results in a complete failure of proximal/distal mandibular arch patterning.

doi:10.1016/j.ydbio.2011.05.291

Program/Abstract # 335
Novel effects of folinic acid and folate supplementation on locomotor development in embryonic Zebrafish, *Danio rerio*
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Periconceptional folate supplementation is suggested to help prevent neural tube defects during early embryonic development as neural crest cells migrate and differentiate. Research has shown that the developing nervous tissue is exquisitely sensitive to endogenous and exogenous chemical signals and that these signals must be interpreted by the developing embryo in a specific temporal, spatial and dose dependent manner. The purpose of the current study was to examine the effect of folate and folinic acid on embryonic development in Zebrafish, *Danio rerio*. After fertilization (0 dpf), embryos were exposed to either 1 mM folate or 10 mM folinic acid for a period of 48 h. After 48 h, embryos were reared using standard procedures and examined everyday for a period of one week. Anatomical and behavioral measures were taken at 2 dpf, 3 dpf, and 5 dpf. Zebrafish exposed to either folate or folinic acid hatched early and showed acceleration in the development of locomotor systems. Consistent with advanced motor system function, exposed Zebrafish were found to have a higher level of swimming activity compared to controls. There was also a change in the startle response, with exposed embryos showing a hypersensitivity to external stimuli. Thus, exposure to either folate or folinic acid accelerates development, increases locomotor activity, and increases anxiety. The results from this study establish the Zebrafish as an excellent model system to examine the role of folate during embryonic development. The effect of folate exposure on embryonic Zebrafish is similar to what has been demonstrated in other species and adds further evidence that folate plays an important role in embryonic development.

doi:10.1016/j.ydbio.2011.05.292

Program/Abstract # 336
Endomesoderm segregation involves cross talk between Notch and Wnt pathways through multiple intersecting regulatory circuits
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Formation of primary germ layers restricts the developmental capacities of cells in early embryos. While this has been studied extensively, germ layer segregation is poorly understood. Using sea urchin embryos, we show that after simultaneous activation of endoderm and mesoderm gene regulatory networks (GRNs) in the same blastomeres, these GRNs segregate in their progeny in response to cross talk between Notch and canonical Wnt (cWnt) signaling through a series of intersecting regulatory circuits. As in other deuterostomes, anisotropic cWnt signaling is required in the sea urchin embryo for endoderm specification in vegetal cells. Also in response to cWnt, micromere descendants at the vegetal pole activate the nonskeletogenic mesoderm (NSM) gene regulatory network (GRN) through a Notch signal to adjacent cells, where the cWnt-dependent endoderm GRN operates. After these endomesoderm (EM) precursors divide, their vegetal progeny, the NSM precursors, continue to receive the Notch signal from the micromeres, which represses operation of a key Hox11/13b-dependent endoderm circuit in them. In contrast, each factor of this circuit, which remains active in descendants that no longer transduce the Notch signal, is essential for endoderm fate. Subsequently, Hox11/13b and its target, Brachyury, maintain cWnt activity in the endoderm through Wnt1 transcription, which reinforces the endoderm GRN. Later, Notch also promotes export of TCF out of NSM nuclei, further insulating this territory from cWnt signals. Thus Notch initiates EM segregation by repressing an endoderm circuit in the NSM and reinforces it by establishing distinct cWnt signaling environments: high cWnt activity in the endoderm and low cWnt function in the NSM.

doi:10.1016/j.ydbio.2011.05.293

Program/Abstract # 337
Elucidating the molecular mechanisms underlying cell movements in the Visceral Endoderm
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The Anterior Visceral Endoderm (AVE) is a specialised group of cells in the simple epithelium of the Visceral Endoderm (VE). Stereotypic migratory movement of the AVE is responsible for properly orienting the anterior–posterior axis of the embryo. AVE cells migrate by intercalation within the VE, which retains epithelial integrity. Using time-lapse microscopy, we have demonstrated that regional differences in cell behaviour in the VE regulate AVE migration. Using 3D imaging of protein localisation patterns and various mutants with AVE migration defects, we have shown that signalling through the Wnt–PCP pathway is required for the proper migration of AVE cells. Moreover, the TGF-β family member Nodal is required for PCP signalling in the VE1. The mechanisms by which Nodal controls PCP signalling and influences the migratory behaviour of cells in the VE have yet to be established. We are using time-lapse microscopy of various mutants with AVE migration defects to visualise the entire VE, in order to address how these genes might influence AVE migration by altering the cellular behaviour of the VE in general. We have found that mutants for Lefty1 (an inhibitor of Nodal) show an abnormal reduction in the regional differences in VE cell motility and morphometrics. We are also using atomic force microscopy to probe the physical characteristics of VE cells in regions showing differences in behaviour. To address how Nodal signalling may regulate PCP signalling we are using confocal microscopy to assess the sub-cellular and regional differences in localisation of key down-stream effectors in Nodal pathway mutants and embryos cultured in pharmacological inhibitors of the Nodal receptor. Results from these experiments will be presented.

