Program/Abstract # 65
Contact-mediated radial polarization of the early *C. elegans* embryo
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Our lab uses the *C. elegans* embryo as a model to understand how gastrulation movements organize the animal embryo. During gastrulation, cells destined to produce internal tissues and organs move from the surface of the embryo to its interior. *C. elegans* gastrulation movements are thought to be driven in part by a constriction of the outer surface of ingressing cells, which is mediated by an asymmetric accumulation of activated myosin at the outer cortex. Normal gastrulation movements and the accompanying asymmetries in myosin require that early embryonic cells polarize along their inner–outer (contacted/contact-free) axis. During polarization, conserved PAR polarity proteins develop inner–outer asymmetries that are needed for both myosin asymmetry and normal gastrulation movements. We have identified a signaling pathway that induces inner–outer PAR asymmetries in early embryonic cells. A central regulator of inner–outer PAR asymmetry is PAC-1, a RhoGAP that is recruited to inner surfaces of cells by cell–cell contact. Our data suggest that PAC-1 inhibits the Rho GTPase CDC-42 at inner surfaces, thereby creating an inner–outer asymmetry in CDC-42 activity that is needed to restrict PAR proteins to the outer cortex. Inner–outer polarization of *C. elegans* embryos shares many similarities with compaction in mouse embryos, raising the possibility that a similar contact-induced pathway polarizes the early mammalian embryo.

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Program/Abstract # 66
Evolutionary plasticity of developmental mechanisms: Evidence from the asymmetric second cleavage of the *Helobdella* (leech) embryo
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Spiral cleavage is a unique cell division program seen in the embryos of animals as diverse as annelids, molluscs and flatworms. Comparing spiral cleavage in extant species provides insight into how changes in the program have influenced body plan evolution. Among the monophyletic clitellate annelids many steps of development are undoubtedly homologous. In the leech *Helobdella* and the oligochaete *Tubifex* the unequal first and second divisions are critical for establishing bilateral symmetry by segregating developmental determinants (teloplasm) exclusively to cell D at the 4-cell stage. Even though D-quadrant specification is homologous in clitellates, significant modification to the mechanisms that control it has been documented. We investigated the mechanisms underlying asymmetric second cleavage of the teloplasm-containing CD cell in *Helobdella*. At metaphase a symmetric bi-astral spindle attaches via both asters to the basolateral cortex. The spindle then shifts toward the right side of the cell inducing an eccentrically located cytokinetic furrow. Various treatments demonstrate that an intimate connection between the spindle poles and the basolateral cortex is necessary for asymmetric cleavage. The localization of leech homologs of the PAR proteins also suggests that the basolateral cortex is uniquely capable of influencing asymmetric CD cleavage. HRO-PAR1 localizes uniformly on the apical and basolateral cortices, while HRO-PAR6 localizes to the apical cortex, but is absent from the basolateral cortex. These data reveal D-quadrant specification divergence between *Helobdella* and *Tubifex*.

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Program/Abstract # 67
Spermidine and spermatid differentiation of *Marsilea vestita*
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The microspore of the water fern, *Marsilea vestita*, contains a single cell that undergoes nine mitotic division cycles to produce 32 spermatids and 7 sterile cells. Cytoplasmic movements of stored proteins and stored mRNAs precede the first division in the transcriptionally-quiet gametophyte, creating zones that later become spermatogenous initials. Asymmetric divisions produce jacket cells, and then, symmetric divisions produce spermatids, which differentiate into ciliated gametes with condensed, coiled nuclei. We developed RNAi strategies to target mRNA degradation and arrest development, and have shown that translation is controlled both spatially and temporally. We have studied the role of spermidine in spermatid differentiation. RNAi and drug treatments were employed to deplete spermidine in the spores. dsRNA probes were made from cDNAs encoding spermidine synthase (SPDS) and spermidine transporter (SPDT) proteins. dsRNA treated spores had fewer but larger spermatogenous cells than controls. Cells were labeled with anti-centrin and anti-α-tubulin antibodies to localize basal bodies and the microtubule ribbon, respectively. In dsRNA-treated spermatogenous cells, the nuclei elongated but did not condense, basal body positioning was altered and the microtubule ribbon was in disarray. An SPDS inhibitor was added at 6 h, the start of spermatid differentiation. The spermatid nuclei remained round, centrin failed to localize into basal bodies and the...
Program/Abstract # 68
Noncanonical Frizzled dependent signaling controls chondrocyte polarity during cartilage morphogenesis
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A central question in developmental biology is how morphologically distinct structures are generated from similar cell types. The skeleton provides a good model system to answer this question due to the highly diverse and complex morphological differences displayed by individual elements. Analysis of the growth plate cartilage by Dodds suggested that longitudinal growth of long bones might result from the specific arrangement of chondrocytes. In the proliferative zone, chondrocytes become discoid and arrange in columns, like stacks of coins, which are parallel to the long axis of the cartilage. Although well-organized chondrocytes derive from a relatively unstructured pool of progenitor cells, it is not known whether this arrangement of cells is the result of a regulated process or is in response to physical constraints of the cartilage matrix. Here we test Dodds’ model that cell columns in the growth plate cartilage form from a single progenitor cell by a process in which cells divide orthogonal to the stack then rearrange. Our work has uncovered a central role of noncanonical Frizzled dependent signaling in the regulation of chondrocyte polarity and morphogenesis in the growth plate cartilage. Funding for these studies was provided by the Searle Leadership Fund of the Chicago Community Trust.

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Program/Abstract # 69
Polarising migrating tissues during organogenesis
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Most complex organ systems arise from the directed migration of cohesive cell groups, such as sheets, chains and clusters. In this morphogenetic context, migration achieves significantly more than the simple displacement of cells from one location to another, rather it allows these cell groups to build three-dimensional shape. Interestingly, recent studies using genetic mosaics have shown that while extracellular cues clearly guide these migration events their receptors need only be expressed by cells at the very leading edge of the tissue. Here, we address the mechanisms that coordinate cell migration and polarity within one such cohesive tissue, the zebrafish lateral line primordium. This is transient migrating structure, comprising of some 100 cells, whose function is to deposit a series of mechanosensory organs throughout the skin of the fish. By combining in vivo imaging with a number of functional approaches – such as genetic mosaics, laser nanosurgery and small molecule inhibitors – we have begun to address the chemical and mechanical cues that regulate coordinated cell polarity and organization across this migrating tissue. This work reveals that many aspects of this tissue morphogenesis, including the direction of migration and the formation and deposition of organs, are based on self-organising principles.

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