Foxb1 plays an essential role during development of the ventral diencephalon in mammals. Interestingly, depletion of Zic2a or Foxb1.1, or compromised Hh signaling, also results in a deficit of the prethalamus, a part of the ventral diencephalon, by 1 day post-fertilization. Zic and Gli proteins are hypothesized to physically interact *in vivo*. Although Zic2a and Gli1 both function during gastrulation to control development of the same forebrain precursors, we have preliminary evidence that suggests they function via different mechanisms. Live imaging analyses examining the dynamic morphological rearrangements in the anterior neural plate of Zic2a and Hh depleted embryos will be presented. These data provide novel insight into the roles of Zics, Foxb1 and Hh signaling in forebrain development. Our findings will help shed light on the genesis of holoprosencephaly (HPE), a prevalent forebrain defect that is caused by mutations in Hh signaling and ZIC2, and establish zebrafish as a model for HPE.

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Program/Abstract # 317
Lipoic acid synthetase is specifically required for forebrain formation in the mouse embryo
Xin Zhou [1,2], Kathryn Anderson [1]
[1] *Developmental Biology Program, Sloan-Kettering Institute, New York, NY 10021, USA*
[2] *Cell Biology and Genetics Program, Weill Graduate School of Medical Sciences, Cornell University, New York, NY 10021, USA*

Many human birth defects involve abnormal brain development. Studies of normal brain development in mouse embryos should elucidate both the origin and possible treatments of these human diseases. Here we describe the ENU-induced mouse mutant *nearly headless* whose most obvious phenotype is a severe truncation of the anterior brain. The initial specification of the forebrain is affected in *nearly headless* mutants. As development proceeds, the anterior truncation phenotype becomes more evident. As the formation of head region of mouse embryos depends on signals from the anterior visceral endoderm (AVE), the axial mesendoderm (AME) and the anterior definitive endoderm (ADE), we have examined marker gene expression of these embryonic structures. Our current data suggest that AVE and ADE are properly patterned in *nearly headless* mutants but there is a defect in axial mesendoderm specification. We have found that the *nearly headless* phenotype is caused by a partial loss of function mutation in *Lipoic acid synthetase* (*Lias*). Lias catalyzes the synthesis of lipoic acid, which is a crucial cofactor for several multienzyme complexes required for oxidative metabolism. This mutation causes a global defect in the cell cycle possibly by activating a cellular energy gauge, AMP-activated protein kinase (AMPK). We are currently investigating how a general defect in metabolism has a differential effect on forebrain patterning in the early embryo.

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Program/Abstract # 318
Analysing the role of *Hoxa1* in mammalian hindbrain, inner ear and cardiovascular development
Nadja Makki, Mario R. Capecchi
Dept. of Human Genetics, University of Utah, Salt Lake City, UT, USA

Hoxa1 is one of the earliest and most anterior *Hox* genes expressed during embryogenesis. It plays an important role in regulating the development of the brainstem, inner ear and cranial ganglia in humans and mice. Patients with homozygous mutations in *HOXA1* (Bosley–Salih–Alorainy Syndrome) exhibit severe defects in these structures. In addition, some patients display cardiovascular abnormalities. To analyse the function of *Hoxa1* in the development of the brainstem, inner ear, cranial ganglia and the heart we performed genetics lineage analysis followed by conditional mutagenesis. Lineage analysis was carried out using a mouse line expressing Cre recombinase from the *Hoxa1* locus. This technique allows us to genetically label all *Hoxa1*-expressing cells in the embryo and to follow their developmental fate. Our results demonstrate that *Hoxa1*-lineage is found in the caudal hindbrain with an anterior border in rhombomere 3. Additionally, we show that *Hoxa1*-expressing cells give rise to rhombomere 4-derived neural crest cells, which populate the second branchial arch and contribute to the VII/VIIIth ganglion complex. *Hoxa1* lineage was also seen in a specific pattern in the otic epithelium and the outflow tract of the heart. In order to inactivate *Hoxa1* function in each of these structures, we generated a *Hoxa1*-conditional allele. This allele permits conditional inactivation of *Hoxa1* function in one tissue at a time using Cre drivers specifically expressed in neural crest cell precursors, the otic placode and different hindbrain rhombomeres.

