Bmp2 caused embryonic lethality at midgestation with defects in head, heart and extra-embryonic mesoderm. We identify and describe here a previously uncharacterized anterior morphogenesis defect of *Bmp2*−/− embryos. The head, heart and foregut tissues are specified properly, but their spatial relationships and thus the morphogenesis of the embryo are perturbed. Using tissue specific ablation we show that *Bmp2* in the epiblast lineage is necessary and sufficient to rescue the extra-embryonic mesoderm defect, and *Bmp2* in the visceral endoderm is necessary and sufficient to rescue the anterior morphogenesis defect. Together with the Bmpr1a phenotype, these findings potentially identify a signaling cascade wherein Bmp2 in the visceral endoderm signals to its receptor in the epiblast to direct anterior morphogenesis during mouse gastrulation.

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Program/Abstract # 119
Cell behaviors during endoderm morphogenesis in the mouse gastrula

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We have used live imaging to investigate the cell behaviors underlying endoderm morphogenesis during mouse gastrulation. Our observations lead us to propose a model of egression-mediated cell dispersal whereby the emerging epiblast-derived definitive endoderm (DE) intercalates with the pre-existing visceral endoderm (VE) epithelium and causes its dispersal in the distal region of the embryo overlying the epiblast. Mechanistically, this phenomenon can be explained by elevated rates of cell proliferation in the epiblast compared to the VE. A rapidly proliferating epiblast could promote an egression of epiblast-derived cells into the overlying VE on the surface of the embryo. Once egressed, rapid division of these cells leads to further dispersal and dilution of VE cells and an increase in the surface area required to accommodate the rapid growth of the embryo. We have used digital quantification to corroborate the sequential steps of our model. Furthermore, we have investigated the molecular identity and cell behavior of both VE-derived and epiblast-derived DE cells overlying the epiblast in wild type embryos and in mutants.

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Program/Abstract # 120
From the tilted mouse to the otopetrin gene family: Molecular insights into the development of the vestibular mechanosensory system

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Otoconia are complex calcium carbonate biominerals that are required for the sensation of gravity. Degeneration, displacement or ectopic formation of otoconia results in benign paroxysmal vertigo (BPV). In addition, aminoglycoside antibiotics can disrupt otoconial structure and function. Despite the prevalence of balance disorders, little is known about the mechanisms regulating the development and pathology of the vestibular mechanosensory apparatus. *Tilted* mice have a severe balance disorder due to the congenital absence of otoconia. By positional cloning we identified missense mutations in *Otopetrin 1* (*Otop1*) as the genetic etiology of the *tilted* phenotype. *Otop1* encodes a multi-transmembrane domain protein required for the initiation of extracellular biomineralization. To establish a null allele and to mark cells expressing *Otop1*, we have generated a LacZ knockin allele that inactivates all splice forms of *Otop1*. Using $Otop1^{lacZ}$ mice we demonstrate specific expression of *Otop1* in the macular epithelium and altered protein subcellular localization in *tilted* mice. Biochemical analysis demonstrates that *Otop1* can modulate purinergic mediated Ca^{2+} homeostasis and defines a unique set of biochemical activities including specific inhibition of the purinergic receptor, P2Y, and regulation of the influx of extracellular Ca^{2+} in response to ATP, ADP and UDP. Together, these studies demonstrate a direct role for Otop1 in the formation and growth of otoconia and the potential to regulate other biomineralization processes.

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Program/Abstract # 121
The Ig superfamily protein Lrig3 controls inner ear morphogenesis by regulating Netrin-1 expression

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Proper morphogenesis of the three inner ear vestibular canals is essential for proper balance. Canal development begins when defined regions of otic vesicle epithelium meet to form the fusion plate. In the fusion plate, the basal lamina breaks down as epithelial cells intercalate to form a single layer of cells. Fusion plate cells eventually disappear and the region is filled with mesenchyme, forming a canal out of an initial pouch-like structure. Netrin-1 is secreted from fusion plate cells and promotes basal lamina breakdown, permitting epithelial–mesenchymal interactions known to drive fusion plate formation for each of the three canals. Therefore, when and where Netrin-1 is produced fundamentally affects the complex structure of the vestibular apparatus. We report that novel protein Lrig3 controls the timing and extent of lateral canal fusion by regulating the expression of *Netrin-1*. *Lrig3* is present in non-fusing epithelium of the otic vesicle, complementary to *Netrin-1* in the fusion plate. In *Lrig3* mutant mice fusion is premature and expanded, resulting in lateral canal truncation and circling behavior in adult mice. The fusion defect is accompanied by ectopic breakdown of the basal lamina and expansion of *Netrin-1* expression. The canal truncation is rescued when one copy of the *Netrin-1* gene is removed, confirming that the *Lrig3* mutant phenotype is a result of increased levels of Netrin-1. Lrig3 is a transmembrane protein with leucine-rich repeats and Ig-motifs in the extracellular region and a short cytoplasmic tail. Current studies are aimed at uncovering the molecular functions of Lrig3.

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