differentiated endoderm cells sorted circumferentially while the undifferentiated F9 cells remained predominantly internal. This indicates that the acquisition of a surface position is an intrinsic property of endoderm epithelia. Disabled-2 (Dab2), an endocytic adaptor protein that mediates the directional transport of clathrin-coated cargos, is required for the spontaneous surface sorting and positioning of the endoderm cells. When Dab2 expression was compromised, the differentiated F9 cells no longer localized correctly and were distributed throughout the interior of the EBs. These results support a model where primitive endoderm cells are first formed within the interior of the inner cell mass of the preimplantation mouse blastocyst and are subsequently sorted to the surface by a Dab2-dependent mechanism. We propose that the autonomous property of epithelial cells to generate polarity is the factor responsible for surface positioning of epithelia.

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Program/Abstract # 308

Thyrotropin-releasing hormone precursor—A novel marker of the mouse definitive endoderm

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Gastrulation is one of the most critical events of embryogenesis, generating the three primary germ layers (endoderm, mesoderm and ectoderm) that will give rise to the tissues of the developing embryo. Of the germ layers the least is known about the definitive endoderm (DE), which gives rise to the lungs, digestive tract, liver and pancreas. This is due in large part to the lack of genetic markers specific for the DE as many of the current markers, including *Cerl*, *Foxa2* and *Sox17*, are also expressed in the visceral endoderm (VE), an extraembryonic tissue. Using Affymetrix GeneChips and Serial Analysis of Gene Expression (SAGE) we have identified a novel marker of the mouse DE—*Thyrotropin-releasing hormone precursor* (*Trh*). We have characterized the expression of *Trh* throughout mouse gastrulation and early organogenesis stages using whole mount *in situ* hybridization. Our expression data shows that *Trh* is expressed in newly formed DE cells and is subsequently expressed in the entire DE before becoming downregulated as the DE is patterned. The dynamic expression pattern of *Trh* is in accordance with recent fate mapping experiments detailing the movement of the DE during gastrulation. Preliminary experiments suggest that *Trh* is absent from the VE, being expressed in a mutually exclusive pattern with *Pem* (a marker of the extraembryonic visceral endoderm). These results point to *Trh* being an exclusive DE marker.

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Program/Abstract # 309

FoxD3 regulation of mesoderm induction in the zebrafish embryo

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Nodal ligands are required for germ layer induction in vertebrates. During zebrafish gastrulation, the expression domain of the Nodal-related genes, Cyclops (Cyc) and Squint (Sqt), overlaps that of FoxD3 in the shield, suggesting a possible role for FoxD3 in mesoderm development. Overexpression of FoxD3 results in expansion of Cyc expression and dorsal mesoderm markers. Knockdown results in reduced expression of these markers and 24-h embryos show a phenotype similar to Nodal pathway mutants. To determine the functional interaction of FoxD3 with the Nodal pathway we examined Antivin-overexpressing and MZoep mutant embryos. FoxD3 does not rescue or induce ectopic mesoderm indicating that FoxD3 is dependent on a functional Nodal pathway for dorsal mesoderm induction. A FoxD3 mutant, Sym1, where the mutation inactivates the FoxD3 gene has been reported. The phenotype shows craniofacial defects and delayed/reduced development of chromatophores. From our results and our model for FoxD3 activity we predict early gastrulation deficiencies and defects in tissues derived from dorsal mesoderm. Our preliminary results indicate that the sym1 protein retains partial function as its overexpression induces Cyc and dorsal mesoderm markers. Future work will examine sym1 embryos for unappreciated defects in mesodermal gene expression and axial development. Results suggest that dorsal mesoderm induction is regulated, at least in part, by FoxD3. We hypothesize that FoxD3 regulates Nodal expression in the zebrafish shield by repressing a negative regulator of Nodal expression, thus indirectly promoting Nodal expression and mesoderm development.

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Program/Abstract # 310

Genetic analysis of Fgf gene function in the limb

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The tetrapod limb emerges from the flank of the embryo as a bud of mesenchyme encased in an epithelial hull. Substantial growth and differentiation of this bud gives rise to a scaffold of skeletal elements that can vary among species in their size, number and shape. At the distal edge of the bud the apical ectodermal ridge (AER), produces signals essential for limb development. In the mouse, four *Fgf* genes, *Fgf4*, *Fgf8*, *Fgf9* and *Fgf17*, are expressed specifically in the AER and may be the critical genes that provide these essential signals. Using a genetic