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Abstracts

Morphogenesis

Program/Abstract # 406
Dynein is required for epithelial polarity and the apical localization of stardust mRNA

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Intense investigation has identified an elaborate protein network controlling epithelial polarity, however little is known about how the individual apical and basolateral determinants become polarized in the cell. Through a screen for epithelial defects in the *Drosophila* ovary, we have found that the Cytoplasmic Dynein motor is an essential regulator of apico-basal polarity. Since Dynein is known to ferry cargoes toward the apical surface in epithelia, we hypothesized that the loss of apico-basal polarity observed in Dynein-deficient cells could result from the mis-trafficking of one or more of the known apical determinant proteins. Our data indicate that Dynein acts through the cytoplasmic scaffolding protein Stardust (Sdt) to localize the transmembrane protein Crumbs (Crb) during early oogenic stages, likely through the apical targeting and localized translation of the *sdt* mRNA. We have mapped the *sdt* localization signal to an alternatively spliced coding exon and have observed a developmental switch during which the *sdt* mRNA becomes uniform at later oogenic stages. These results introduce a new class of gene products, mRNAs, to the existing paradigm for apico-basal polarity regulation and suggest that *sdt* mRNA localization may play a key role in the establishment of epithelial polarity. We will discuss how these two modes of *sdt* mRNA localization contribute to the maturation of epithelia during development.

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Program/Abstract # 407
Rho GTPase is required for distinct steps in epithelial tube morphogenesis

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Many organs consist of epithelial tubes, yet the mechanism by which tubes form is not well understood. Here, we report that the small GTPase, Rho, is required for three distinct steps in morphogenesis of the *Drosophila* embryonic salivary gland, which consists of a pair of epithelial tubes. Gland morphogenesis begins by invagination of the primordial cells from the embryo surface followed by cohesive migration of the internalized gland. We show that Rho GTPase is required to maintain apical polarity in the early gland epithelium, invagination and posterior migration. We show that Rho maintains apical polarity through stabilization of Crumbs (Crbs) at the apical lateral membrane and epistasis analysis places Crbs downstream of Rho. Crbs in turn, is required for apical localization of non-muscle myosin II during Rho-mediated invagination of gland cells. After invagination is complete, Rho is again required for contraction of the proximal tip of the gland that results in the posterior migration of the gland. Our studies provide the first evidence for Rho in maintenance of apical polarity and of a functional link between Rho and Crbs. Our studies also reveal that Rho-mediated contraction is necessary both for invagination and migration of the salivary gland.

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Program/Abstract # 408
turtle, a novel immunoglobulin superfamily member, is required for dendrite morphogenesis in a subset of *Drosophila* PNS sensory neurons

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Dendrites function as the primary sites of synaptic and/or sensory input and integration in the developing nervous system, thus, elucidating the molecular mechanisms governing dendrite morphologies is critical to our understanding of how diverse cell-type-specific dendritic morphologies arise and further, how these morphologies may affect biological events such as sensory perception, learning, memory, aging, and nervous system disease pathologies. Here we report the molecular genetic characterization of *Drosophila turtle* (*tutl*), a gene encoding a nervous system-

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specific putative receptor molecule of the immunoglobulin superfamily. Analyses of Tutl PNS expression revealed localization to Class III and IV dendritic arborization (da) neurons, suggesting that *tutl* may regulate class-specific dendritogenesis. Loss-of-function (LOF) analyses revealed cell-autonomous functions for *tutl* in promoting Class III and IV dendritic arborization and receptive field innervation. Conversely, gain-of-function (GOF) studies revealed ectopic Tutl expression in Class I da neurons results in increased dendritic complexity. The *tutl* LOF and GOF phenotypes, as well as Tutl PNS expression patterns, are similar to those observed for the Cut homeodomain transcription factor, recently demonstrated to mediate class-specific da neuron dendritogenesis. We therefore examined the potential that Cut may transcriptionally regulate Tutl expression in da neurons. Both LOF and GOF cut analyses suggest Tutl represents the first known downstream Cut transcriptional target in the regulation of class-specific da neuron dendrite morphogenesis.

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Program/Abstract # 409

The zebrafish calpain system – expression and role of calpain and calpastatin during early development

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The calpain superfamily is a large group of highly conserved calcium-dependent cysteine proteases that have been implicated in regulating a wide variety of biological processes such as cell adhesion, migration and intracellular signaling. Two heterodimeric typical family members, μ-calpain and m-calpain, have been studied extensively in vitro and in cell culture but few studies have been aimed at determining the function of calpain and its endogenous inhibitor calpastatin in vivo. Recently, calpain knockout mice have revealed that both m-calpain subunits are indispensable for survival of the pre-implantation embryo; however, the precise role the calpain system plays during early development has yet to be determined. We have cloned and characterized the temporal and spatial expression of four zebrafish genes encoding typical calpain catalytic subunits (capn1a, 1b, 2a, 2b), two genes encoding common regulatory subunits (cpns1a, 1b) and calpastatin (cast). RT–PCR and whole-mount in situ hybridization analysis reveals that these genes are expressed in distinct, yet overlapping, spatiotemporal patterns during the first 24 h of development. Preliminary loss-of-function experiments, employing chemical calpain inhibitors, cpns-directed morpholinos and calpastatin over-expression, suggest the calpain system might be necessary for the successful completion of morphogenetic movements, such as epiboly, and proper patterning of the dorsal–ventral axis.

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Program/Abstract # 410

Crip2 has dual functions in the cytoplasm and nucleus, induces non-canonical Wnt signaling during convergent extension movement in zebrafish notochord

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In notochord development, ntl induce intercalation of the cells, but their target or signaling cross-talks were unclear. We performed microarray experiment using ntl knockdown embryo of zebrafish and identified *Cysteine-rich protein 2* (*crip2*) as a transcriptional target of ntl. Crip2 expressed specifically in the notochord and regulate convergent extension cell movement of gastrulation. By molecular and cellular assay, Crip2 was localized in the nucleus on Wnt stimulation. In the nucleus, Crip2 bind to β-catenin and inhibited β-catenin/Tcf-dependent transcription. Moreover, Crip2 was also localized in cytoplasm, directly interacted with Dishevelled 2 and formed a complex with Crip2/Dvl2/Daam1/Diversin on non-canonical Wnt stimulation. Moreover Crip2 recruited this complex to the focal adhesion complex near the leading edge of cell and control cell morphology and migration. This is a first report elucidating the molecular mechanism of intercalation and directly interactions between ntl and canonical and non-canonical Wnt signaling.

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Program/Abstract # 411

Cadherin-based adhesion cooperates with non-canonical Wnt signaling to mediate morphogenesis in the zebrafish

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A critical step in central nervous system development is the shaping of the neural tube (or neurulation). *N-cadherin* (*N-cad*), a calcium-dependent, homophilic-binding cell adhesion molecule, has a conserved role in this process in vertebrates as disruption of *N-cad* results in a variety of neural tube defects. However, the role of *N-cad* in regulating the cellular and molecular mechanisms underlying neurulation has not been clearly elucidated. By direct analysis of cell behaviors, we have shown that although *N-cad*-depleted cells are not defective in their ability to form protrusions, they are not able to maintain them stably. Here, we begin to address whether *N-cad* functions solely as an adhesion molecule