Serotonin (5-HT) is a neuromodulator that plays many different roles in adult and embryonic life. Among the 5-HT receptors, 5-HT2B is one of the key mediators of 5-HT functions during development. We used *Xenopus laevis* as a model system to investigate the role of 5-HT2B in embryogenesis. By means of gene gain- and loss-of-function approaches and tissue transplantation assays, we demonstrated that 5-HT2B modulates, in a cell-autonomous manner, postmitatory skeletogenic cranial neural crest cell (NCC) behavior. 5-HT2B overexpression induced the formation of an ectopic visceral skeletal element and altered the dorsal-ventral patterning of the branchial arches. Loss-of-function experiments revealed that 5-HT2B signaling is necessary for jaw joint formation and for shaping the mandibular arch skeletal elements. In particular, 5-HT2B signaling is required to define and sustain the Xbp1 gene expression necessary for jaw joint formation. We also showed that the phospholipase C beta 3 (PLC) is the effector of the transduction pathway acting downstream of 5-HT2B. The in vivo experiments also revealed that serotonin signaling, via 5-HT2B receptors, results in the formation of defective eyes, characterized by irregular form, position and coloboma. Interestingly, we showed that the 5-HT2B gene is expressed in periocular mesenchyme that represents a key signaling center required for a correct eye morphogenesis. These results contribute to the understanding of the interactive networks of patterning signals that are involved in the development of the vertebrate craniofacial and ocular structures.

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Program/Abstract # 143
Pharyngeal endoderm and FGF signaling in induction and patterning of the chick middle ear columella condensation
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Development of the middle ear skeletal element, the columella, is a complex process involving multiple tissue interactions coupled with a diverse set of molecular signals. We investigated these tissue interactions using explants to determine which tissue recombinations are able to induce and maintain the pre-chondrogenic cell marker Sox9. We find that the pharyngeal endoderm adjacent to the neural crest derived mesenchyme of the proximal 2nd arch, which will give rise to the columella condensation, is able to perform this function at HH14. By HH24, Sox9 expression is unaffected by lack of the endoderm indicating that the mesenchyme is specified by this stage. We hypothesize that FGF signaling from the pharyngeal endoderm is required for the induction of pre-chondrogenic identity in cells that will give rise to the columella condensation. We analyzed Fgf gene expression patterns in the region, identifying Fgf3, 4, 8, 18 and 19 expressions in the pharyngeal endoderm between HH9 and HH19. Sox9 expression is no longer detected following application of FGF receptor inhibitor SU5402 at HH14, demonstrating that FGFs are required for its expression. Conversely, application of FGF8 protein beads in the putative middle ear forming region in ovo results in expansion of the extracolumella cartilage. To determine if FGF signaling is sufficient for Sox9 expression in pre-chondrogenic tissue we are applying FGF protein beads to explants of the putative middle ear mesenchyme. These studies will allow us to determine which individual FGF proteins or combination of FGFs is sufficient to specify pre-chondrogenic identity.

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Program/Abstract # 144
Mechanism of mesenchymal condensation during chick middle ear morphogenesis
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The middle ear is an excellent model system for exploring fundamental principles of organ morphogenesis. A critical event of middle ear morphogenesis is the condensation of cranial neural crest cells to form the middle ear skeletal primordium. Our objective is to understand the mechanism of mesenchymal condensation during middle ear development in order to understand the physical processes necessary for correct skeletal size and shape. Our model is the chick embryo, containing a single middle ear bone — the columella. The columella spans the middle ear cavity from the proximal inner ear to the distal tympanic membrane. The columella forms by endochondral ossification of cranial neural crest cells in the second pharyngeal arch. Mesenchymal condensation is followed by overt differentiation into chondrocytes. The mature columella is a composite bone, comprising a proximal ossified columnella, from replacement cartilage and a persistent cartilaginous extracolumella, situated distally. Our central hypothesis is that dynamic cell shape changes drive condensation during morphogenesis of the columella. We have visualized temporal cell shape changes in the putative columella region using fluorescence immunohistochemistry. Our results demonstrate that dynamic cytoskeletal reorganization and cell shape changes occur over several days, resulting in a cartilage template of correct size and shape. Currently, we are undertaking experiments to inhibit the cell shape changes in our region of interest to determine if dynamic cell shape changes drive mesenchymal condensation. Our experiments will be helpful in understanding the general principle of self-assembly of multi-potent progenitor cells to form a specific cartilage template.

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Program/Abstract # 145
Expression dynamics of PAR proteins during establishment of the chick lens placode
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Vertebrate lens originates from pre-lens ectoderm, a simple cuboidal epithelium that overlies the optic vesicle. After induction by the optic vesicle, the pre-lens ectoderm cells elongate at their apico-basal axis, becoming columnar and forming a pseudostratified epithelium known as the lens placode. We have shown previously that actomyosin contractile filaments are homogeneously distributed along the apico-basal sides of the pre-lens ectoderm and become enriched apically at later stages of the lens placode and during lens invagination. Therefore, the actomyosin filaments redistribution to the apical side of the pre-lens ectoderm is necessary for correct skeletal size and shape. Currently, we are undertaking experiments to inhibit the cell shape changes in our region of interest to determine if dynamic cell shape changes drive mesenchymal condensation. Our experiments will be helpful in understanding the morphological transition from pre-lens ectoderm to lens placode. Here, we investigate the role of PAR proteins in the establishment of the placode’s apico-basal polarity. In particular, we focus on PAR3, PAR6 and aPKC. PAR3 is a scaffold protein that establishes and maintains epithelial cell polarity in a diverse set of models. PAR3 is initially homogeneously distributed along the apico-basal side of pre-lens ectoderm cells. At lens placode stage, it becomes restricted to the cell apical domain prior to the completion of actomyosin and beta-catenin apical enrichment. In contrast, the segregation of PAR6 into different membrane domains during placodal elongation is less clear. Finally, aPKC is concentrated at the apical domain even at the pre-placodal stage and remains so during
invagination. Taken together, these data suggest that the dynamics segregation of components of the PAR complex differs during the establishment of apico-basal polarity in lens cells during lens morphogenesis.

