Program/Abstract # 37

Redefining brain serotonergic neurons by genetic lineage and selective in vivo silencing

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Central serotonin-producing neurons are heterogeneous – differing in embryonic origin, final location, morphology, firing properties, and associated clinical disorders – but the underpinnings of this heterogeneity are largely unknown, as are molecular markers capable of distinguishing among functional subtypes. To examine this heterogeneity, we have generated genetic tools for use in mice that allow multiple features of a neuron type to be delineated and linked in vivo, for example, its origin in the embryo, fate in the adult, and function in particular circuits as relates to behavior and physiology. Our starting point has been development of a dual recombinase-based molecule delivery system with plug-n-play modularity such that most any genetically-encoded lineage tracer or effector molecule can be incorporated and delivered in vivo to most neuron types. Neuron types are defined by combinatorial gene expression, making cell-type specificity high. Using these tools, we have generated a new classification scheme for serotonin neurons that is based on genetic programs differentially enacted among serotonergic precursor cells and which represents a more mechanistic view of serotonergic neuron heterogeneity than offered by anatomical segregation. Neuronal silencing tools to plot cellular functions to these different serotonergic lineages will be presented. Through these approaches, we are redefining the roles served by specific serotonin neuron subtypes in the embryo that produce hematopoietic stem cells: the allantois, yolk sac, and AGM regions. Mutating Tbx4, a T-box gene thought to be expressed throughout the allantois, causes defective vasculogenesis in which endothelial cells differentiate but do not coalesce into tubes. We examined Tbx4 expression at cellular resolution and traced the fate of Tbx4-expressing cell lineages using a Cre knock-in of Tbx4 with a Cre reporter line. We observe that a subset of cells in the interior of the allantois do not express Tbx4, and comparison of these cells to endothelial markers suggests that Tbx4 is expressed in a reciprocal pattern with endothelial genes. Double staining with endothelial markers and the Tbx4 lineage reporter reveals that the endothelium is entirely derived from cells that never express Tbx4, which represent a cryptic compartment within the allantois. This work is the first observation that vasculogenic mesenchyme, presumed to be naïve, is actually prepatterned. This work also shows that the vascular phenotype of the Tbx4 knockout is due to defects in perivascular tissue, as the endothelium does not express Tbx4. Tbx4 expression has also been reported in the hindlimb and external genitalia. We have examined this expression and Tbx4 lineage and we observe that, rather than separate domains, the allantois, hindlimb, and genital tubercle all arise from a single contiguous mesenchymal Tbx4-positive domain. We also note that while Tbx4 has been described as hindlimb-specific, we observe two domains of Tbx4 expression in the forelimb that contribute to distinct structures.

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Program/Abstract # 38

Lineage tracing of Tbx4-expressing cells reveals cryptic developmental decisions

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Vasculogenesis is the de novo formation of blood vessels out of undifferentiated mesenchyme, which occurs only in the regions of the embryo that produce hematopoietic stem cells: the allantois, yolk sac, and AGM regions. Mutating Tbx4, a T-box gene thought to be expressed throughout the allantois, causes defective vasculogenesis in which endothelial cells differentiate but do not coalesce into tubes. We examined Tbx4 expression at cellular resolution and traced the fate of Tbx4-expressing cell lineages using a Cre knock-in of Tbx4 with a Cre reporter line. We observe that a subset of cells in the interior of the allantois do not express Tbx4, and comparison of these cells to endothelial markers suggests that Tbx4 is expressed in a reciprocal pattern with endothelial genes. Double staining with endothelial markers and the Tbx4 lineage reporter reveals that the endothelium is entirely derived from cells that never express Tbx4, which represent a cryptic compartment within the allantois. This work is the first observation that vasculogenic mesenchyme, presumed to be naïve, is actually prepatterned. This work also shows that the vascular phenotype of the Tbx4 knockout is due to defects in perivascular tissue, as the endothelium does not express Tbx4. Tbx4 expression has also been reported in the hindlimb and external genitalia. We have examined this expression and Tbx4 lineage and we observe that, rather than separate domains, the allantois, hindlimb, and genital tubercle all arise from a single contiguous mesenchymal Tbx4-positive domain. We also note that while Tbx4 has been described as hindlimb-specific, we observe two domains of Tbx4 expression in the forelimb that contribute to distinct structures.

