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 Misshapen (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, can 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

mutants, *akirin* mutant embryos have misattached or missing muscles and severely altered muscle morphology. *Akirin* interacts genetically and physically with Twist and is localized to Twist-dependent enhancers *in vivo*. Accordingly, Twist target gene expression is highly reduced in *akirin* mutants. While Akirin has been identified as a component of other transcriptional pathways, its mode of action in these pathways remains unclear. We determined that *akirin* colocalizes and genetically interacts with subunits of the Brahma SWI/SNF-class chromatin remodeling complex at Twist-target genes. This suggests that *akirin* mediates a novel link between Twist and chromatin remodeling complexes to facilitate Twist-regulated transcription during *Drosophila* myogenesis. These results also provide a common mechanism by which *akirin* regulates the activities of multiple TFs during development, the immune response and homeostasis.

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Program/Abstract # 263
The Drosophila estrogen-related receptor is required for the transition from embryonic to larval metabolism
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Drosophila larval metabolism is exquisitely tuned to promote exponential growth. This growth state is in contrast with embryonic metabolism, which is dependent on intrinsic energy reserves. Despite these differences, little is known about how a developing animal transitions between embryonic and larval metabolic states. We have discovered that the *Drosophila* ortholog of the vertebrate estrogen-related receptor, dERR, directs this developmentally-regulated metabolic switch. dERR null mutants die as second instar larvae with abnormally low ATP levels, diminished triglyceride stores, elevated levels of circulating sugars, and decreased concentrations of lactate, α-ketoglutarate, and malate. These defects can be attributed to reduced expression of key genes in several metabolic pathways, including all of the genes that act in glycolysis. dERR appears to directly regulate these pathways as there are putative dERR binding sites in nearly all of the misregulated genes examined. Intriguingly, the metabolic pathways induced by dERR at the onset of larval development are similar to the Warburg effect, by which cancer cells use glucose to support biomass production and rapid proliferation. Our results demonstrate that the Warburg effect can be utilized in the context of normal developmental growth, indicate that dERR establishes a metabolic state that supports larval development, and implicate the ERR receptor family as central regulators of the metabolic parameters that support cancer. This work was supported by the NIH (1R01DK075607). JMT was supported by the NIDDK (F32DK083864).

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Program/Abstract # 264
Novel animal model for studying the roles of the upstream open-reading frames
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Regulation of gene expression may be achieved at multiple levels. Among the regulatory mechanisms, translational control is an immediate early response that becomes crucial in the absence of transcription. It is now known that the upstream open reading frames occur in approximately 10%–25% of 5′-UTRs and have been generally found to repress translation of the downstream open reading frame. However, these upstream open reading frames mediated translational inhibition has less been studied *in vivo*. In this report, we developed an *in vivo* system to study the upstream open reading frame mediated translational inhibition by using model animal, zebrafish (*Danio rerio*). We generated a transgenic line *Tg*(*CMV*:*uGFP*), in which the upstream open reading frame from human CCAAT/enhancer-binding protein homologous protein is fused with GFP and driven by cytomegarovirus (CMV) promoter. We found that the GFP signal was not apparent under normal condition, although the *gfp* mRNA was transcribed throughout the embryos. These indicate that the translation of *gfp* is completely inhibited by the upstream open reading frame cassette. Interestingly, when *Tg*(*CMV*:*uGFP*) embryos were treated with thapsigargin, the GFP was greatly detected in the brain, indicating that environmental stimulus may direct the upstream open reading frame cassette to abolish its translational inhibition. Thus, zebrafish is an excellent animal model for studying the upstream open reading frame mediated translational inhibition.

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Program/Abstract # 265
The molecular structures and expression patterns of two distinct zebrafish Dickkopf 3 genes
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The Wnt signaling pathway is a cellular communication pathway that plays critical roles in development and disease. A major class of Wnt signaling regulators is the Dickkopf (Dkk) family, which is a secreted glycoprotein. The DKK family has been identified in birds and mammals, and has been known that it consists of *dkk1*, *2*, *3*, *4* and a *dkk3*-related gene (*soggy*). However, in low vertebrates, only *dkk1* has been defined, the others are still unknown. Here, we cloned two zebrafish *dkk3* genes, which were *dkk3* and *dkk3*-related genes *(dkk3r*, also named the long-isoform *dkk3*). Based on the unrooted radial gene tree analysis of the *dkk* genes among vertebrates, the zebrafish *dkk3* and *dkk3r* we cloned were homologous of the *dkk3* of other higher vertebrates. Using reverse transcription-polymerase chain reaction and whole-mount *in situ* hybridization, we demonstrated that both *dkk3* and *dkk3r* were maternally expressed. In addition, *dkk3* and *dkk3r* were ubiquitously expressed during 16 h post-fertilization (hpf). However, they were expressed in the head, somite and spinal cord at 24 hpf. Interestingly, while *dkk3* was particularly detected in the craniofacial neuron tissue after 24 hpf, *dkk3r* was restricted in craniofacial arch muscles and pancreas. These evidences suggested that *dkk3* and *dkk3r* shared the same expression patterns before 24 hpf, but displayed different patterns after 24 hpf. Thus, using zebrafish as our system model, it is suggested that the results, as noted above, may provide more insight into the molecular structures and expression patterns of the lower vertebrate *dkk3* genes.

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Program/Abstract # 266
B1 SOX coordinate cell specification with patterning and morphogenesis in the early zebrafish embryo
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The B1 SOX transcription factors SOX1/2/3/19 have been implicated in various processes of early embryogenesis. However,