression of CNP and NPR-C is being studied in cranial foramina. Initial results suggest a role for CNP signalling in development of cranial foramina.

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Program/Abstract # 253
Mechanical and mesenchymal mechanisms of secondary chondrogenesis
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The evolutionary presence or absence of secondary cartilage reflects species-specific differences in functional anatomy. Secondary cartilage arises in the head skeleton at articulations, sutures, and muscle attachments after formation of the primary cartilaginous skeleton and subsequent to osteogenesis. To investigate mechanisms that regulate secondary cartilage formation, we conducted experiments using quail and duck embryos, which exhibit distinct craniofacial morphologies. Quail have small pointed beaks that peck at seed, while duck have large wide bills that filter heavy mud. In duck, jaw muscles attach to a pronounced secondary cartilage that is absent in quail. Neural crest mesenchyme (NCM), which gives rise to all skeletal and connective tissues of the jaw, contains information for species-specific pattern. We hypothesize that NCM-dependent mechanisms also generate species-specific differences in the local mechanical environment that can either promote or inhibit secondary chondrogenesis. To test our hypothesis we employ two approaches. First, we alter the mechanical environment in embryonic duck by paralyzing skeletal muscles, and by inhibiting stretch-activated channels. Second, we re-pattern the duck jaw complex to resemble that found in quail by transplanting NCM. Both approaches inhibit secondary cartilage, as evidenced by 3D reconstructions, histological analyses, and changes in expression of genes associated with musculoskeletal development including sox9, col2a1, runx2, fgfr2, and bmp4. We conclude that NCM controls molecular pathways underlyng musculoskeletal patterning, which in turn establishes mechanical forces necessary to induce secondary cartilage.

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Program/Abstract # 254
How to make a new appendage: Invasive dermal skeleton contributes to a novel fin in a zebrafish mutant
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Dermal skeleton (bony fin rays, lepidotrichia) is a major component of fin skeleton in both paired and median fins of bony fish. Recent developmental evidence suggests that fin patterning mechanisms might originate in median fins, whereas fossil evidence suggests that dermal skeleton formation preceded endoskeleton in paired fins. This raises an intriguing possibility that dermal skeleton-generative mesenchyme may play an important role in fin development. However, due to the lack of homology with tetrapod limb endoskeleton, the developmental origin of dermal skeleton in bony fish fins is largely overlooked. Here we present the zebrafish mutant Lewis, which exhibits ectopic dermal skeleton formation in the ventral midline fin fold resulting in a novel fin. Isolated from an X-ray mutagenesis screen, Lewis homozygous mutants possess an ectopic fin between two pelvic fins. We observed that the pre-anal fin fold, which normally degenerates in wild type individuals, appears to be invaded by mesenchymal cells and transformed into a midline-positioned fin with paired fin morphology in the Lewis mutant. We further demonstrate that the dermal skeleton of the ectopic fin is derived from the invasive mesenchymal cells and is patterned...
similarly to that in normal fins. We believe that further investigations in the Lewis mutant will both elucidate the developmental origin of dermal skeleton in bony fish fins, and shed light on role of dermal skeleton in developmental and evolutionary origin of vertebrate appendages.

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Program/Abstract # 255
The odd-skipped family transcription factors play essential roles in synovial joint development
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Synovial joints play crucial roles in skeletal function, but the mechanisms regulating joint development are still poorly understood. The odd-skipped family transcription factors Osr1 and Osr2 are expressed in the developing synovial joint tissues during limb development in both chick and mice. However, no joint defects have been reported in the Osr2−/− mutant mice whereas Osr1−/− mutant mice died during midgestation. We have generated mice lacking Osr1 in the developing limb using Cre/loxP-mediated tissue-specific gene inactivation. Mice lacking Osr1 gene function during limb development did not exhibit obvious joint defects. However, inactivation of both Osr1 and Osr2 in the developing limb caused aberrant fusion of multiple synovial joints. At the cellular level, lacking Osr1 and Osr2 prevented programmed cell death of the joint cells. At the molecular level, expression of Gdf5 and Wnt9a, signaling molecules involved in the regulation of joint formation, was significantly down-regulated in the Osr1/Osr2 double mutant mice. Moreover, while inactivation of the beta-catenin gene using the Osr2−/− heterozygous mice caused fusions of synovial joints, expression of Osr1 and Osr2 was unaffected in the beta-catenin-mutant presumptive joint cells. These data indicate that Osr1 and Osr2 function redundantly and upregulate the canonical Wnt signaling pathway to regulate formation of the synovial joints.

