mediate, caudal hypothalamus and pituitary tissue at 24 and 48hpf. Expression was also seen in the postoptic commissure, optic chiasma, otic vesicles, neural tube and extensively in brain tissue. Immunohistochemistry also indicated expression of CaMKII within developing zebrafish neuroendocrine tissues. These data strongly implicate the CaMKII proteins in the development of the hypothalamus and pituitary. Supported by a Small Project Grant from the Society for Endocrinology.

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Program/Abstract # 332
Zebrafish retinoic acid receptors function as context-dependent transcriptional activators during axial patterning
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Retinoic acid (RA) signaling is used during patterning and organogenesis in vertebrate embryos. At the transcriptional level, RA signaling is proposed to occur through a biphasic activator-repressor model. In the absence of ligand, RA receptors (RARs) are thought to act as transcriptional repressors. When RA binds to the RARs, they are converted to transcriptional activators. However, this biphasic model, which is derived primarily from cell culture studies, has not been tested in most developmental contexts. Therefore, we wanted to better understand the functional capabilities of zebrafish RARs during early development. Surprisingly, we found that overexpression of zebrafish RARs does not affect development nor sensitize embryos to RA treatment, despite the ability of RARs to be strong transcriptional activators in a cell culture reporter assay. Thus, it seems that RARs themselves are not limiting RA signaling in the early zebrafish embryo and that other co-factors contribute to RAR function. Consistent with this notion, hyperactive RAR-VP16 fusion proteins can overcome these limitations and ectopically activate the expression of endogenous RA target genes and a transgenic RA signaling reporter. In contrast, zebrafish dominant negative RARs are not able to act as transcriptional repressors in the early embryo, even though they can act as transcriptional repressors in a cell culture reporter assay. Therefore, our studies suggest a model in which the zebrafish RARs function primarily as context-dependent transcriptional co-activators. Together with studies of RA signaling in tunicates, we propose an evolutionary model of RAR function.

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Program/Abstract # 333
Cell fate decisions and expanded progenitor niches in the zebrafish patched2 retina
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The roles of the Hedgehog pathway in controlling vertebrate retinal development have been studied extensively; however, species- and context-dependent findings have provided conflicting information. To gain further insight into Hedgehog pathway function in the retina, we have analyzed the retinal phenotypes in patched2 mutant zebrafish embryos. Hedgehog signaling has previously been shown to control differentiation and cell fate decisions by controlling the timing of cell cycle exit. While patched2 mutants possess more cells in their retinas, all cell types, except for Müller glia, were present at identical ratios as those observed in phenotypically wild-type siblings. Interestingly, a reduction in differentiated Müller glia was accompanied by an upregulation of GFAP, a marker for reactive glia, and coupled with localized abnormalities in the vitreo-retinal interface. These pheno-types are similar to ocular abnormalities found in human patients suffering from Basal Cell Naevus Syndrome (BCNS), a disorder that has been linked to mutations in the human PTCH gene, and point to the utility of this mutant line as a model for the study of BCNS ocular pathologies. In addition, our analysis of an over-proliferation phenotype at the ciliary marginal zone (CMZ) revealed that the number of proliferating progenitors, but not the rate of proliferation, was affected in patched2 mutants. This finding suggests that, at least in this context, the Hedgehog pathway may not affect cell cycle kinetics, but rather regulates the size of the retinal progenitor niche in the CMZ.

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Program/Abstract # 334
Uncovering the roles of BMP signaling during mouse embryogenesis
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Bone Morphogenetic Proteins (BMPs) are growth factors that participate in highly conserved signaling modules and influence various developmental processes. Previously, we reported the identification of a conserved BMP-responsive element (BRE) that has been identified in the regulatory regions of many Xenopus and Drosophila genes (von Bubnoff et al., 2005; Yao et al., 2006). The BRE reporter gene specifically responds to BMP2/4 but not to other members of the TGF-b superfamily. We have recently generated a new BRE-LacZ reporter mouse line from murine ES cells (E14 line) that contains a single integrated copy of the BRE(7X)-LacZ transgene. Many of the expression domains of our BRE-LacZ mice are consistent with known BMP activity in development. However, it is clear that our BRE reporter does not mark all sites of BMP signaling. Given the various ways Smad proteins interact with gene promoters, this is not surprising. What is unexpected is how well BRE-LacZ expression marks BMP signaling in many organs and developing tissues, suggesting that this BRE-dependent mode of BMP signal regulation is an evolutionarily conserved process. We have also implicated a vertebrate ortholog of the Drosophila zinc finger protein Schnurri as a critical transcription factor in mediating BMP signaling via the BRE site. We are therefore currently examining the roles of BRE sites and Schnurri proteins in BMP signal transduction.

