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Abstracts

Patterning and Transcription Factors

Program/Abstract # 399

Regulatory elements encoded in the first intron are necessary for proper expression of the MADS-box transcription factors AGL6 and AGL13 in *Arabidopsis thaliana*

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Screening enhancer detector lines in *Arabidopsis thaliana* has identified genes that are specifically expressed in the diploid maternal sporophytic tissue of the ovule. One such gene is the MADS-box transcription factor *AGAMOUS-LIKE6* (*AGL6*), which is expressed asymmetrically in the endothelium layer of the ovule, adjacent to the developing haploid gametophyte. *AGL13*, the other member of the *AGL6* subfamily present in *Arabidopsis*, has an overlapping expression pattern in the ovule and both are likely to be functionally redundant, as neither null mutant has a phenotype. For the *AGL* genes where it has been examined, the large first intron contains key *cis*-regulatory elements. Analysis of the transcriptional regulation of both *AGL6* and *AGL13* indicates that Intron 1 is critical for proper expression for both genes: encoding an endothelium specific enhancer element in *AGL6* and encoding a silencer element in *AGL13*. Using a duel enzymatic gene reporter system, the overlapping expression of *AGL6* and *AGL13* was localized only to the developing nucellus. Analysis of the amino-acid structure of members of the *AGL6* subfamily from related *Brassicaceae* indicates considerable divergence in the MADS-box DNA binding domain between *AGL6* and *AGL13* that is not present in the other conserved domains.

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Program/Abstract # 400

OsMADS1 as a transcriptional regulator of rice floral organ fate affects auxin and cytokinin signaling pathways

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The SEPALLATA genes encode a functionally diverse group of MADS domain transcription factors including redundant floral organ fate determinants in *Arabidopsis* and non-redundant factors like *OsMADS1* in rice. Earlier functional studies show that *OsMADS1* regulates the identity of all floret organs and controls floret meristem determinacy. To investigate *OsMADS1* as a regulator of floret development we have identified its likely target genes using microarray analysis. Many of the affected genes in *OsMADS1* knockdown panicles belong to auxin and cytokinin signaling pathways. Transcripts for 11 of the 25 rice auxin response factors (ARFs) including OsETTIN1 and 2; for a third of all predicted rice Aux/IAA proteins that are repressors of ARFs and nearly half of all GH3 family of proteins involved in hormone homeostasis are affected. Further, genes encoding cytokinin biosynthetic enzymes (e.g. *LOG*) and response regulators (*OsRR1* and *OsRR9*) are up-regulated. The consequences of mis-expression of an *OsMADS1* target, *OsMGH3*, an auxin responsive member of GH3 family were examined in transgenic plants. Its ectopic over expression creates extreme dwarfs with no apical dominance, while its targeted over expression during panicle branching reduces panicle length—a phenotype also seen on *OsMADS1* overexpression. The enlarged carpel phenotype created on its partial knockdown may arise from loss of floral determinacy and could provide a plausible mechanism for the determinacy defects of *OsMADS1* mutants. Overall our data reinforce the role of hormone homeostasis and its transcription regulation during floral organ development.

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Program/Abstract # 401

Pattern formation in leaves via small RNA mobility

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Small RNAs, including microRNAs (miRNAs) and *trans*-acting short interfering RNAs (ta-siRNAs), have important regulatory roles in development. In plants, members of these classes of small RNAs act to pattern the adaxial–abaxial (dorsal–ventral) axis of leaves. The *AUXIN-RESPONSE FACTOR* (*ARF*) family members *ARF3* and *ARF4* are together necessary to establish abaxial (ventral) fate in leaves. *ARF3* and *ARF4* are targets of ta-siRNAs that are termed "tasiR-ARFs." To begin to understand the possible role of tasiR-ARFs in leaf polarity, we have localized the biogenesis components of the tasiR-ARF pathway, including the microRNA miR390, the activity of the *ARGONAUTE* gene required for miR390 activity (*AGO7*), and the activity of tasiR-ARFs themselves. We provide evidence that the tasiR-ARF pathway in *Arabidopsis* acts non-cell autonomously to maintain the polarized accumulation of *ARF3* in leaf primordia. Small RNAs (both miR390 and tasiR-ARFs) in this

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specialized RNAi pathway may contribute to its non-cell autonomous activity, as they accumulate outside their discrete regions of biogenesis. We propose that small RNAs can possibly function as mobile inductive signals to direct patterning events during development.