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Program/Abstract # 146
A TRIO-RhoA-Shroom3 pathway is required for apical constriction during lens pit invagination
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Early embryonic lens morphogenesis is, in part, driven by epithelial cell shape changes in the lens placode. The placodal cells change from a columnar to a conical shape causing invagination of the lens pit in a process that is dependent on the cytoskeletal protein Shroom3. This cell shape change, termed apical constriction (AC), occurs when the apical circumference of an epithelial cell is reduced via Rho-kinase (Rock1/2) dependent activation of non-muscle myosins and their contraction of the apical actin–myosin-junctional complexes. Analysis of chicken embryos treated with a Rock1/2 chemical inhibitor revealed that invagination of the lens pit requires Rock1/2 function. Although it has been established that the ability of Shroom3 to induce AC is Rho-kinase dependent, it is unclear how Shroom3 activates Rock1/2. To investigate this, a cell culture-based AC assay was used to determine that Shroom3 induced AC is dependent on RhoA, a Rock1/2 activator, and Trio, a guanine exchange factor (GEF) that activates RhoA. Utilizing chicken embryos overexpressing a Trio inhibitor and Trio-deficient mice we demonstrate that Trio is required for AC during lens morphogenesis. Furthermore we determined that RhoA functions downstream of Trio during lens pit AC. Together these data support a role for Trio in mediating the RhoA/Rock1/2 dependent activity of Shroom3 during AC of the cells within the invaginating lens pit.

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Program/Abstract # 147
Optic tectum morphogenesis: A step-by-step model based on the temporal–spatial organization of the neuroepithelial cell proliferation
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Appropriately organized in space, cell proliferation contributes to morphogenesis. Morphometric analyses on the spatial distribution of the mitotic neuroepithelial cells (mNEcs) in the developing chick optic tectum (OT) allow understanding how cell proliferation contributes to model the OT morphology. Step 1: after the establishment of the IsO (19–21 somites), a medial zone of high mitotic density (medial ZHMD) appears at the cephalic border of the IsO node. Step 2 (ED2): the medial ZHMD extends cephalically; the dorsal midline (future roof plate) elongates and forms a transient “dorsal crest” of dorsal convexity. Step 3 (ED2–ED3): the medial ZHMD expands over the lateral–dorsal regions of each hemitectum; the mNEc density decreases along the dorsal midline and the ZHMD duplicates into two bilateral ZHMDs. The “dorsal crest” regresses while the lateral–dorsal regions grow acquire regular semicircular contours. Step 4 (ED3–4): planar expansions of the OT lateral walls form two semi-ovoid prominences on both sides. During this planar expansion the bilateral ZHMDs move cephalically and ventrally towards the central region of each hemitectum. Step 5 (ED4–ED5): the decrease in mNEc density along the dorsal midline together with the re-localization of the ZHMDs at center of the OT surfaces produces a medial groove separating both hemitectum into two bilateral tectal hemispheres. Step 6 (ED6 onwards): a relative displacement of the ZHMDs towards the caudal region, accompanying an intense expansion of the cephalic regions, produces the two zones of high curvature corresponding to caudal pole of the OT observed at ED8–10. A “moving” ZHMD, by means of a sequence of simple changes, contributes to model the global OT morphology.

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Program/Abstract # 148
Retinal developmental defects in the barely started and good effort mutant zebrafish correlate with elevated cell death
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Two zebrafish ENU-induced mutants, barely startednt1 (bas) and good effortnt2 (gef), were found by screening for abnormal eye development. Both mutants are characterized by successful initiation of the optic primordium, but a failure to mature. The bas phenotype is more severe and is grossly detected by 2 days post fertilization (dpf), whereas gef is observed at 3 dpf. Both mutants have disrupted retinal lamination. The photoreceptor array in gef mutants is disorganized and contains markedly fewer cells, whereas bas fry fail to generate any photoreceptors. At the time of retinal disruption, both mutant lenses seem unaffected. The gef mutant lens develops normally through 6 dpf, whereas the bas lens degenerates noticeably by 5 dpf. Both mutants are homozygous lethal and have no obvious dominant phenotype. While both mutants showed no obvious change in cell proliferation, based on PCNA immunohistochemistry, they both exhibited elevated levels of cell death throughout the CNS prior to gross morphological phenotypes, which may be the cause of lethality. Meiotic recombination placed the bas mutation on chromosome 1 and the gef mutation in a 1 Mb region on chromosome 9. Of the 11 genes in the chromosome 9 interval, none contained any coding region defects relative to wild-type. However, quantitative real-time PCR of these 11 genes showed a markedly decreased expression of only the integrin, beta-like 1 (itgb1) gene in gef mutants relative to control siblings. Morpholino-mediated knockdown of itgb1 and rescue of the gef mutant phenotype by injection of in vitro-transcribed itgb1+ mRNA to confirm that itgb1 corresponds to gef are underway. These data suggest a role for the novel gene itgb1 in maintaining neuronal viability.

Program/Abstract # 149
Role of glycosaminoglycans in murine primary spinal neurulation
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