Program/Abstract # 19
Apoptosis controls the speed of looping morphogenesis in *Drosophila* male terminalia
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During animal development, dynamic cellular behaviors, including cell movement, divisions and death, are precisely orchestrated. The execution mechanisms of complex behaviors has been mainly deciphered through the identification of conserved signaling pathways that control each cellular movement, however, how cellular behaviors regulate morphogenesis according to the developmental timetable is elusive. To understand the mechanisms of complex morphogenesis, we have investigated the development of *Drosophila* male terminalia. The *Drosophila* male terminalia is an asymmetric looping organ; the internal genitalia (spermiduct) loops dextrally around the hindgut. Mutants for cell death signal have the orientation defect of their male terminalia, indicating that cell death may contribute to looping morphogenesis. We studied the role of cell death in the organogenesis of male terminalia using time-lapse imaging. In normal flies, genitalia rotation accelerated as development proceeded, to complete the full 360° rotation, however, the acceleration was impaired by suppression of cell death. The acceleration was produced by two distinct rotations of the A8 segment that surrounded the male genitalia (A9 segment): an inner ring primarily rotates with genitalia, and an outer ring additionally rotates later to accelerate the primary rotation. Inhibition of cell death suppressed this additional rotation. Thus, we found that cell death coordinates two independent rotations, which drives the acceleration of genitalia rotation, enabling the complete morphogenesis of male genitalia within a limited developmental time window.

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Program/Abstract # 20
MicroRNA regulation of lunatic fringe is essential for proper vertebrate segmentation
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Somites are the embryonic precursors of the vertebrae, ribs, and skeletal muscles. They form from the presomitic mesoderm (PSM) by a periodic segmentation process called somitogenesis. This process is controlled in part by a segmentation clock that requires the oscillatory expression of genes such as Lunatic fringe (*Lfng*). Proper clock function requires that both the mRNA half-lives of oscillatory genes and the translational efficiency of these transcripts be tightly controlled during the rapid time period of the clock. We propose that microRNAs (miRs) have a conserved function in the segmentation clock through post-transcriptional regulation of oscillatory genes. We used miRNA microarray analysis to identify miRs enriched in the PSM. Of the 32 miRs we identified as >1.5 enriched in the mouse PSM, 3 (miR-200b, miR-200c, and miR-125a-5p) are predicted to target the 3′UTR of *Lfng*, a critical component in the segmentation clock. Whole mount in-situ hybridization confirms that these miRs are specifically enriched in the PSM of mouse embryos during somitogenesis. Cell culture analysis demonstrates that both miR-200b/c and miR-125a-5p directly target conserved sequences in the *Lfng* 3′UTR of both mouse and chicken. We further find that preventing interactions between these miRs and the *Lfng* 3′UTR *in vivo* leads to abnormal segmentation and perturbs cyclic gene expression in the PSM, indicating that miR regulation of *Lfng* is important for the clock mechanism. These data provide the first evidence supporting a role for miRs in the segmentation clock and enhance our understanding of post-transcriptional regulation of oscillatory genes.

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Program/Abstract # 21
Early neural crest development and skeletogenic potential
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We have investigated the specification of chick neural crest cells (NCCs) in early pre-gastrula embryos through a specification assay on isolated fragments of the epiblast from stage XII (Eyal Gilaldi) embryos explanted in isolation in collagen gels under non-inducing conditions. This assay identified a restricted region in pregastrula embryos, able to generate NCCs (Pax7, AP2, Msx1, Sox9, Snail2, Sox10, and HNK-1) in the absence of mesodermal and neural markers. Through DiI/DiO mapping experiments we identify neural folds and NCCs originating from the same region identified in the specification experiments. Thus, at this early stage, a set of cells in the epiblast of the chick embryo is poised to generate NCCs, strongly suggesting an earlier time-table of neural crest development. The implications of early events in the differentiation potential of NCCs have not been investigated. We tested the capacity of the early environment to induce skeletogenic differentiation in trunk NCCs, which were thought to lack skeletogenic capacity in vivo. Grafted trunk NCCs downregulate Hox expression, migrate to branchial arches, launch early chondrogenic markers and generate a wide range of skeletogenic derivatives when exposed to the early prospective cranial NCC environment. These results suggest a critical effect of early events in neural crest development on their differentiation capacity.

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Program/Abstract # 22
Elucidating the genetic network that controls the initiation of the mammalian respiratory lineage
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The mammalian respiratory system, consisting of both trachea and lung, initiates from the foregut endoderm. Defects in either the specification of the respiratory progenitors, or the subsequent morphogenesis events that separate trachea/lung from the digestive tract, result in failure to breathe at birth. The embryonic foregut is a hub for developmental signals. Here we will present genetic data from conditional knockout mice suggesting that WNT/*b-Catenin* signaling as well as BMP signaling promote the respiratory fate and inhibit the digestive fate in the ventral foregut endoderm. Characterization of compound mutants further elucidated the hierarchical relationship not only between these signaling pathways, but also with transcription factor genes, *Nkx2.1* and *Sox2*, that are important for early foregut development. These findings unveil an initial gene network that dictates the establishment of the respiratory lineage in mice.

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