During gastrulation, epiblast cells undergo epithelial to mesenchymal transition (EMT) as they delaminate and ingress through the primitive streak to form the definitive endoderm and mesoderm. The dissociation of tight junctions between cells of the epiblast is essential for EMT to occur. Tight junctions are localized to the apical pole of epithelial cells and the claudin component of the tight junction is responsible for determining the ion and size selectivity of the junction. We are examining the expression and function of the claudin family of integral tight junction proteins during chick gastrulation. We found that 14 claudins exhibit unique and overlapping expression patterns during gastrulation. From these expression analyses, we have identified a novel molecule, Claudin-10, that plays a role in directing asymmetric organ positioning in the chick. We report that Claudin-10 mRNA is asymmetrically expressed on the right side of Hensen's node, the site where the asymmetric gene expression is first observed, and is maintained throughout gastrulation. We demonstrate that overexpression of Claudin-10 on the left side of the node significantly randomizes the direction of heart-looping, the earliest morphological sign of disrupted left–right patterning, and disrupts normal expression of Pitx2. In addition, morpholino knock-down of Claudin-10 results in a significant randomization of heart-looping. We are currently examining the effects of manipulating Claudin-10 expression on other classical left–right patterning genes, as well as understanding how asymmetric Claudin-10 expression is regulated, and identifying the functional domains necessary for the role that Claudin-10 plays during left–right patterning. These data suggest that asymmetric expression of Claudin-10 is required for normal left–right patterning, perhaps through regulation of the permeability of the ions that have been proposed to be involved in the initial symmetry-breaking event that occurs at Hensen's node.

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Program/Abstract # 358
Detection of dynamic fucosylation at cellular level during Zebrafish development

Lei Feng[a], Hao Jiang[b], Tianqing Zheng[b], Peng Wu[b]
[a]*Albert Einstein College of Medicine Department of Biochemistry, Bronx, NY, USA*
[b]*Albert Einstein College of Medicine, Bronx, NY, USA*

Protein fucosylation plays critical roles in neural development and function. However, due to the non-template nature of glycosylation and lack of effective detection methods, little is known about the molecular details of proteins fucosylation during development. Here, we report a new detection method based on bioorthogonal chemistry for in vivo characterization of the molecular and cellular function of fucosylation in Zebrafish embryos. In this strategy, an alkyne-bearing analog of GDP-fucose, the universal fucosyl donor, is first introduced into Zebrafish embryos by micro-injection. Then the alkyne-tagged fucosides on the cell surface is treated with a complementary azido probe, which allows the imaging or enrichment of the labeled glycoproteins. Using this approach, we successfully visualized fucosylated glycans in the enveloping layer of live Zebrafish embryos and the inner organs of fixed embryos. Our study provided the first sketch of the spatiotemporal expression of fucosylation in developing Zebrafish, which is probably linked to the specific distribution of fucosylation enzymes during development. The chemical tools reported here can be directly applied to study sectors of the glycome and be generalized for dynamic in vivo imaging or profiling of other bio-molecules in living systems.

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Program/Abstract # 359
Loss of mouse Porcupine homolog recapitulates multiple embryonic Wnt signaling defects

Steffen Biechele[a], Brian Cox[b], Janet Rossant[b]
[a]*Sickkids Research Institute Developmental & Stem Cell Biology, Toronto, ON, Canada*
[b]*The Hospital for Sick Children Research Institute, Toronto, ON, Canada*

In mammals, 19 Wnt ligands activate canonical and non-canonical pathways, which play important roles in development and disease. Biochemical evidence shows that the X-chromosomal gene Porcupine homolog (Porcn) is required for lipid modification of several, if not all, Wnt ligands, which is essential for Wnt secretion or function. We hypothesized an embryonic requirement for Porcn based on its important function for Wnt secretion and the embryonic lethality of several single Wnt mutants. Here we present evidence for the requirement for Porcn and Wnt signaling during mouse embryonic development. Using embryonic stem (ES) cells carrying a null allele, we confirmed in Luciferase reporter assays that Porcn is exclusively required in the Wnt ligand secreting cell and in vitro differentiation as embryoid bodies further demonstrates a requirement for Porcn in the generation of Flk1+ mesoderm and CXCR4+ endoderm. In vivo, aggregation of Porcn null ES cells with wildtype embryos as well as zygotic deletion of a conditional Porcn allele is lethal in hemizygous males due to gastrulation defects. Morphology, marker gene and canonical Wnt reporter expression are reminiscent of the earliest single Wnt knock-out phenotype (Wnt3). Female heterozygous embryos carrying a deletion of the maternal Porcn allele have functionally mutant extra-embryonic tissues due to imprinted X chromosome inactivation. These embryos display defects in chorio-allantoic fusion consistent with the previously described requirement for Wnt7b in the extra-embryonic chorion. Our data demonstrate a critical role for Porcn in mouse embryonic development and in the functions of Wnt3 and Wnt7b, but do not exclude a role for other Wnts. The generated conditional Porcn allele is thus a tool with the potential to abrogate all Wnt ligand secretion, thereby allowing for a better understanding of Wnt ligand redundancy in development and disease.

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Program/Abstract # 360
The P4 ATPase TAT-5 prevents the budding of extracellular vesicles and phosphatidylethanolamine exposure during *Caenorhabditis elegans* embryogenesis

Ann M. Wehman[a], Barth Grant[b], Jeremy Nance[c]
[a]*NYUMC, Skirball Developmental Genetics, New York, NY, USA*
[b]*Piscataway, NJ, USA*
[c]*NYU School of Medicine, Skirball Institute, New York, NY, USA*

During cytokinesis and morphogenesis, embryos undergo dramatic changes in cell shape. While much is known about the role of the cytoskeleton, we understand less about the role of the lipid bilayer in modulating cell shape. For example, the asymmetric partitioning of phosphatidylserine (PS) and phosphatidylethanolamine (PE) to one leaflet of the bilayer can affect membrane curvature and influence dynamic membrane events such as cytokinesis and endocytosis. We identified TAT-5, a P4 ATPase predicted to flip phospholipids to the cytoplasmic leaflet, in an RNAi screen for regulators of contact-induced polarity in *Caenorhabditis elegans*. Loss of TAT-5 results in defects in cell shape, adhesion, cytokinesis, and morphogenesis. GFP-tagged TAT-5 localized to the plasma membrane and TAT-5 prevented the externalization of PE, but not PS, on the surface of cells. We used electron tomography to examine the 3D structure of the plasma membrane at