inversion in both cases is inferred and has never been shown directly. We have used 3D reconstructions and cell tracing in chick embryos to show that the cardiogenic mesoderm is organized such that lateral-most cells are incorporated into the cardiac inflow (atria and left ventricle) while medially placed cells are incorporated into the cardiac outflow (right ventricle and outflow tract). This happens because the cardiogenic mesoderm is inverted concomitant with movement of the anterior intestinal portal caudomedially to form the foregut pocket. The bilateral cranial cardiogenic fields fold medially and ventrally and fuse. After folding the seam made by ventral fusion will become the greater curvature of the heart loop. The caudal border of the cardiogenic mesoderm which ends up dorsally coincides with the inner curvature. Physical ablation of selected areas of the cardiogenic mesoderm based on this new fate map confirmed these results and, in addition, showed that the right and left atria arise from the right and left heart fields. These findings provide a unified concept of heart fields and heart tube formation for avians and mammals.

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Program/Abstract # 387
The function of the mammalian Pumilio gene, *Pum1*, in early embryonic development of mice

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The mammalian Pumilio genes are members of a conserved family of RNA-binding proteins called the Puf protein family, which act as translational repressors. Puf proteins are characterized by their consensus RNA binding motif; the Pum-homology domain (Pum-HD). They are key regulators in stem cell and germ cell maintenance in diverse organisms from invertebrates to vertebrates. In this study we explore the role of mammalian Pumilio1, which has been identified in mice and humans. However, its function remains unresolved. Our initial characterization demonstrates that *Pum1* is widely expressed in human and mice tissues and in ES cells. To further investigate the role of *Pum1* in mammals, we used a gene-trap strategy to generate mice that lack a functional Pum-HD and express lacZ under the endogenous control of *Pum1*. Loss of *Pum1* leads to preimplantational embryonic lethality. However, heterozygous mutant mice are viable and fertile. Therefore, our results suggest that at least one copy of *Pum1* is essential for early embryonic development in mice. Future directions will elucidate the mechanism behind the early embryonic lethality.

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Program/Abstract # 388
Tcf3 regulation of pluripotency for lineage commitment during gastrulation

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The primary germ layers of the mammalian embryo are specified from pluripotent epiblast cells during gastrulation. While considerable effort has been dedicated to elucidating the patterning signals mediating lineage commitment, the mechanisms regulating the pluripotency of epiblast cells remain poorly defined. We propose that cell intrinsic mechanisms exert tight control over transcriptional circuits of pluripotency to allow for rapid and appropriate differentiation during gastrulation. Recent discoveries have revealed a network of transcription factors (Nanog, Oct4, Sox2) required for self renewal of pluripotent embryonic stem cells (ESC) in vitro, and for expansion of the precursors of the epiblast. Tcf3, a transcription factor required for correct gastrulation, has been identified as a limiter of this pluripotency network that acts by repressing Nanog expression in ESC. To determine whether a similar network controls pluripotency within the epiblast, we examined the function of Tcf3 in gastrulating mouse embryos. We show that Tcf3 is required to restrict Nanog expression to the site of primitive streak formation. Furthermore, the ectopic expression of Nanog in Tcf3−/− embryos coincides with delayed or defective processes of lineage commitment as determined by altered expression patterns of Brachyury, Sox1 and Otx2. Using novel Tcf3ΔN knock-in mice, we show that Tcf3 is required to act as a transcriptional repressor independently of Beta-catenin. These findings provide new insight into the importance of negative regulation of pluripotency for lineage commitment during development and suggest new mechanisms for the patterning of the embryo during gastrulation.

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Program/Abstract # 389
Role of paracrine Furin activity during gastrulation

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Axis and germ layer formation in vertebrates are orchestrated by the secreted subtilisin-like proprotein convertases (SPC) Furin and PACE4. Genetic evidence in the mouse suggested that Furin and PACE4 are provided by the extraembryonic ectoderm (ExE) to activate the Nodal precursor in adjacent epiblast during gastrulation, but soluble forms of these proteases and their distribution have never been directly observed in vivo. In addition, we hypothesized that Nodal signaling may be stimulated already after implantation by an early wave of transient Furin expression in the visceral endoderm (Mesnard et al., 2005), possibly to achieve maximal Nodal signal duration (BenHaim et al., 2005). To visualize ExE-derived Furin, and to monitor its effect on Nodal signaling, we expressed a Furin-GFP transgene in the ExE of wild-type and Furin−/−;Pace4−/− double mutant embryos. We show that GFP-tagged Furin suppresses the precocious neural differentiation in Furin−/−;Pace4−/− double mutant embryos, and stimulates Nodal signaling.

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Program/Abstract # 390
Role of Rac1 in the regulation of axis specification and cell migration during early mouse development

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