Program/Abstract # 256
A sulfotransferase (SpSult) is required for mesoderm and endoderm developments in sea urchin
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The sulfotransferase (suit) gene family generally catalyzes the sulfate conjugation of a broad range of substrates. In development, suit has been shown to have an important role in regulating Notch (N) and FGF signaling. In sea urchin, a suit gene (SpSult) expressed exclusively in pigment cells was previously isolated. SpSult transcription starts at the blastula stage in pigment cell precursors and it is maintained throughout the pluteus stage. SpSult belongs to a differentiation gene battery positively regulated by the 7th–9th cleavage N signaling. In this study we analyzed the SpSult function in sea urchin development. SpSult knock-down embryos showed abnormalities in both endoderm and mesoderm developments. Pigment cell number was higher, approximately doubled. Moreover, differentiated pigment cells showed an abnormal morphology and distribution. Spiculogenesis was also abnormal and the gut was absent or severely under-developed. Gene expression analysis showed a down-regulation of key pigment cell specific transcription factors that are directly regulated by N. The down-regulation of this transcription factors occurred within 2 h from the onset of SpSult transcription during the blastula stage. Their expression was back to normal levels by late gastrula. Our data suggest that SpSult negatively regulates the development of the sea urchin embryo.

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Program/Abstract # 255
The phylogenetically conserved C. elegans T-box factor TBX-2 is SUMOylated
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T-box transcription factors are crucial developmental regulators in all multicellular organisms, and mutations in T-box genes are implicated in both cancer and congenital diseases. Despite the importance of these regulators, remarkably little is known regarding the mechanisms they use to regulate target gene expression. SUMOylation has recently been implicated in the function of T-box factors in humans and C. elegans. We are using the C. elegans TBX-2 subfamily member TBX-2 as a model to study T-box factor SUMOylation. TBX-2 interacts with the C. elegans E2 SUMO conjugating enzyme UBC-9 and the E3 SUMO ligase GEI-17 in yeast-two-hybrid assays, and it is SUMOylated in in vitro reactions using human SUMOylation enzymes and when expressed in mammalian cells. We have identified two consensus SUMOylation sites located in the T-box and near the C-terminus, respectively, that mediate TBX-2 interaction with UBC-9 and GEI-17 in two-hybrid assays. The C-terminal site is the primary SUMOylation site in vitro and in mammalian cell assays, although the T-box site may also be SUMOylated. In C. elegans, UBC-9 (RNAi) strongly enhances the lethality of a hypomorphic TBX-2 mutant, consistent with the hypothesis that TBX-2 functions as a SUMO-dependent transcriptional regulator. We are currently asking if TBX-2 is SUMOylated in C. elegans and characterizing the effect of SUMOylation on TBX-2 function.

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Program/Abstract # 257
A genome-wide study of the maternal-to-zygotic transition transition
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The maternal-to-zygotic transition (MZT) is a critical developmental transition following fertilization and is characteristic of a dramatic epigenetic regulation of gene expression. Prior to the MZT, the zygotic genome is globally silenced by a number of epigenetic regulators, including DNA methylation, histone modifications, and nucleosome remodeling. During the MZT, maternal gene products are destructed in a well-controlled manner, allowing the proper activation of the zygotic genome. Recent studies in Drosophila begin to uncover molecular mechanisms governing Drosophila MZT. In contrast, only a few vertebrate MZT regulators have been studied so
far. To identify genes and pathways regulating vertebrate MZT, we have performed a unique phenotypic screen in Xenopus embryo. A number of potential MZT regulators have been identified. These include RNA binding proteins, protein kinase, epigenetic regulators, and signaling molecules. Our work thus builds up an important foundation for studying epigenetic regulation of gene expression during vertebrate MZT.