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Program/Abstract # 256
The evolution and development of the mammalian dentition: Insights from the marsupial Monodelphis domestica
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to understand developmental mechanisms of evolutionary change, we must first know how different morphologies are formed. The vast majority of our knowledge on the developmental genetics of tooth formation derives from studies in mice, which have only molars and incisors and only one tooth generation. In contrast, the marsupial Monodelphis domestica has incisors, canines, premolars, and molars on both the upper and the lower dentition. As in other metatherian mammals, Monodelphis has a deciduous dentition, in which the last upper and lower premolars are replaced. Here, data is presented on the development of the teeth in M. domestica that reveals the normal program of development of these teeth as compared to mouse wild-type and mutant dentitions. I show that the tooth germs of M. domestica express fibroblast growth factor (FGF) genes and Sprouty genes in a manner similar to wild-type mouse molar germs. I also show that the replacement dentition of M. domestica does not follow a similar developmental program as the deciduous premolar.

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Program/Abstract # 257
Building a single-cusped tooth: Shh signalling during tooth morphogenesis in snakes and lizards
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Single-cusped teeth are possessed by members of all toothed amniote taxa and likely constituted the dentition of their last common ancestor. We know little about their development, however, as the prevailing experimental model of amniote odontogenesis has been the multicusped molar of the mouse. Molar morphogenesis is controlled by enamel knots, transient structures that signal to the enamel epithelium via growth factors such as Shh. Single-cusped squamate teeth lack obvious enamel knots, but retain expression of Shh in the inner enamel epithelium (IEE). To gain insight into unicuspid tooth morphogenesis, we characterized Shh’s role in the developing conical teeth of lizards and snakes (Squamata). Gene expression studies of pathway read-outs Patched1 and Gli2 reveal that Shh, produced by the IEE, signals in a paracrine fashion to neighboring cells of the squamate enamel organ. Outer enamel epithelial cells respond to Shh by undergoing cell proliferation, driving outgrowth of the enamel organ from the dental lamina. Stellate reticulum (SR) cells instead seem to hear Shh as a ‘cell survival’ signal. Exposing developing squamate teeth to Hh antagonist cyclopamine prevents formation of the cervical loop and SR, causing teeth to flatten in shape and appear fused with the adjacent epithelium. This phenotype evokes that of Hh-abrogated mouse teeth as well as vestigial, first-generation teeth in the bearded dragon Pogona vitticeps. Our data then suggest a conserved role for Shh across different tooth morphologies and a role for the pathway in the vestigialization of primary teeth in amniote evolution.

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Program/Abstract # 258
Endothelin signaling in the lamprey head and the evolution of the jaw
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The elaborate head skeleton of jawed vertebrates is thought to have evolved from the unjointed gill bars seen in fossil jawless vertebrates. How this occurred is an outstanding question in vertebrate evolution. In the modern jawless vertebrate, lamprey, the pharyngeal skeleton forms an unjointed cartilaginous basket symmetrical along the DV axis. In lamprey embryos, uniform expression of Dlx genes is observed throughout the DV extent of the pharyngeal arches rather than in the nested pattern seen in gnathostomes. These observations have led to speculation that the lamprey pharyngeal skeleton lacks significant DV patterning and that the appearance of such patterning drove the evolution of the jointed gnathostome head skeleton and jaw. We have tested this hypothesis
ABOVE: An image of dissected zebrafish pectoral fin bones demonstrates that fish with a gain-of-function mutation in waslb have two more long bones (right, arrowheads) than wildtype fish (left). M.b. hawkins et al., cell, 2021. They exposed zebrafish males to a mutagen, then crossed those males with wildtype females, and then crossed the mutant offspring with wildtype fish again. The researchers examined the skeletons of heterozygous mutant fish over the course of development and found that the extra bones appeared around three to five weeks after fertilization, with an initial lengthening and subsequent joint formation separating two of the existing long bones into four segments. Development of the appendicular skeleton, fish fins, and tetrapod limbs provides an ideal model for understanding patterning mechanisms in vertebrates (Zeller et al. 2009). Though fish fins and tetrapod limbs share a common evolutionary origin, their distal portions have experienced independent morphological diversification (Clack 1994). However, recent findings have shown that somite cells contribute to fin ray development, suggesting a common mesodermal developmental origin for both fin rays and digits (Lee et al. 2013a, b; Mongera and Nusslein-Volhard 2013; Shimada et al). These findings reveal a novel function of dermomyotome bioelectricity in zebrafish fin patterning. Materials and Methods. Single mutations cause zebrafish fins to transform into complex limb-like structures. Date: February 4, 2021. Researchers examine what's happening at genetic level to drive patterns in fin skeleton versus limb skeleton and find mutants with modified fins in a more limb-like pattern by adding new bones, complete with muscles and joints. The results reveal the ability to form limb-like structures was present in the common ancestor of tetrapods and teleost fishes and has been retained in a latent state which can be activated by genetic changes. Share: FULL STORY. Fin-to-limb transition is an icon of key evolutionary transformations. Many studies focus on understanding the evolution of the simple fin into a complicated limb skeleton by examining the fossil record.