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Program/Abstract # 319
Functional analysis of novel genes differentially expressed genes in heart/hemangioblast precursor cells (H/HPC)
Jose A. Belo [1], Margaret C. Bento [1], Ana T. Tavares [2]
[1] *IBB, CBME, Univ. do Algarve, Faro, Portugal*
[2] *Instituto Gulbenkian de Ciência, Oeiras, Portugal*

Genetic evidence has implicated several genes as being critical for heart development. However, the inducers of these genes as well as their other targets and the pathways they constitute, remain largely unknown. The heart precursor cells are generated within bilateral fields in the lateral mesoderm, which consequently converge toward the midline to form a

beating linear heart tube. In the avian embryo, *Caronte* (*Car*) transcripts are detected in the anterior mesendoderm including the heart precursor cells and in the left lateral plate mesoderm. We have identified a promoter element of chick Car able to drive EGFP expression into the heart and the hemangioblast precursor cells. More importantly, these EGFP-positive H/HPC were able to be traced back to a population of cells that consistently exit from the anterior primitive streak region from as early as stage 4. In order to identify and study novel genes expressed and involved in the correct development and differentiation of the vertebrate H/HPC lineages, a differential screening using Affymetrix GeneChip system technologies was performed. Remarkably, this screening led to the identification of more than 200 new genes potentially expressed in these hematopoieses, angiogenesis or cardiogenesis precursor lineages. An in-depth study of the novel proteins CHEP33 and CHEP36 will be presented. By performing developmental, genetic, biochemical and functional studies in chick, *Xenopus* and mouse models we aim to unravel the roles and the mechanisms of these novel genes in early vertebrate heart/hemangioblast induction and organogenesis.

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Program/Abstract # 320
Endogenous alkaline phosphatase expression during developmental angiogenesis in chick embryo chorioallantoic membrane capillary plexus

Alejandro Gorustovich [1], Luis Haro [1], Gabriela Vargas [1], Rosa Vera [3]
[1] *Res. Lab., CNEA*
[2] *CONICET, Argentina*
[3] *Dept. Dev. Biol., Salta Natl. Univ., Salta, Argentina*

The chick embryo chorioallantoic membrane (CAM) capillary plexus provides an excellent model to study capillary formation *in vivo*. The development and structure of the CAM capillary plexus has been previously described. However, the histoenzymological contribution to the study of the CAM microvessels is lacking. In the present investigation, we have employed alkaline phosphatase (AP) enzyme histochemistry to study the CAM capillary plexus. Fertilized eggs (*Gallus sp*) were used. These were incubated *in ovo* at 37 °C with 60% relative humidity for 72 h. The *ex ovo* embryo development was carried out in a polystyrene container (4×7 cm, 0.2 mm thick) placed inside another high density polyethylene container (8.5×9 cm) containing 50 mL of distilled water. All culture chambers were maintained at 37 °C at saturation humidity in a standard laboratory incubator. Embryos were monitored every 24 h and staged. The embryos were killed at 7 and 12 days of total incubation. The CAM was removed entirely, and appropriate samples of the full thickness CAM were processed for histochemical staining of native AP using naphthol AS-BI phosphate as the substrate (BioGenex, USA) and examined by light microscopy. Endogenous AP activity has been localized in the microvessels of CAM capillary plexus both 7 and 12 days of embryonic development. This study present a reliable and simple method whereby en face evaluation of CAM capillary plexus, that is particularly useful in studying capillary growth patterns during developmental angiogenesis, as well as in response to both stimulation and inhibition of angiogenesis.

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Program/Abstract # 321
Embryonic vascular stabilization in zebrafish requires Pix/Pak and integrin pathways

Jing Liu, Sherri Fraser, Johannes Von Berg, Evvi-Lynn Rollins, Olivera Starovic-Subota, Sarah Childs
Department of Biochemistry and Molecular Biology, and Smooth Muscle Research Group, University of Calgary, Calgary AB, Canada T2N4N1

Little is known about the causative genes of developmental vascular malformation. We are studying a genetic mutant in zebrafish called bubblehead (*bbh*^{m292}). It is characterized by multiple hemorrhages in the brain at a predictable developmental stage. By positional cloning, we found a point mutation in bPix, a guanine nucleotide exchange factor, which results in a truncated bPix protein. Injection of low dose morpholino against bPix phenocopies the *bbh*^{m292}, while high dose morphants show a curled body axis, severe pericardial and ventricular swelling. According to previous work, Pak (p21-activated kinase) strongly associates with bPix. We hypothesized that Pak was likely to be involved in embryonic vascular stability. Through an expression screen of Pak genes in zebrafish, I found that Pak2 is expressed in the major trunk vessels (dorsal aorta and posterior cardinal vein) as well as in the head mesenchyme. Knockdown of Pak2 by morpholino leads to brain hemorrhage. Further, I have been able to rescue *bbh*^{m292} with a constitutively active Pak2 RNA, suggesting that these two genes function in the same pathway promoting vascular stability. According to mouse models, integrin av has been suggested to be a good candidate to stabilize the embryonic vasculature. Knockdown of integrin av by morpholino in *bbh*^{m292} background causes more intracerebral hemorrhages than in wild-type background ($p<0.05$). It suggests that integrin signaling is involved in Pix/Pak mediated vascular stabilization in zebrafish embryonic development.

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Program/Abstract # 322
Axial patterning in the polychaete annelid, *Capitella sp. I*

Kariena K. Dill, Elaine C. Seaver
Kewalo Marine Laboratory, Pacific Bioscience Research Center, University of Hawaii at Manoa, Honolulu, Hawaii, USA