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Program/Abstract # 259
Serine protease activation of the epidermal wound response in Drosophila
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Following injury, breaches in the skin or cuticle are repaired by the epidermal wound response to restore barrier integrity. However, the manner by which nearby unwounded epidermal cells sense the wound and begin the process of repair is largely unknown. Here we show that treatment of embryonic epidermal cells with the serine protease trypsin can activate a global wound response. Part of the evidence for this is that wound enhancers from four different genes, originally identified by their abilities to be activated and localized around epidermal puncture wounds, are activated throughout the epidermis by serine protease treatment. The genes activated by this treatment include Dopa decarboxylase (Ddc) and tyrosine hydroxylase (ple), chitin synthase (kkv), and Missshapen (msn). Serine protease activation of the epidermal wound response can be activated by body cavity injection. This serine protease activation can also be effected by injection into the perivitelline space, which is not associated with a loss of epidermal integrity. Injections of the serine protease inhibitor aprotinin resulted in highly reduced expression levels of the wound response gene msn surrounding the wound site. Proteases from other families, such as the cysteine protease papain, do not activate the epidermal wound response as robustly. Serine protease treatment is likely to generate widespread activation of a wound response ligand, initiate a signaling pathway, and activate genes necessary for restoring epidermal integrity. We have used the trypsin-mediated wound response to screen Drosophila microarrays to determine the genomic response to epidermal wounding in late embryos/early larvae.

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Program/Abstract # 260
Functional analysis of a UBX-responsive regulatory element
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Although Hox proteins play a major recognized role in establishing anterior–posterior pattern in developing animals, identification of direct target genes is challenging. Most Hox proteins can bind the DNA sequence TAAT, and co-factors, such as Extradenticle, increase the DNA binding specificity. However, in many instances specific co-factors are not known, so the mechanisms for discriminating between target and not-target sequences are poorly characterized. Only a single conserved Ultrabithorax (UBX) binding site is necessary for the activation of a cis-regulatory element (CRE) for the CG13222 gene in the developing Drosophila melanogaster haltere. Here we have identified an additional sequence important for the activation of this gene through characterization of a minimal CRE and mutagenesis of specific sequences flanking the critical UBX site. Additionally, we have introduced homologous CRE sequences from several species of Drosophila into D. melanogaster. Changes in the expression pattern driven by the D. ananassae CRE suggest alterations in the cis-sequences regulating expression. In addition, the D. pseudoobscura CRE when introduced into D. melanogaster drives an expression that does not match the endogenous D. pseudoobscura pattern, suggesting changes in the trans-regulatory landscape between the two species. Therefore, at this single CRE, we are able to observe changes in both cis- and trans- that affect regulation of a UBX-target gene.

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Program/Abstract # 261
Investigating the regulatory sequences of dpp required for negative feedback of dpp transcription
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Patterning a uniform field of cells can be achieved by positional information provided by morphogen gradients. In the Drosophila wing disc, the BMP signaling pathway acts as a morphogen through a graded distribution of phosphorylated transducer, Mad. The pMad gradient, formed in response to the ligands dpp and gbb, directs distinct transcriptional responses of target genes to specify cell fates. As a morphogen system, it is vital that cells receive proper levels of signaling and that both generation and maintenance of the pMad gradient are highly regulated. Work in our lab has demonstrated that a negative feedback loop exists in the wing disc on dpp transcription. We believe that this serves to “fine tune” signaling in the event of altered activity levels. Expression of dpp reporters respond to BMP signaling, showing increased expression when signaling is reduced and decreased expression when signaling is increased. These experiments seek to identify the dpp enhancer sequences required for negative feedback. I have tested several reporters containing different regions of dpp enhancer sequence for their response to BMP signaling levels. I have also tested the requirement for binding of known dpp regulators by examining these reporters with mutated transcription factor binding sites. I have determined that BMP signaling regulates both the level and domain of dpp expression. In addition, feedback is not due solely to any one of the factors known to regulate the wing disc dpp expression that I have tested so far. Further experiments aim to identify the factors required for negative feedback of dpp and the mechanism by which this is achieved.

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Program/Abstract # 262
Akirin links Twist transcription factor activity with the Brahma chromatin remodeling complex during embryogenesis
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The activities of developmentally critical transcription factors (TFs) are regulated via interactions with accessory proteins. Such interactions either directly influence TF activity through binding and dimerization or indirectly promote gene activation by promoting a favorable chromatin environment for gene activation. Using a modified yeast two-hybrid screen, we identified akirin, a highly conserved nuclear protein, as a novel cofactor of the Drosophila muscle transcriptional regulator, Twist. Like twist hypomorphic