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Concurrent session 5: Systems and network biology

Program/Abstract # 23
Mapping spatiotemporal gene regulatory networks in the *Arabidopsis* root stele

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Arabidopsis root development provides a remarkably tractable system to delineate tissue-specific, developmental gene regulatory networks and to study their functionality in a complex multicellular model system over developmental time. Tightly controlled gene expression within tissues is a hallmark of multicellular development and is accomplished by transcription factors (TFs) and microRNAs (miRNAs). We present an automated, enhanced yeast one hybrid (eY1H) assay using a tissue-specific TF resource to comprehensively map gene regulatory networks in the *Arabidopsis* root stele. These gene regulatory networks are robust and highly combinatorial in nature. Using these methods and computational modeling, we have additionally modeled a gene regulatory network that regulates distinct transcriptional events in developmental time. Distinct regulatory modules were identified that temporally drive the expression of genes involved in xylem specification and in the subsequent synthesis of secondary cell wall metabolites associated with xylem differentiation.

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Program/Abstract # 24
Web based algorithms EvoPrinter and cis-Decoder reveal functional sequences in enhancers and complex networks of transcription factor interactions required for gene regulation

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A hierarchical system of activators and repressors operates to establish temporally derived pan-neural cell layers that constitute the embryonic CNS. We have developed web-accessed algorithms EvoPrinter and cis-Decoder and a *Drosophila* genome-wide conserved sequence cluster database to identify functionally related enhancers for temporal determinants. The comparative genomics tool EvoPrinter shows that enhancers consist of clusters of conserved sequence blocks that are essential for their function. Using the enhancer search and alignment program cis-Decoder, we have identified early and late CNS neuroblast temporal network enhancers that reveal the following properties: 1) most developmental enhancers contain multiple binding sites for a signature factor(s) that defines enhancers of a particular temporal window, and they also contain repeated or unique sites for additional factors; sequences of many of these sites are not novel but instead are variants of either the signature and/or repeat elements, 2) different combinations of transcription factors bind to sites overlapping of the target sites of the signature site-binding determinates or are positioned independently of these sites, 3) Coordinately regulated enhancers can often be identified by possessing shared sites in the same proportionate balance, 4) in many cases multiple sub-pattern enhancers are found associated with developmental genes that drive expression in overlapping, non-identical subsets of cells, 5) many enhancers are multipurpose, functioning in embryo, larva and/or adult, suggesting that it might be that it is easier to incorporate novel functions into a pre-existing enhancer than to create developmentally specific enhancers anew.

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Program/Abstract # 25
Transcriptional mechanisms underlying Sonic hedgehog mediated regulation

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The Sonic hedgehog (Shh) pathway plays critical roles in development and cancer. All Shh activity is mediated by Gli proteins, which act as context-dependent transcriptional activators or repressors in the presence or absence of Shh pathway stimulation. According to current models, the ratio of Gli-activator to repressor forms gives rise to a quantitative transcriptional output. While several studies have persuasively demonstrated a quantitative role for Gli-activator activity, the role of Gli-repressors remains poorly understood. We identified a long-range Gli binding site in the Gremlin locus that has Gli-dependent enhancer activity in transgenic embryos, and exhibits a spatiotemporal pattern consistent with control by Shh. To determine how this domain is regulated by Gli activator and repressor forms, we examined its activity in a number of different Shh pathway mutants. Our results suggest that the enhancer domain requires Gli-activator activity but is not repressed anteriorly by Gli-repressor forms. In light of these results, we were surprised to find that Gli repressor binding to the enhancer does play a direct role in preventing ectopic Gremlin transcription by repressing activity from other cis-regulatory domains. Together, our results

suggest a model for Shh-directed transcription where the Gli-bound cis-regulatory domain acts as a toggle switch to impose Gli-dependent control over genes with multiple cis-regulatory domains.

