Establishing left-right (LR) asymmetry is required for correct position and function of internal organs. Although remarkable progress has been made in understanding the molecular basis of LR establishment, how this molecular information is translated into morphogenetic events is unknown. To elucidate the cellular mechanisms that control asymmetric morphogenesis, we have analyzed the initial LR asymmetric morphogenesis of the early heart rudiment, a process called C-looping, in chick and mouse embryos. Our detailed analysis with time-lapse microscopy revealed that this process is achieved by two independent, asymmetric morphogenetic events: rightward rotation of the rostral portion of the primitive heart tube and asymmetric growth of its caudal portion. Interestingly, we found that cell shape and cell arrangements undergo similar changes during asymmetric morphogenesis of both parts. This finding suggests that common molecular and cellular mechanisms function to generate different LR asymmetric morphological features in the rostral and caudal regions of the looping heart tube. Based on our histological and genetic analyses, we will discuss what molecules and cell behaviors are regulated by LR signals and how common molecular machinery could control differential morphogenesis in these two regions of the heart tube. Our study sheds light on the mechanisms that enable LR signals to control complex morphogenesis.

doi:10.1016/j.ydbio.2010.05.121

Program/Abstract # 83
The KRAB Zinc Finger Protein ZFP568 is Required in the Extraembryonic Mesoderm for Yolk Sac and Placental Development
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In mammals, extraembryonic tissues are critical for sustaining embryonic life inside the uterus, providing nourishment and secreting factors to maintain pregnancy. chato, an ENU allele disrupting the mouse Kruppel-associated box (KRAB) zinc finger protein ZFP568, causes unique defects in the morphogenesis of extraembryonic tissues. The yolk sac of chato mutants contains bubble-like protrusions, accompanied by a detachment of the visceral endoderm from the underlying extraembryonic mesoderm, and defective extraembryonic mesoderm migration. Additionally, the placenta fails to develop properly in chato mutants. Most chato embryos have an expanded chorionic ectoderm that, in extreme cases, prevents the closure of the ectoplacental cavity. Also, development of the labyrinth layer of the placenta is disrupted in all chato mutants. Interestingly, we found that the severity of yolk sac defects correlated with the placental malformations, suggesting that all extraembryonic defects in chato mutants have a common developmental origin. To address the requirements of Zfp568 in different extraembryonic lineages, we analyzed chimeric embryos generated by both tetraploid complementation assays and by the use of a reversible allele of Zfp568 in combination with Cre lines. Our results indicate that ZFP568 is required in the extraembryonic mesoderm to regulate the development of the yolk sac and placenta. Results will be presented that support a previously undescribed role of the extraembryonic mesoderm in the morphogenesis of extraembryonic tissues.

doi:10.1016/j.ydbio.2010.05.122

Program/Abstract # 84
Live-imaging analysis of apoptosis and caspase activation reveals that apoptosis is a facilitator of the neural tube closure
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Neural tube closure (NTC) is a fundamental process to construct the brain and the spinal cord in vertebrates. The neural plate develops bilateral neural folds, which in turn come into contact in the midline and fuse together to create the neural tube. Programmed cell death (PCD), especially apoptosis, is widely observed during the process. Several lines of evidence suggested that apoptosis is important for NTC. However, it is still unclear how apoptosis contributes to the completion of NTC. To examine the relationship between dying cells and morphogenetic movement such as neural plate bending, fusion of neural ridge, and epithelial remodeling after the fusion, direct visualization of dying cells during NTC could be effective. We have conducted a live-imaging analysis of apoptosis during NTC with the mouse embryo expressing SCAT3, a fluorescent indicator protein to monitor caspase-3 activation in living cells. In these embryos, caspase activation was observed in dying cells of the pre-fusion neural ridge, fusion point, and the midline. Thus, this system allowed us to observe the dynamics of NTC when caspase activation and apoptosis were inhibited. We found that the speed of closure tended to become slow under caspase-inhibited condition, suggesting that caspase activation and apoptosis contribute to the progression of NTC within a limited developmental time window.