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Program/Abstract # 335
Comprehensive analysis of molecular signals operating at the medial fusion site of mouse palatal shelves
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In mammalian palatogenesis, medial edge epithelia (MEE) of the opposing secondary palatal shelves contact each other and give rise to the midline epithelial seam (MES), which subsequently disintegrate before the mesenchyme confluence. We focused on the molecular regulation and associated gene expression profiles at three discrete developmental stages, i.e., horizontal growth, contact and fusion of MEE, and MES disintegration. Tissue specimens were dissected out of the medial portion of palatal shelves of ICR mouse embryos at E14.014.5. For comprehensive gene expression analysis, cDNA microarray was conducted and data was processed using GeneSpring. To gain further insight into the localization of gene expression, we collected the MEE population uniquely from the shelves with the aid of a laser-capture microdissection. The present microarray analysis unveiled
spatio-temporally altered gene expression profiles during the contact and subsequent fusion of mouse palatal shelves. A total of 882 genes were identified as candidates that were differentially expressed at the medial portion of palatal shelves. The KEGG pathway analysis showed that Wnt (Canonical, Ca²⁺-PCP, Tgfβ (Tgfβ, Bmp, Activin), Notch and Retinol signalings were involved in palatal fusion. The results of GO analysis in BiNGO underlined the functional categories (i.e., JNK activity, integrin-mediated signaling, cellcell adhesion and apoptosis) specifically related to the MEE contact and fusion. The present results also proved that a significant number of the documented cleft palate response genes displayed distinct spatio-temporal expression patterns.

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Program/Abstract # 336
Numb differentially regulates the function of Notch1 and Notch3
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Numb, a cytosolic adaptor protein, is a negative regulator of Notch signaling. In the vertebrate embryo, skeletal muscle is derived from the myotome of the somites. Notch13 demonstrate overlapping expression in mouse somites. Numb is limited in expression to dividing cells of the dorsal medial and ventral lateral lips and the myotome. Notch1 and Notch2 have been shown to inhibit skeletal myogenesis and we demonstrate that Notch3 is an effective inhibitor as well. The focus of these studies was to determine if there was Notch receptor specificity demonstrated by the four Numb protein isoforms during myogenesis. In transcription and myogenesis assays, Notch1 was consistently negatively regulated by all four Numb isoforms. Notch2 was variably affected and Notch3 was not a target for Numb. Subsequent analyses showed that unlike Notch1, that Notch3 was not polyubiquitinated, nor degraded when co-expressed in cells with Numb. These data provide the first observation that Notch receptors are variably affected by Numb and will be important for the interpretation of the function of Notch and Numb interactions during development. This work was funded by the American Heart Association.

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Program/Abstract # 337
Wnt/ β-catenin pathway activation and myogenic differentiation are induced by cholesterol depletion
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Skeletal muscle differentiation is a multi-step process that begins with the commitment of mononucleated precursors that withdraw from cell cycle. These myoblasts elongate while aligning to each other, guided by the recognition between their membranes. This step is followed by cell fusion and the formation of striated multinucleated myotubes. Myogenic differentiation is influenced by a number of growth factors and determination factors, such as the proteins of the Wnt family, that are required for the induction of embryonic myogenesis. The main interest of the present work was to study the effects of cholesterol depletion in the Wnt/β-catenin signaling pathway during muscle differentiation. We used primary cultures prepared from breast muscles of 11-day-old chick embryos, and treated them with MCD after 24h. We analyzed the expression and distribution of beta-catenin as a downstream component of the Wnt pathway. Cholesterol depletion increased the expression of beta-catenin, its translocation to the nuclei, and activation of Wnt pathway. Moreover, we show an enhancement in the expression of the Troponin-T and Sarcomeric-actin in MCD-treated cells. Frizzled, the receptor Of Wnt proteins, was co-localized with the GM1 ganglioside in membrane micro-domains. Taken together, the data here presented provide evidence that cholesterol depletion from myoblasts membranes induces the activation of Wnt signaling pathway, the enhancement of β-catenin expression and its nuclear translocation, resulting in myoblast recognition and fusion into multinucleated myotubes.

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Program/Abstract # 338
WID, a novel negative regulator of the WNT signaling pathway, is important for kidney development
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Mammalian kidney development is regulated by distinct activities of the Wilms tumor gene, WT1, and the WNT/β-catenin pathway, but how these two pathways converge on renal development is not well understood. Here we identified a novel gene WID (WT1-induced Dishevelled) as a WT1 transcriptional target that is both critical for kidney development and negatively regulates WNT/β-catenin signaling. WID interacts with Dishevelled via its C-terminal CXXC zinc finger and Dishevelled Binding domains and potently inhibits WNT/β-catenin signaling in vitro and in vivo. In the developing mouse kidney, Wid and WT1 expression overlap closely and Wid−/− mice exhibit glomerular defects with proteinuria and early postnatal lethality. In addition, a subset of Wid+/− and Wid−/− mice displayed duplicated kidneys and ureter hydrourephrosis. Remarkably, knockdown of wid expression in zebrafish also interferes with development of the embryonic kidney. Taken together, our results demonstrate that the WT1 target gene WID plays an important role in kidney development, and implicate WT1 in the negative regulation of WNT signaling during nephrogenesis, via WID.

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Program/Abstract # 339
ERK1/2-signaling is required for cell differentiation during ocular lens development
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The ocular lens is a simple and polarized tissue that is made of two cell types, epithelial and fiber cells. During lens development, the lens epithelial cells at the periphery are induced to proliferate and then differentiate into the lens fiber cells. Growth factor signaling has been