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Program/Abstract # 402
Early zygotic gene regulatory network for epidermis in the ascidian *C. intestinalis*
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The epidermis is the principal interface between an animal and its environment, and generally the largest organ system. Basic epidermal cell specification in ascidians is initiated by maternal determinants. However, the early zygotic homeobox transcription factor *CiDll-B* has been shown to regulate a number of epidermal target genes, suggesting that it is a pivotal element in the epidermal cell specification network. *CiDll-B* is also one of the earliest genes to be expressed throughout, but restricted to, non-neural ectoderm. A misexpression transgene, using *CiFoxA-a* regulatory DNA to drive expression in non-epidermal territories downregulates the expression of a reporter transgene driven by the same regulatory DNA. This suggests that an additional function of *CiDll-B* may be to repress transcription of genes, such as *FoxA-a*, inappropriate to epidermis. *CiDll-B* upstream and intergenic *cis*-regulatory elements have been located which drive robust expression of reporter transgenes in the endogenous *CiDll-B* territory. This *cis*-regulatory DNA is being used to manipulate expression of a dominant negative form of *CiDll-B* to test the effect of downregulation of Dll-B on putative target genes, the aim being to build an understanding of the epidermal gene regulatory network in this simple chordate.

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Program/Abstract # 403
The *C. elegans tailless* ortholog *nhr-67* functions in uterus and tail development
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The *tailless* family of nuclear receptors is highly conserved among animals. In *Drosophila*, *tailless* (*tll*) functions in terminal embryonic patterning and central nervous system development. In vertebrates, Tlx functions in the maintenance of neural stem cell identity, but does not play a known role in terminal patterning. The *C. elegans tll* ortholog, *nhr-67*, is expressed in a dynamic pattern in pre-uterine cells. *nhr-67* is initially expressed in the 4 pre-VU cells, whose progeny form much of the uterus. *nhr-67* is upregulated in one of these four cells, the anchor cell (AC), in response to a *lin-12/Notch* reciprocal signaling system. During the L3 stage, *nhr-67* expression is maintained at high levels in the AC and at low levels in the six π cells whose twelve progeny form cells of the adult ventral uterus. Development of the π cells also depends on a *lin-12/Notch*-based signal from the AC. The development of the π cells is defective in *nhr-67* mutants, but the AC appears normal. Genetic analysis demonstrates that *nhr-67* functions downstream of *lin-12* in the ventral uterus. We are characterizing the nature of the *nhr-67* uterus defect by electron microscopy. Strong alleles of *nhr-67* arrest development in the L1 after hatching. The arrested larvae display tail defects in the hyp10 epithelial cell similar to those caused by mutations in the cadherin gene *cdh-3*. This observation suggests that a function for *tailless* in terminal development may be conserved among the ecdysozoa.

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Program/Abstract # 404
***Drosophila* CtBP causes local inhibition of Dorsal and dCBP that regulate neuroectoderm genes**
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Transcriptional repression mediated by *Drosophila* C-terminal Binding Protein (dCtBP) together with the DNA-binding repressor Snail specifies mesoderm by excluding the neuroectodermal fate in the early embryo. dCtBP interacts with Snail through the PxDLS amino acid motifs and acts as a corepressor. The Snail/dCtBP repressor complex is only able to repress adjacent activators located within 100 bp. In the last meeting, we presented the first analysis of the molecular mechanisms by which dCtBP mediates this short-range repression at the chromatin level. Particularly, we showed using chromatin immunoprecipitation (ChIP) assays that (a) the Snail/dCtBP complex locally inhibits the DNA-binding of the Dorsal activator to a neuroectodermal enhancer that are regulated by both Snail and Dorsal, but (b) recruitment of Dorsal's coactivator dCBP is not prevented, and that (c) histone H4 is, however, hypo-acetylated. These data suggested that dCtBP acts by locally preventing DNA-binding of adjacent activators, possibly by inhibiting dCBP's HAT activity. We are currently testing whether this is employed for regulation of other neuroectodermal enhancers. In addition, we are extending ChIP assays to core promoter factors to understand how the neuroectodermal enhancer when repressed by the Snail/dCtBP complex communicates with its core promoter. We are testing promoter-enhancer interaction using Chromosome Conformation Capture assay. These experiments should provide details on the molecular mechanisms of dCtBP-mediated short-range repression.

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Program/Abstract # 405
Dispensable function of the B′ regulatory subunit of Protein Phosphatase 2A (PP2A) in *Drosophila melanogaster*
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PP2A is a major serine/threonine phosphatase that has roles in diverse processes from gene regulation, protein syntheses to cytoskeleton organization. PP2A is proposed to dephosphorylate and activate the HOX protein Sex combs reduced (SCR). SCR is required for development of the larval first thoracic and labial segments, and the adult first thoracic segment and proboscis formation in *Drosophila melanogaster*. PP2A activity is composed of a set of enzymes that are composed of common catalytic and core subunit, and distinct regulator subunits. One of the regulatory subunits, B' interacts with the homeodomain of SCR (Berry and Gehring 2000). Using FLP-mediated site specific recombination I created a complete deletion of the *PP2A-B′* coding sequence. To our surprise, flies homozygous for the deletion were viable and wild type in appearance. We did not observe a large