Hedgehog (Hh) signaling controls gene transcription through the Gli family of transcription factors, including Drosophila Ci. In the absence of Hh, Ci acts as a transcriptional repressor, but upon signaling activation, Ci becomes a transcriptional activator. Two Hh target genes, patched (ptc) and decapentaplegic (dpp) contain enhancers with Ci binding sites. The ptc enhancer has three high-affinity Ci sites, whereas the dpp enhancer has only low-affinity sites. In the developing wing, ptc is expressed in a narrow stripe of cells that receive the highest levels of Hh, while dpp is expressed in a broader stripe in a region of moderate signaling. We found that dpp requires low-affinity sites for optimal activation by Hh, as replacing the low-affinity binding sites of dpp with high-affinity sites from ptc caused repression of dpp in cells that receive moderate signaling. Because higher binding affinity correlates with increased Ci occupancy of these enhancers, our results are consistent with the idea that Ci may act more cooperatively as a repressor than as an activator in cells that have both forms of this transcription factor. To test this hypothesis, we quantified the expression of synthetic enhancers with three high-affinity sites versus one high-affinity site. Our data showed that having a single high-affinity site abolishes the transcriptional repression observed in enhancers with multiple high-affinity sites. These results are consistent with the cooperative repression model, thus we propose a novel transcriptional mechanism to interpret the Hh signaling gradient.

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Program/Abstract # 556
The mutational basis for the repeated evolution of a cis-regulatory element generating morphological diversity
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A central goal of evolutionary developmental biology is to elucidate the gradual progression of mutational steps by which development, and thereby traits evolve. Empirical and theoretical studies implicate mutations in cis-regulatory element (CRE) sequences, which control gene expression, as a prominent route by which development evolves. However, few studies have determined both the mutational (the identity of the evolutionarily relevant mutations) and molecular (biochemical property altered) basis of CRE evolution. Hence, this type of evolutionary path remains poorly understood. One excellent model trait to study CRE evolution is the diverse abdominal pigmentation patterns exhibited by species of the Drosophilinae subfamily. These patterns have evolved by modifications to a well-characterized gene regulatory network. Male-specific sexually dimorphic pigmentation of *Drosophila melanogaster* is a particularly tractable trait controlled by the Bric-à-brac (Bab) transcription factor proteins. Previously, we identified a CRE controlling sexually dimorphic Bab expression, and elucidated how it functions and evolved in one lineage. Here we show that alterations in this CRE contribute to pigmentation variation within a species and furthermore differences in orthologous dimorphic elements similarly correlates to pigmentation differences between closely-related species. Using ancestral reconstruction methods, we determined the sequence and gene regulatory activity of the dimorphic element possessed by various ancestors at key phylogenetic nodes. Moreover, here we present data that has begun to trace the mutational and molecular mechanistic path by which descendant CREs with distinct activities evolved.

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Program/Abstract # 557
Premature differentiation and reversal of imprinted X-Chromosome inactivation in extraembryonic ectoderm lacking paternally derived Xist
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Through the process of X-Chromosome Inactivation (XCI), somatic cells of mammalian females inactivate one of their two X-Chromosomes in order to balance X-linked gene dosage with their male counterparts. During mouse embryogenesis, two forms of XCI are observed, imprinted and random. Imprinted XCI inactivates the paternally inherited X-Chromosome (Xp) and occurs in the extraembryonic lineages. Random XCI occurs in the embryonic lineages where either the Xp or the maternally inherited X-Chromosome (Xm) can be inactivated. The process of XCI is dependent upon the long non-coding RNA Xist, which is expressed from and coats the inactivated X-Chromosome (Xi) in cis. Consequently, females harboring a paternally derived Xist mutation (Xist+/−) die due to failure of imprinted XCI and poor trophoblast development. Here, we investigate the consequence of two active X-Chromosomes in the extraembryonic ectoderm (ExE) of Xist+/− female embryos. At embryonic day 6.5, we find that the Xist+/− ExE no longer proliferates and lacks the transcriptional regulator Cdx2, a factor required to maintain the ExE in a progenitor state. Curiously, we observe an Xi in a few cells of the ExE. When grown in culture, Xist+/− embryo outgrowths retain Cdx2 and harbor an Xi in some Cdx2+ cells. Trophectodermal stem cells derived from Xist+/− embryos harbor an inactive Xm, consistent with a reversal of imprinted XCI. Taken together, our data suggests that poor trophectoderm development in Xist+/− embryos is due to premature differentiation of the trophoblast progenitors. Furthermore, the capability of Xist+/− ExE cells to reverse imprinted XCI suggests that the ExE no longer retains the initial imprint required for imprinted XCI.

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Program/Abstract # 558
A role for Xenopus Zygote Arrest 2 (Xzar2) in the regulation of key cell cycle mRNAs
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Zygote Arrest proteins, Zar1 and Zar2 (aka Zar1-like) have been implicated in the oocyte to embryo transition, zygotic genome activation, preimplantation development and epidermalization, and disruption of these proteins causes zygotic arrest at the 1 or 2-cell stage in mouse embryos. However, the mechanism of action of Zar proteins is unknown, transcriptional regulation, chromatin remodeling and RNA metabolism, have all been suggested. Early development is regulated by maternal mRNAs that have specific combinations of cis-elements in their 3′ untranslated regions (UTR) that determine where, when and to what extent each mRNA is translated. The mRNA of a key cell cycle regulator of embryogenesis, Wee1, is translated during oocyte maturation. The Wee1 mRNA 3′ UTR contains a cis-element called the Translation Control Sequence (TCS) that regulates mRNA translation during meiotic maturation of Xenopus oocytes. The protein that binds to the TCS is unknown. Here, we show that

Xenopus Zygote Arrest 2 (Xzar2) binds to the TCS in the 3′ UTRs of the key cell cycle mRNAs, Mos and Wee1. Dominant inhibitory Xzar2 also attenuates the accumulation of Mos and Wee1 proteins during meiotic maturation of Xenopus oocytes. We propose that one role of the Zar proteins in early development may be to regulate the synthesis of maternal cell cycle proteins in the maturing oocyte in anticipation of their roles in fertilization and embryogenesis.