doi:10.1016/j.ydbio.2010.05.123

Program/Abstract # 85
Apoptosis is dispensable for the control of cell number during early brain morphogenesis
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Apoptosis is essential for normal brain morphogenesis. Mice lacking genes required for apoptosis (caspase-3, caspase-9, and apaf-1) exhibit severe brain malformations such as small brain ventricles and folded neuroepithelium. Because these morphological changes led to the idea that the supernumerary cells are generated in these mutants’ brain, it has been thought that apoptosis is essential to control the brain cell number. However, quantitative changes in total cell number of these mutants’ brain have not been investigated. So, we performed quantitative morphological analysis of apaf-1 mutants’ brain. We measured the area of neuroepithelium and the cell density in serial sections and counted the total cell numbers of dissociated brain tissue. To our surprise, there was no detectable increase in the cell number of apaf-1 mutants’ brain, even when the typical brain malformations were apparent. The influence of apoptosis inhibition on the brain cell number in apaf-1 mutant embryos might be diminished by some other cell number-controlling mechanisms, such as alternative cell death programs or suppression of cell proliferation. In contrast, apaf-1 mutant embryos failed to expand its brain ventricles, and this may be the cause of neuroepithelial deformations. Completion of neural tube closure is thought to be required for the drastic brain ventricles expansion. Indeed, apaf-1 mutant embryos exhibited neural tube closure defects, suggesting that the primary
The cerebellum is derived from dorsal rhombomere 1 in the embryo. Previous studies have demonstrated the importance of FGF signals for the maintenance and patterning of r1 in the early embryo. Three Sprouty genes (Spry1, Spry2 and Spry4), which encode feedback antagonists of FGF signalling, are expressed in the mid-hindbrain (MH) organiser and MH-specific Spry1;2 and Spry1;2;4 knockout embryos exhibit enlarged cerebellar primordia (r1) due to increased FGF signalling. We also observed FGF and Sprouty gene expression during later stages of cerebellar development, implying roles for these genes in precursors of various cerebellar cell types such as Bergmann glia, granule and Purkinje neurons. In support of this idea, MH-specific Spry1;2 and Spry1;2;4 knockout cerebella analysed at postnatal day (P)21 have fewer granule neurons and abnormal foliation. The expression of FGF target genes, Pea3 and Erm are increased, and SHH target genes, Gli1 and Ptc1 are decreased in P7 mutant cerebella. The phenotype of Sprouty mutant can be rescued by reducing FGF receptor 1 and Ptc1 gene dosage, providing genetic proof that the cerebellar defects in Sprouty knockouts are due to deregulated FGF and SHH signalling. Cell type-specific conditional gene knockout experiments suggest that Sprouty gene function is required in several cerebellar cell types during development.

doi:10.1016/j.ydbio.2010.05.125

Program/Abstract # 87

LRP2/megalin is required for the FGF-dependent expansion of the basal telencephalon

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Loss of endocytic receptor LRP2/megalin in mice impairs specification of the developing CNS, leading to holoprosencephaly. This phenotype results from impaired morphogen signalling in the early rostral forebrain organizer indicating an important function for LRP2 in neureulation (see abstract by Annabel Christ). Since expression of Lrp2 persists throughout embryonic development, we wanted to test whether the receptor may also have additional functions in later stages of brain formation. Employing Foxg1 CRE-mediated conditional recombination, we generated mice lacking LRP2 in the telencephalon from embryonic day 9.5 onwards. Conditional Lrp2 mutants showed a reduced thickness of the basal telencephalon and an expansion of the telencephalic vesicles. We did not observe loss of distinct differentiated cell types but an overall reduced proliferation in the ventral telencephalon. The rate of apoptosis was unchanged. Remarkably, loss of LRP2 after neureulation in the embryonic telencephalon did not affect the SHH or BMP4 pathways, which are severely compromised in constitutive Lrp2 mutants. Rather, conditional LRP2 deficiency specifically exhibited dorsal expansion of Fgfr8 and Fgf17 gene expression domains in the rostral forebrain. FGF signalling has been previously implicated in the regulation of proliferation in rostroventral telencephalon. We thus identified a late developmental role for LRP2 in maintaining proliferation and correct expansion of the basal telencephalon, likely mediated by FGF signalling.

doi:10.1016/j.ydbio.2010.05.126

Program/Abstract # 88

Disruption of Apaf1 leads to defective craniofacial development in the mouse embryo

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Using a mouse strain isolated during an ethyl-nitrosourea (ENU) screen for recessive point mutations affecting midgestation morphology, we identified a role for apoptotic peptidase activating factor 1 (Apaf1) during craniofacial development. Apaf1 is a key component of the mitochondria-mediated apoptotic pathway, functioning upstream of caspases 9 and 3. Interestingly, mice lacking caspase 9 or 3 do not exhibit craniofacial defects in their phenotypes, demonstrating a novel contribution of Apaf1 to facial development. Twenty percent of our mutant mice show craniofacial abnormalities (wide frontonasal prominence, cleft face, short/broad nasal region) by embryonic day 12.5. We observe these defects as early as embryonic day 10; they vary in severity, and persist until birth – at which point we see skull defects (in the frontal, nasal, and maxilla/premaxilla bones). Our mutant allele is due to a T to C transition in exon 8 of Apaf1, resulting in a proline substitution at a highly conserved leucine (L375P). We found this is a critical residue as protein levels are unaffected in our mutants. Furthermore, our Apaf1 allele phenocopies targeted Apaf1-null alleles and fails to complement a spontaneous mutant that is functionally deficient in Apaf1. Taken together our analyses of this novel allele of Apaf1 identify a previously unrecognized residue that is important for Apaf1 function, and provide evidence for a caspase-independent role of Apaf1.

doi:10.1016/j.ydbio.2010.05.127

Program/Abstract # 89

ABSTRACT WITHDRAWN

doip:10.1016/j.ydbio.2010.05.128

Program/Abstract # 90

Aglossia in Hand2 conditional knockout mutants results from misregulation of Dlx5/6

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The development of the lower jaw is regulated spatiotemporally by signaling cascades, and is refined through both permissive and inhibitory signals. We have shown that endothelin-A receptor (Ednra) signaling is a central regulator of lower jaw identity, establishing the identity of cranial neural crest cells (NCCs) in the mandibular arch through a mechanism involving Dlx5 and Dlx6