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Program/Abstract # 39

Genetic and genomic dissection of a cell specification pathway in Arabidopsis

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The Arabidopsis root epidermis provides a simple model for studying the fundamental problem of cell fate specification. Root-hair cells are specified in the space between underlying cortical cells (the H cell position) and non-hair cells are specified over a single cortical cell (the N cell position). This simple relationship between cell position and cell type differentiation implies that cell-cell communication events are important in the establishment of cell fates. Cellular, molecular, genetic, and genomic approaches have been used to define and analyze genes and their corresponding proteins that are used for the specification of the hair and non-hair cell types. Some of these genes (e.g. GLABRA2 (GL2), TRANSPARENT TESTA GLABRA (TTG), WEREWOLF (WER), GLABRA3 (GL3), and ENHANCER OF GLABRA3 (EGL3)) encode transcription factors important for non-hair cell specification, whereas others (e.g. CAPRICE (CPC), TRIPHYCHON (TRY), and ENHANCER OF TRY AND CPC (ETC1)) help to specify the hair cell type. By studying the expression and interactions...
between these genes, we have found that transcriptional feedback loops acting within and between adjacent cells are important in establishing the cell type pattern. Specifically, the WER MYB-type protein, the GL3/EGL3 bHLH-type proteins, and the TG WD-protein appear to interact in a transcriptional complex to positively regulate the GL2, CPC, TRY, and ETC1 genes. The GL2 homeodomain transcription factor is involved in regulating genes that generate the non-hair cell type. The CPC, TRY, and ETC1 proteins are structurally-related small MYB transcription factors that appear to move between cells and inhibit the formation of the WER-GL3/EGL3-TTG complex; this negative regulation represents a type of lateral inhibition mechanism. The position-dependent pattern relies on a leucine-rich-repeat receptor-like kinase (SCRAMBLED (SCM)), that appears to influence WER gene expression causing an unequal distribution of the transcriptional regulators in the N and H cell positions. Current research is focused on using genomics, bioinformatics, and math modeling to further dissect the regulatory network. These studies are likely to provide new insights into the basic mechanisms of regulatory gene networks and cell-type specification during development.

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Program/Abstract # 40
Neurons develop in situ in foregut endoderm of sea urchin embryos
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Here we report the surprising discovery that some cells in the foregut endoderm of sea urchin embryos develop into neurons, challenging the dogma that neurons are strictly ectodermal derivatives. Neurons are specified in the foregut after it has been specified as endoderm by a well-defined regulatory network (Peter IS and Davidson, EH (2010) Dev Biol 340:188–199) during early blastula stages. However, the foregut is a unique part of the gut that also expresses SoxB1, an ortholog of transcription factors known to support neural precursor states. It also transiently expresses Six3 during mesenchyme blastula stages, a factor that we previously showed to be required for development of all neurons in this embryo, including those in the foregut. As well, foregut cells express the transcription factor, Nkx3-2, which we show is a regulatory intermediate that links Six3 to the development of foregut neurons. Several lines of evidence indicate that these neurons develop in situ in the foregut. They appear in embryos in which the foregut does not fuse with the stomodeal ectoderm and they are not derived from migrating ectodermal precursors, as shown by lineage tracing using the photo-activatable protein, KikGR. Furthermore, the foregut behaves as a neurogenic field in which lateral inhibition operates, as additional neurons develop when Notch signaling is inhibited. Thus, both endodermal and neural gene regulatory networks operate in cells of foregut lineages during gastrulation, a condition we propose is supported by a SoxB1-dependent pluripotent state.

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Program/Abstract # 41
Sensory neuron specification in the neural crest lineage
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The neural crest is a migratory, multipotent cell lineage that contributes to myriad tissues, including the sensory neurons and glia of the dorsal root ganglia (DRG). To identify genes that affect cell fate specification in neural crest, we performed a forward genetic screen in zebrafish for mutations causing DRG deficiencies. This screen yielded a mutant lacking all DRG and associated satellite glia; we named this mutant sensory-deprived (sdp). We identified a total of four alleles of sdp, all of which possess lesions in the gene coding for reversion-inducing cysteine rich protein containing Kazal motifs (reck), an inhibitor of metalloproteinases implicated in cancer metastasis. We found reck function to be both necessary for DRG formation and sufficient to rescue the sdp phenotype. Reck acts upstream of neurogenin1, a transcription factor previously known to be the earliest indicator of sensory neuron development. Reck is expressed in a subset of neural crest cells and is required in a cell-autonomous fashion for DRG sensory neuron formation. We present a model where loss of reck function in the neurogenic precursor cells of the neural crest causes these cells to migrate inappropriately; as a result DRG do not differentiate. Our results suggest that control of migration is the earliest critical step in sensory cell fate specification.

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Program/Abstract # 42
Jagged-Notch, Edn1, and Bmp signaling define discrete preskeletal domains along the dorsoventral axis of the vertebrate face
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The shaping of organs depends on the specification of discrete functional domains along the major body axes, yet how signaling pathways interact to define organ domains remains poorly understood. Here we show that Jagged1-Notch2, Edn1, and Bmps define the dorsal, intermediate, and ventral domains, respectively, of the facial skeleton in zebrafish. In animals deficient for Jagged1b or Notch2 the dorsal facial skeleton adopts a ventral morphology, and conversely the ventral face adopts a dorsal morphology in transgenic embryos that misexpress human Jagged1. Surprisingly, misexpression of just the intracellular domain of Jagged1 results in similar ventral to dorsal transformations, suggesting a role for “reverse signaling” from Notch2 to Jagged1 in dorsal facial patterning. In contrast, we find that overactivation of Bmp signaling ventralizes the dorsal and intermediate preskeletal domains, whereas Bmp inhibition dorsalizes the ventral skeleton. Moreover, we show that Bmps and Edn1 have overlapping yet distinct roles in ventral patterning, with Bmps promoting hand2 and msxe expression in the ventral domain and Edn1 promoting dlx3b/5a/6a and nkk3.2 expression in the intermediate domain. Cross-inhibitory interactions also play a major role in defining discrete preskeletal domains. Jagged1 and Edn1 restrict Bmp signaling to the ventral face by inducing the expression of a Bmp antagonist, Gremlin2, in dorsal and intermediate precursors, and Edn1 and Bmps restrict dorsal signaling by repressing expression of jag1b. Our genetic analysis thus provides an integrated framework for how discrete preskeletal identities are generated along the dorsoventral axis of the face.

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