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Program/Abstract # 559
Asian Sand Dust (ASD)—Particle Matter (PM) effect on overexpress of tissue Transglutaminase2

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During springtime in the East Asia, Asian Sand Dust (ASD)—Particular Matter (ASD-PM) from China and Mongolia desert areas over to East Asia on the westerlies and is generally thought to threaten the East Asian health by provoking respiratory illness like bronchitis and asthma and conjunctivitis. And tissue Transglutaminase (tTG) are enzymes that are widely used in biological systems and can contribute to various pathophysiologies. tTG participates in posttranslational modification reactions and affect to blood coagulation, skin barrier formation, inflammatory, autoimmune and tissue repair. In this study, we examined how ASD-PM associates lung fibrosis and hepatocyte. C57BL/6 mice were exposed to saline suspensions of ASD particle 3 times a week for 4 weeks, 8 weeks, and 12 weeks. Following exposure with ASD, the liver was analyzed by immunochemistry using hematoxylin and eosin (H&E) and Masson's trichrome (MT) staining. We studied Transglutaminase mRNA (Tg mRNA) and tTG expression, using Real-Time PCR and Western Blot in mice hepatocyte treated with ASD. Long term exposure to ASD showed significant collagen accumulation in the liver as compared with short term mice. And long term exposed sample also overexpress Tg mRNA and tTG in hepatocyte. As a result, ASD-PM accumulates collagen and causes overexpression of Tg mRNA and tTG. Our results suggest that if people or animals are exposed to ASD, ASD will damage the lung and liver and result to fibrosis.

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Program/Abstract # 560
The expression of urokinase-type plasminogen activator is induced in cultured mouse blastocyst by the high glucose concentration

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During embryo implantation in mammals, the blastocyst penetrates the uterine wall at different depths by an invasive process, involving proteases that degrade the extracellular matrix (ECM), including matrix metalloproteinase 9 (MMP9) and urokinase-type plasminogen activator (PLAU). Plasminogen is activated to plasmin by PLAU and plasmin degrades ECM and activates some MMPs, like MMP-9. PLAU and MMP-9 are expressed in primary trophoblast cells of mouse blastocyst in vivo and in vitro and they are secreted abundantly during embryo implantation. High concentration of glucose affects the synthesis and degradation of the ECM in different cell types, because it induces the formation of reactive oxygen species (ROS) that alter the expression of PLAU and MMPs. Therefore the effect of glucose on the expression of PLAU in cultured mouse blastocysts was evaluated. Gestation fourth blastocysts were cultured in HAM-F-10, and high glucose 25 mM, was added in different schedules, glucose 6 mM was used as a control, the expression of PLAU was evaluated using real time RT-PCR and amidolytic assay. Glucose 25 mM inhibits hatching (−25%) and induces a higher activity of PLAU in the conditioned medium and enhanced the level of PLAU mRNA in embryo extracts obtained after four days of culture. Hydrogen peroxide (10 mM) induces similar increase in PLAU activity in the conditioned medium. High concentrations of glucose promote oxidative stress, due to increased formation of ROS, which probably increased the expression of PLAU in trophoblast of mouse blastocysts. Supported by PAPIT, DGAPA, UNAM, grant IN230611.

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Program/Abstract # 561
Comprehensive survey and perturbation of the transcriptional control of Ptf1a

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Pancreatic transcription factor 1a (Ptf1a) participates in the formation of the ternary complex Ptf1, that has a critical role in pancreas specification and is involved in cell fate choices within the pancreas and additionally in the retina, cerebellum, hindbrain and dorsal spinal cord. The proximal promoter of mouse Ptf1a can partially recapitulate pancreatic expression; in addition, an autoregulatory element positively regulates Ptf1a expression in the pancreas, dorsal spinal cord and hindbrain, while a conserved immediate downstream area has dorsal spinal cord activity. Analysis of a spontaneous deletion of downstream Ptf1a non-coding sequence suggests that necessary regulatory elements for proper cerebellar and pancreatic development remain to be identified. We sought to assess the role of Ptf1a autoregulation in a tractable genetic system. As a first step towards this direction we comprehensively characterized cis-regulatory elements tiling the entire sequence of a BAC that recapitulates endogenous expression in zebrafish. We discovered previously uncharacterized regulatory elements with activity in the hindbrain, retina and spinal cord, and also identified a zebrafish autoregulatory enhancer with comparable activity to the known mouse enhancer. Using the BAC transgene that contains all necessary Ptf1a regulatory sequences, we mutated the Ptf1 binding sites within the autoregulatory enhancer, thus perturbing the autoregulatory loop. We are currently testing the ability of the mutated transgene to rescue the Ptf1a null phenotype, to determine the role of autoregulatory control in the dynamics of allocation between opposing cell fates and the stability of terminal differentiation.

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Program/Abstract # 562
Multiple cis-acting enhancers regulate temporal and spatial expression of the human LHX3 gene in the developing pituitary

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LHX3 is a LIM homeodomain transcription factor necessary for proper development of the pituitary and central nervous system.