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Abstracts

Concurrent session 1: Germ and embryonic stem cells

Program/Abstract # 3
Asymmetric stem cell division ensured by anaphase spindle repositioning
Yukiko M. Yamashita[a], Hebao Yuan[a], Jun Cheng[b], Alan J. Hunt[b]
[a]*Life Sciences Institute, Center for Stem Cell Biology, USA*
[b]*Department of Biomedical Engineering, Center for Ultrafast Optical Science, USA*

Many stem cells divide asymmetrically to balance self-renewal and differentiation. In *Drosophila* testes, two stem cell populations, germline stem cells (GSCs) and somatic cyst stem cells (CySCs, or historically called cyst progenitor cells), cohere and regulate one another. CySCs not only generate cyst cells (CCs) that support differentiating germ cells, but also encapsulate GSCs to maintain GSC identity. Therefore, the balance between CySC self-renewal and differentiation must be tightly controlled to maintain the corresponding balance of GSCs and sustain spermatogenesis. Here, we report that CySCs divide asymmetrically through spindle repositioning in anaphase. This is in striking contrast to their neighbor GSCs, whose spindle is rigidly oriented toward the niche throughout mitosis. CySC spindle repositioning in anaphase requires functional centrosomes and the actin-associated membrane protein, Moesin. We demonstrate that anaphase spindle repositioning is required to achieve high-fidelity asymmetric divisions in CySCs, thus maintaining both GSC and CySC numbers. We propose that dynamic spindle repositioning allows CySCs to divide asymmetrically while accommodating encapsulated GSCs.

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Program/Abstract # 4
A travelling niche: Steel factor controls primordial germ cell survival and motility throughout their migration
Ying Gu[a,b], Chris Runyan[a], Amanda Shoemaker[a], Azim Surani[c], Chris Wylie[a]
[a]*Div. of Dev. Biol., Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, USA*
[b]*Graduate Program in Mol. and Dev. Biol., Univ. of Cincinnati, Cincinnati, OH, USA*
[c]*Gurdon Institute, Univ. of Cambridge, Cambridge, UK*

Primordial germ cells (PGCs) are the embryonic founders of adult gametes. In mouse, they arise around E7.25, and migrate through different tissues in an embryo which is undergoing rapid growth and organogenesis, to colonize the genital ridges by E11.5. How are PGC behaviors, including proliferation, survival, motility and homing, controlled in a rapidly changing environment? By studying the expression pattern and functions of Steel factor, we propose a mechanism in which the expression of essential signals accompanies the migrating PGCs in a kind of “travelling niche”, to regulate PGC behaviors. We show first that PGCs are surrounded by Steel factor-expressing cells from their first appearance in the allantois to the time they enter the genital ridges. Second, fewer PGCs are found in the allantois in *Steel*-null embryos, but this is not due to a failure of PGC specification. Third, the analysis of cultured *Steel*-null early embryos shows that Steel factor is required for normal PGC motility, both in the allantois and in the hindgut. Altogether, our data show that PGCs are Steel factor dependent throughout their migration, and suggest the existence of a “traveling niche” in which the Steel factor-expressing cells provide a spatio-temporal environment along the migratory route to retain the normal properties of this important pluripotential cell population.

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Program/Abstract # 5
Specifying root/shoot stem cells during *Arabidopsis* embryogenesis
Jeff A. Long
Plant Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA

In both animals and plants, the formation of a polaraxis is one of the first steps during embryogenesis. Cell fate must then be specified and maintained along thisaxis in order for proper development to occur. In plants, a basic body plan is set up during embryogenesis, with a shoot pole at the apical half and a root pole at the basal half. We have identified a mutation in the *Arabidopsis TOPLESS* gene (*tpl-1*) that displays a transformation of the apical half of the embryo into a second basal half, resulting in embryos with two root poles. The TPL protein shows structural similarity to known co-repressors and is hypothesized to repress root genes in the shoot half of the embryo. Using the *tpl-1* allele as a tool, we have now identified two classes of transcription factors that either specify the shoot or the root during embryogenesis. Through misexpression studies and genetic analysis, we can now control the identity of either the shoot or the root pole. I will present data on how these transcription

factors are regulated and how they pattern specific cell types in both wild-type and *tpl-1* mutant embryos.