doi:10.1016/j.ydbio.2011.05.294

Program/Abstract # 338
Understanding inter-strain differences in pre-implantation mouse development
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By implantation, the mouse embryo forms three primary lineages: the trophectoderm, the primitive endoderm (PE) and the epiblast (EPI). The PE and EPI are derived from the inner-cell mass (ICM) of the E3.5 blastocyst, which is used for ES cell derivation and gives rise to extra-embryonic tissues and the embryo, respectively. While much is known regarding what factors are involved in specification and maintenance of these three lineages, it is unknown whether there is variability in these processes among different mouse strains. Two lines of evidence lead us to hypothesize that there are differences between mouse strains in these

early stages. First, some mouse strains, such as 129 Sv, are more permissive to ES cell derivation compared to others. Second, when generating host/ES cell chimeras, certain mouse strain combinations are significantly more successful than others. We propose that differences in the responsiveness to certain developmental cues may influence the timing or manner of lineage specification, thus causing inter-strain variance. To test this, we study mouse strains (129 Sv, C57Bl/6 and CD1) often used for derivation of ES cell lines and generation of host-ES chimeras. First, we grossly observe embryo division dynamics using time-lapse imaging. Second, we compare the expression of lineage-specific markers. Finally, we ask whether there is variable sensitivity to FGF signaling, which is required for specification of the PE and EPI lineages of the ICM. Our observations will not only help us understand what processes of pre-implantation development are variable between mouse strains, but may also provide strategies for the use of an improved variety of mouse strains in genetic engineering applications.

doi:10.1016/j.ydbio.2011.05.295

Program/Abstract # 339
Investigating the role of the Hippo pathway member Nf2 in trophectoderm/inner cell mass specification

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The first lineage decision that takes place during mouse preimplantation development is the establishment of the trophectoderm (TE) and inner cell mass (ICM) lineages, which are distinct by the early blastocyst stage. The kinases Lats1 and 2 (Lats1/2) and the transcriptional co-activator Yap play a key role in TE/ICM segregation by regulating the expression of the TE-specific transcription factor Cdx2. In outside cells of the preimplantation embryo, Yap accumulates in the nucleus, where it induces Cdx2 expression. In inside cells, Yap is phosphorylated by active Lats1/2 and retained the cytoplasm, preventing Cdx2 expression. It is not clear how Lats1/2 activity is regulated during preimplantation development. Notably, Lats1/2 and Yap are members of the Hippo signaling cascade, suggesting that upstream components of this pathway could regulate their activity in the embryo. Here, we demonstrate that the upstream Hippo signaling component Nf2 is expressed throughout preimplantation development. Additionally, injection of a dominant negative (DN) form of Nf2 cell-autonomously increases nuclear accumulation of Yap and decreases Yap phosphorylation in inside cells of the embryo. DN Nf2 injection also increases Cdx2 expression, indicating that this nuclear-localized Yap is transcriptionally active. Finally, consistent with a role upstream of Lats1/2, the effects of DN Nf2 injection can be rescued by over-expression of wild-type Lats2. This work suggests a novel role for Nf2 in the process of TE/ICM specification during mouse preimplantation development.

doi:10.1016/j.ydbio.2011.05.296

Program/Abstract # 340
Transforming growth factor-beta-related signaling in blastocyst morphogenesis

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Studies of mouse preimplantation development using embryos cultured in vitro establish the importance of autocrine/paracrine growth regulatory pathways in controlling preimplantation development. Signaling molecules of the Transforming Growth Factor beta (TGF-β) superfamily are potent regulators of development and tissue homeostasis. Smad4 is a central mediator of the TGF-beta-related signaling and is important for numerous processes including cellular growth, differentiation, migration, and extracellular matrix production. Mice lacking Smad4 die around peri-gastrulation period due to defects in epiblast proliferation, mesoderm formation and extraembryonic development. Maternal gene products play key roles in shaping the earliest developmental programs in a wide variety of organisms. Relatively little is known about the functions of mammalian maternal gene products. Maternal Smad4 gene products are abundant in unfertilized eggs as well as cleaving blastomeres of preimplantation mouse embryos. We have conditionally inactivated Smad4 in the female germline. Eggs depleted of maternal Smad4 gene products complete meiosis and are fertilized normally. However, preimplantation development is severely compromised in embryos derived from such eggs even when they are fertilized by wild type sperms, demonstrating the importance of maternally derived Smad4 gene products in early mouse development. The consequences of disrupting maternal TGF-β related signaling on blastocyst morphogenesis, including blastomere cleavage, cell polarity establishment and lineage segregation, will be presented.

doi:10.1016/j.ydbio.2011.05.297

Program/Abstract # 341
Wnt8a is a target of miR430 post-transcriptional regulation

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Wnt/β-catenin signaling in early vertebrate embryos patterns the D/V and A/P axes, and Wnt8a is one of the earliest acting genes that interconnects both D/V and A/P patterning. In the Zebrafish, Wnt8a, the earliest zygotically expressed Wnt gene, acts over broad domains to regulate D/V and A/P patterning of the mesoderm and ectoderm. Wnt8a acts in two phases to regulate different developmental choices. In early gastrula stages, Wnt8a signaling prevents expansion of the Dorsal Organizer and induces posterior neural plate fates. During mid to late gastrulation, Wnt signaling promotes growth of the posterior embryo. Because of its critical role during axis patterning, Wnt8a regulation is likely to occur at several levels, including post-transcriptionally. We have used a combination of transgenic and transient sensor assays and target protector morpholinos in the Zebrafish to test the hypothesis that post-transcriptional regulation of Wnt8a occurs through the action of microRNAs. Our results show that Wnt8a is a target of several microRNAs during early development, a major regulator being miR430. We identified two miR430 binding sites in Wnt8a UTR elements, and these sites are necessary and sufficient for Wnt8a post-transcriptional regulation. Protecting Wnt8a transcripts from miR430 regulation results in elevated Wnt8a expression and embryo patterning defects. Thus, microRNA-dependent regulation of Wnt8a is crucial to normal embryonic axis patterning.

doi:10.1016/j.ydbio.2011.05.298

Program/Abstract # 342
A dorsalized and cell migration maternal effect mutant in Zebrafish

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