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Program/Abstract # 26
Hedgehogome: Hedgehog signaling proteome analysis for understanding craniofacial and brain development
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Loss and gain of Hedgehog (Hh) function in humans cause craniofacial and brain abnormalities such as holoprosencephaly and Gorlin and Grieg syndromes respectively. Holoprosencephaly is associated primarily with mutations in SHH, while Gorlin and Grieg syndromes have been mapped to mutations in PATCHED1 and GLI3. Hh signaling therefore is critically required for proper craniofacial and brain development and must be tightly regulated, however our understanding of the gene and protein networks governing Hh signaling remains incomplete. To identify new Hh protein signaling complexes ("Hedgehogome") we performed proteomic analyses of mammalian Hh signaling proteins using (1) flag tagged Hh proteins expressed in human Hek293 and stable mouse NIH3T3 cell line and (2) cell-free protein synthesis together with mouse embryo lysates. These approaches have successfully identified novel candidate proteins that are important for mediating Hh signaling. Here we describe the function of A kinase anchoring protein (AKAP11) which associates with the Hh receptor, Patched1 and Hh transducer, smoothened to modulate cell death, proliferation and dorso-ventral patterning of neural tube via PKA signaling activity.

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Program/Abstract # 27
A computational model reveals the remarkable patterning potential of the Wnt–FGF gene regulatory network in the posterior lateral line primordium
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Interactions between the Wnt and FGF signaling systems contribute to the coordination of cell fate and morphogenesis in the posterior lateral line primordium (pLLp) as it migrates caudally depositing neuromasts from its trailing end. Wnt signaling at the leading end of the pLLp promotes proliferation and drives expression of FGF ligands. However, as the Wnt system locally inhibits activation of the FGF receptor, FGF ligands, secreted in response to Wnt signaling, diffuse away from the Wnt system and activate FGF signaling in an adjacent trailing domain. Activation of FGF signaling locally inhibits Wnt signaling and this facilitates establishment of a stable FGF signaling center. This FGF signaling center initiates formation of proto-neuromasts by promoting formation of epithelial rosettes and by initiating center-biased expression of atoh1a. We have built a computational model of this genetic regulatory network in the pLLp incorporating the local inhibitory relationship between the Wnt and FGF signaling systems, coupled with long distance support of the FGF system by Wnt-dependent FGF ligand production. It reveals the remarkable patterning potential inherent in this simple genetic network. The model shows how a center-biased FGF signaling center could be established in proto-neuromasts, and how the pattern of Wnt and FGF signaling is expected to change in the trailing domain of the pLLp, where FGF signaling dominates and Wnt signaling is weaker. The model also shows how Wnt-dependent proliferation is expected to create the conditions for spontaneous generation of new FGF signaling centers at the leading end of the pLLp. We are now experimentally testing a number of predictions and specific assumptions of this model.

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Program/Abstract # 28
Modulation of hindlimb gene expression patterns by Pitx1
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Despite the characterization of numerous genes required for limb development, remarkably little is known about the mechanisms determining forelimb vs. hindlimb identity. Pitx1 is a homeodomain transcription factor that is differentially expressed in hindlimbs and implicated in the specification of hindlimb morphology. Earlier studies suggest that the hindlimb transcription factors Tbx4, HoxC10, and HoxC11, are likely direct transcriptional targets of Pitx1, but definitive evidence for this regulatory interplay is lacking. We sought to pinpoint the binding sites that mediate Pitx1 function and to identify additional genes that Pitx1 may directly regulate. Using a ChIP-seq approach in embryonic mouse hindlimbs, we identified thousands of putative Pitx1 binding sites and found these to be significantly enriched near genes involved in limb morphogenesis, including Tbx4, HoxC10, and HoxC11. Analysis of Pitx1-enriched ChIP sequences shows significant overlap of Pitx1 binding sites with functionally verified limb enhancers. Notably, we identified a known hindlimb-specific enhancer in the Tbx4 gene as a binding site for Pitx1. Cell culture studies demonstrate that this hindlimb enhancer is up-regulated in the presence of Pitx1, and in vivo studies show that mutation of the highly conserved Pitx1 binding site in this Tbx4 enhancer results in substantially reduced enhancer activity in mouse embryos. Our findings validate the biological relevance of a Pitx1 binding site in the regulation of Tbx4 expression and provide proof of the direct regulation of Tbx4 by Pitx1. Future work will focus on a subset of putative Pitx1 binding sites that exhibit extreme conservation and that are located near key limb-patterning genes.

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