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Program/Abstract # 6

Molecular dissection of germ cell development in the planarian *Schmidtea mediterranea*

Yuying Wang[a], Joel M. Stary[a], Phillip A. Newmark[a,b]
[a]*Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign,, Urbana, IL, USA*
[b]*Howard Hughes Medical Institute, USA*

Freshwater planarians appear to utilize inductive signals to specify their germ cell lineage: germ cells are believed to form post-embryonically from the pluripotent somatic stem cells, known as neoblasts. Previously, we identified a planarian homolog of *nanos* (*Smed-nanos*) and demonstrated by RNA interference (RNAi) that this gene is required for the development, maintenance, and regeneration of planarian germ cells. We have performed microarray analyses to compare gene expression profiles between planarians with early germ cells and those without them. We identified ~300 genes that are significantly down-regulated in animals lacking early germ cells. This data set contains genes implicated in germ cell development in other organisms, conserved genes not yet reported to have germ cell-related functions, and novel genes. Analysis using putative domain functions (Clusters of Orthologous Groups) suggested diverse molecular functions, including cytoskeletal components, metabolism, RNA processing and modification, transcription, as well as signal transduction. Top hits have been validated by in situ hybridization. Functional analyses of these genes via RNA interference are being carried out. Thus far, we have identified several genes that, when knocked down by RNAi, cause various defects in germ cell development, including: impaired testes development; loss of spermatogonial stem cells; meiotic failure; and defects in sperm elongation. This work will contribute to our knowledge of conserved regulators of germ cell differentiation.

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Program/Abstract # 7

Development rooted in interwoven networks

Philip N. Benfey[a], Hongchang Cui[a], Richard Twigg[a], Terri Long[a], Anjali Iyer-Pascuzzi[a], Hironaka Tsukagoshi[a], Rosangela Sozzani[b], Terry Jackson[a], Jaimie Van Norman[a], Miguel Moreno-Risueno[a]
[a]*Biology Department, Duke University, Durham, NC USA*
[b]*IGSP Center for Systems Biology, Duke University, Durham, NC USA*

Specification and maintenance of cell identity are central processes of development. In an effort to understand the regulatory networks that control cell identity, we have profiled all cell types and developmental stages within a single organ, the *Arabidopsis* root. To acquire global expression profiles we developed technology that uses sorted marked populations of cells with subsequent hybridization of the labeled RNA to microarrays. We are using computational methods to infer networks functioning within different cell types and developmental stages and have begun to test the hypothesized relationships. Our current efforts are aimed at understanding the responses to environmental stimuli at high spatio-temporal resolution. We are developing new expression analysis platforms and means of analyzing 4-D data sets. We are also analyzing the dynamics of growth of physical root networks using novel non-invasive imaging methods and developing mathematical descriptors of network architecture.

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Program/Abstract # 8

Cdx2 and FGF cooperate to specify brachial and thoracic spinal identity of mouse embryonic stem cell-derived motor neurons

Mirza Peljto[a], Esteban O. Mazzoni[a], Vladimir Korinek[b], Hynek Wichterle[a]
[a]*Department of Pathology, Center for Motor Neuron Biology and Disease, Columbia University, New York, NY, USA*
[b]*Department of Cell Dev. Biol., Institute of Molecular Genetics AS CR, v. v. i., Prague, Czech Republic*

In the presence of retinoic acid and Sonic Hedgehog, embryonic stem (ES) cells differentiate to Hoxa5+ cervical spinal motor neurons (MNs) with high efficiency. However, the generation of Hoxc8+ brachial and thoracic MNs from ES cells is significantly less efficient. The goal of this study was to identify developmentally relevant extrinsic signals and intrinsic genetic programs that would facilitate high efficiency generation of caudal brachial and thoracic Hoxc8+ MNs from mouse ES cells. Newly born Hoxc8+ MNs expressed high levels of Cdx2 transcription factor whose expression peaks at the neural plate stage when cells are responsive to extrinsic rostrocaudal patterning signals. In addition, we found that Wnt is the principal signal inducing Cdx2 expression during MN differentiation. Using a newly generated ES cell line harboring a doxycycline inducible Cdx2 transgene, we observed that Cdx2 expression by itself was not sufficient to specify Hoxc8+ MNs. However, a brief induction of Cdx2 expression followed by the treatment of differentiating cells with FGF resulted in the high efficiency generation of Hoxc8+ MNs and the complete loss of Hoxa5 expression. We propose that Wnt directed Cdx2 expression followed by the activation of the FGF signaling cascade are prerequisites for efficient specification of Hoxc8+ brachial and thoracic spinal MNs from mouse ES cells.

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Program/Abstract # 9

Vive la difference: The creation of sexual dimorphism in the soma and germline

Mark Van Doren
Department of Biology, Johns Hopkins University, Baltimore, MD, USA

The events that trigger sex determination in animals evolve rapidly, but we now know that the downstream events that translate this information into sexual dimorphism are more highly conserved. Recent work indicates that this conservation may extend between vertebrates and invertebrates, and flies and man. This is likely to be particularly true in gonad development, as the fundamental requirement for creating sperm and eggs is shared across animal species. One interesting aspect of gonad sexual dimorphism that we are currently investigating is the formation of the male vs. female germline stem cells and the niches for these stem cells created by the soma. In some species, such as humans, a germline stem cell population is only thought to exist in males but not females. In *Drosophila*, germline stem cells exist in both sexes, but there are clear differences in the development and regulation of these stem cell populations and their respective stem cell niches. How is the dramatic sexual dimorphism in germline stem cell development