Concurrent Session 6: Generation of Asymmetry

Program/Abstract # 39
The role of PCP signaling, fluid flow and cytoskeletal dynamics in orienting motile cilia
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The ability of ciliated epithelia to generate directed flow is critical to diverse biological processes. To achieve this flow, ciliated cells must generate ~100 cilia that are coordinately polarized along a common axis. Here I present a model for how motile cilia in the Xenopus larval skin are polarized. In this model, the PCP components Vangl2 and Fz3 provide non-cell autonomous cues to orient ciliated-cells. These cues bias cilia and initiate a weak, but directed flow. This flow initiates a positive feedback loop, such that non-aligned cilia respond to the prevailing flow as well as to intracellular hydrodynamic forces to achieve coordinated polarity. This process indicates that cilia orientation is malleable, yet the mechanisms regulating this remain unexplored. EM studies have revealed a close association between ciliary basal bodies and the cytoskeleton. We address this relationship in detail by analyzing the role of cytoskeletal dynamics in regulating cilia orientation. Specifically, we report the effects of cytoskeleton modulating drugs on the process of generating cilia polarity.

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Program/Abstract # 40
Establishment of left–right asymmetry in zebrafish: Surprising predictions from a modeling approach
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Left–right (LR) axis specification leads to the proper arrangement of asymmetric organs in the body. In vertebrates, the Nodal signaling pathway is asymmetricaly expressed and plays an essential role in LR patterning. We have determined that the levels and spatial pattern of the Nodal pathway is asymmetrically expressed and plays an essential role in LR asymmetry. In addition, ectopic expression of oep results in loss of nodal asymmetry, suggesting that oep is not a permissive factor in LR patterning, as was previously believed. We used the profile of nodal expression to determine the relationship between the propagation speed of nodal and the mRNA turnover rate. Using this data we have constructed a two-dimensional mathematical model of zebrafish LR axis establishment. Our model makes several predictions which we have confirmed through additional testing. Surprisingly, our model along with additional experiments from our group, support the idea that there is a prepattern of asymmetric information in the embryo that is strengthened by activity at Kupffer’s vesicle. In mutants where fluid flow is slowed, but not eliminated, proper asymmetric expression occurs correctly more often than expected if fluid flow was the only method of establishing asymmetry. Our model provides clues as to why pkd2 mutants in zebrafish produce bilateral nodal signals, while loss of this gene in mouse is reported to cause an absence of Nodal. Our work generating and confirming the model will be presented.

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Program/Abstract # 41
Asymmetry, fate and self-renewal in stomatal development
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Self-renewing populations of cells are integral to the creation and maintenance of tissues and organs. The overarching regulatory issues for these diverse tissues include establishing the populations of self-renewing (stem) cells in discrete locations and ensuring that these cells divide and create new differentiated cells at the appropriate rate and in the appropriate place. Plants have a remarkable ability to maintain these populations; yet, this ability to self-renew does not seem to come at the price of increased susceptibility to cancerous growth. Stomata (epidermal structures that regulate CO2 and H2O exchange in plants) are a useful genetic model to understand the mechanism(s) by which dispersed self-renewing populations are established and how their division and differentiation behavior is directed by interaction with neighboring cells. Our current studies address control over the asymmetric divisions that generate stomatal stem-cell populations, focusing on the signals and transcription factors that regulate the frequency and position of the divisions and the downstream factors required to carry out the divisions.

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Program/Abstract # 42
The Par6 complex is required for both early and late orientation of the left–right axis in Xenopus
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A three protein signaling complex, composed of Par3, Par6 and atypical protein kinase C (aPKC), is a central part of the mechanism that regulates cell polarity in a wide range of organisms. In C. elegans, this complex is responsible for the establishment of the anterior–posterior axis and regulation of asymmetric cell division. Additionally, a recent in vitro study of neutrophil-like cells revealed a role for Par6 in an intrinsic chirality that allows single cells to reliably distinguish left from right in culture. Because left–right (LR) asymmetry is fundamentally linked to cellular polarity, we probed the roles of Par6 and aPKC in the orientation
of the LR axis in *Xenopus laevis*. Misexpression of dominant negative mutants targeting either protein, including in cells which do not contribute to the embryos’ ciliated organ, randomizes asymmetric Nodal expression and organ *situs*. Using an ablated organizer/conjoined twin model, we recently showed that late-induced organizers (forming when thousands of cells are present) cannot properly initiate asymmetry unless an endogenous organizer is present that received orientation cues during early cleavage stages. We proposed that a planar cell polarity pathway allowed the primary organizer to exert LR-instructive influence over the induced organizer. Our latest functional data implicate Par6, gap junctions, and serotonin in this process. We propose that well-conserved polarity (Par6, aPKC) complexes are required for LR asymmetry and that polarity and physiological signals combine to control the flow of laterality information across the early blastoderm.

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Program/Abstract # 43
Mechanism of asymmetric meiotic divisions in mouse oocytes
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Oocytes undergo highly asymmetric meiotic divisions to produce haploid gametes by discarding half of the chromosomes into polar bodies, thus maximally preserving maternal resources in the gametes. The special asymmetric division is determined by an actin-dependent cortical movement and peripheral positioning of the meiotic chromosomes/spindle in the oocytes. The cortical movement of the meiotic chromosomes/spindle is dependent on the Formin-2 and not the Arp2/3-mediated actin assemblies in the maturing oocytes. Temporal upregulation and downregulation of CDK1 and MAP kinase activities during meiosis contribute to the initiation and cessation of chromosome/spindle cortical movement in the oocytes. Dynamic interactions of the chromosome-induced cortical cap with the meiotic chromosomes/spindle result in proper spindle orientation and polar body extrusion. Microinjection of DNA coated beads into oocytes recapitulates the processes of meiotic chromosome/spindle cortical movement as well as polar body extrusion and can be used to study the mechanism of asymmetric meiotic division in the oocytes. By using the DNA bead technique, it was found that the chromosome/spindle peripheral moving machinery was specifically assembled during meiosis I and disassembled thereafter. This makes the Formin-2-dependent actin driving chromosome/spindle cortical movement a once-in-a-life-time event during oocyte meiosis.

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Program/Abstract # 44
*Wnts regulate asymmetric spindle to generate asymmetric cell fates in* C. elegans
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Asymmetric cell division is a fundamental process that produces cellular diversity during development. In C. elegans, many asymmetric divisions that occur along the A–P axis are regulated by the Wnt signaling pathway. During asymmetric divisions, WRM-1/β-catenin localizes asymmetrically to the posterior nucleus at telophase to regulate asymmetric fates of the daughter cells. However, the mechanism of asymmetric β-catenin nuclear localization is largely unknown. We found two lines of evidence that suggest the importance of microtubules in this mechanism: (1) β-catenin nuclear asymmetry is generated in a microtubule-dependent manner, and (2) the numbers of astral microtubules were asymmetric during telophase; higher at the anterior spindle pole than the posterior one. To know the importance of spindle asymmetry in asymmetric β-catenin localization, we experimentally disrupted the spindle asymmetry using laser irradiation of the microtubule organizing center (MTOC). When the posterior MTOC was irradiated, nuclear β-catenin asymmetry was enhanced. In contrast, when the anterior MTOC was irradiated, nuclear β-catenin asymmetry was disrupted. These results strongly suggest that β-catenin asymmetry is controlled by microtubule number asymmetry. We also found that the kinesin is required for β-catenin asymmetry. In our poster, we will present a model that kinesin regulates nuclear export of β-catenin differently between the anterior and posterior nuclei by utilizing asymmetric spindle.

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