programs and are necessary for cardiac and skeletal muscle functions in zebrafish.

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Program/Abstract # 270
Regulation of endoderm development by zebrafish Nipbl
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Heterozygous loss of the *Nipped-B-like* gene (*Nipbl*) is the most common cause of Cornelia de Lange Syndrome (CdLS). Although *Nipbl* is a cohesin-associated protein conserved among all eukaryotes, recent studies suggest that it regulates gene expression through mechanisms independent of cohesin's established role in chromatid cohesion. There are 2 *Nipbl* genes in zebrafish, *zNipbl-1* and *zNipbl-2*, and embryos injected with morpholinos targeting either gene (*zNipbl*-morphants) show defects in formation of the gut/visceral organs and heart with a range of severity from looping defects to organs duplication. Such morphants show significant reductions in *sox32*, *sox17* and *foxa2* expressions in endoderm cells, and we found that combined *sox17*/*foxa2*-double morphants exhibited gut bifurcations similar to *zNipbl*-morphants. Interestingly, the degree of *sox17* and *foxa2* suppression in *zNipbl*-morphants was greater than could be explained by the reduction in *sox32* (a known positive regulator of these genes), and the response of these genes to ectopic *sox32* was significantly blunted. These data suggest that *zNipbls* may influence *sox17* and *foxa2* transcription directly. In *zNipbl*-morphants, we also observed abnormal spatial expression of the left–right patterning genes, *lefty2* and *southpaw*, although Kuppfer's vesicle (KV), a critical organ for left–right patterning that depends on *sox32* function, formed normally, implying that *zNipbls* act downstream of KV formation. These results support the idea that *zNipbls* regulate embryonic development through modulating gene expression at multiple levels. Supported by NIH P01-HD052860.

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Program/Abstract # 271
Transcriptional regulation of GlyT2 and GAD67 in *X. laevis*
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GABA and glycine serve as the two most prevalent inhibitory neurotransmitters in the vertebrate CNS. Elucidating the processes involved in the adoption of the glycinergic and GABAergic phenotypes is critical to understanding overall CNS development. To this end we are examining the transcriptional regulation of the terminal differentiation genes glutamic acid decarboxylase (GAD67) and glycine transporter 2 (GlyT2) in GABAergic and glycinergic neurons respectively in *Xenopus laevis*. Many of these terminal differentiation genes demonstrate little regulatory sequence conservation across the vertebrate lineages. *In silico* analysis of the GAD67 and GlyT2 sequences was used to compare the genes to other members of the vertebrate lineages to identify areas of conservation that could potentially represent putative regulatory elements revealed little conservation in the GAD67 upstream region but several conserved sequences in the GlyT2 upstream region. Using this information we constructed a series of EGFP reporter constructs driven by different regions of the GAD67 or GlyT2 upstream regions. Embryos produced with constructs driven by 4.3 kb of upstream DNA revealed expression patterns comparable to that of endogenous GAD67, while GAD67 expression in the retina was observed with 111 bp of upstream DNA. Despite significant conservation between the *X. laevis* and other vertebrate GlyT2 upstream regions, no expression was observed using up to 5 kb of upstream DNA. This analysis will further our understanding of the mechanisms during expression of neural terminal differentiation genes.

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Program/Abstract # 273
Regulation of nervous system development by F-box mediated ubiquitination
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Development of the central nervous system (CNS) is a dynamic process during which the abundance of proteins is regulated temporally and spatially at the transcriptional level and by the degradation of proteins no longer needed. While much is known about the regulation of gene expression during neural development, little is known about the role of protein degradation. Studies of cell cycle regulation show that a major mechanism of protein degradation is through F-box proteins, which function in the recognition and recruitment of specific targets for the ubiquitin/26S proteasome pathway. To determine whether F-box proteins function in CNS development, we identified 53 putative F-box genes in the *Xenopus tropicalis* genome and annotated the family using phylogenetic approaches. We performed a genome-wide search for the neuron restrictive silencing element (NRSE), a motif required to silence neuronal genes in non-neuronal tissues, and identified 3 candidate F-box genes; Fbxl7, Fbxo16, and Fbxo41, all of which are expressed in neuronal tissues. Furthermore, we showed that overexpression of Fbxo16 causes loss of neurons suggesting an important role in neurogenesis. We are studying the role of each F-box protein with gain- and loss-of function analysis. We will also identify targets of F-box proteins using mass spectrometry and a candidate target approach.

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Program/Abstract # 274
hnRNP K: An essential element of a posttranscriptional regulon of multiple cytoskeletal mRNAs involved in axon outgrowth
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Axon outgrowth is controlled by extracellular cues and intrinsic factors that regulate the synthesis and cytoarchitecture of the neuronal cytoskeleton. Previously, we demonstrated that hnRNP K, an RNA binding protein, is essential for axon outgrowth in Xenopus and hypothesized that the axonless phenotype arising from hnRNP K knockdown was caused by dysfunctional posttranscriptional control of multiple mRNAs associated with the cytoskeleton. To identify potential neuronal hnRNP K targets in vivo, we analyzed mRNAs from juvenile Xenopus brain that co-IP'd with hnRNP K using microarrays. We observed significant enrichment in genes associated with intracellular transport and localization (37%) and with the cytoskeleton and its regulation (15%). Select mRNAs were further validated by qRT-PCR, most of which increased in expression coincidentally with axon outgrowth. Among these validated targets, three mRNAs representing components of each of the neuronal cytoskeletal polymers (microfilaments, microtubules, and neurofilaments) were