and a scaffolding protein. It is required for mouse yolk sac vasculogenesis, chorioallantoic fusion, and axis elongation. To better define the role of YAP in early embryonic development, we used MO-mediated YAP loss-of-function and injected mRNA gain-of-function assays in frog and zebrafish. In the absence of YAP, mesoderm induction was delayed and embryos did not progress normally through gastrulation. YAP gain-of-function assays expanded the neural plate (Sox2) and the paraxial mesoderm (Pax3), while inhibiting the induction and/or expansion of the neural crest and the preplacodal ectoderm. While YAP gain-of-function assays maintained and expanded the neural and muscle progenitor fields, neural and muscle differentiation were inhibited. YAP loss-of-function resulted in a complete loss of Pax3 expression, suggesting that Pax3 may be a direct target of YAP. Co-expression of YAP with a known interacting transcription factor, TEAD1, enhanced the expansion of Pax3 paraxial mesoderm expression. Chromatin immunoprecipitations for endogenous YAP, showed that it localizes to a conserved, previously undescribed, TEAD binding site within the 5′ regulatory region of Pax3. Thus, YAP is an important regulator of cellular differentiation, and Pax3 is a direct transcriptional target of TEAD and YAP within the paraxial mesoderm.

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Program/Abstract # 463
A new "Twist" on an old transcription factor
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Transcription factors are critical regulators during development. Execution of tissue-specific developmental programs depends on modulation of target genes by these factors, making it important to clearly understand transcription factor regulation. One factor that plays a role in mesoderm specification and differentiation is the basic helix–loop–helix (bHLH) transcription factor, Twist. In *Caenorhabditis elegans,* CeTwist plays two critical developmental roles. In a subset of undifferentiated mesodermal cells, CeTwist is important for patterning, including regulating the orientation and number of cell divisions. CeTwist is also required for the differentiation of a subset of muscle cells including the enteric and the egg-laying muscles. In order to address these very different roles for a single transcription factor, we investigated whether CeTwist has different dimer partners and unique cell-type-specific gene regulation in the mesoderm. To investigate CeTwist's cooperation with the bHLH partner CeE/DA, we used RNAi and loss-of-function mutations to show that CeE/DA functions in a smaller subset of cells than CeTwist. To define a role for CeTwist homodimers, we engineered tethered CeTwist dimers, which partially rescued a null CeTwist mutant. Finally, to understand how the regulation of the CeTwist gene contributes to its function, we identified intron enhancer elements that mediate autoregulation in differentiated tissues. Altogether, the studies indicate that CeTwist provides an interesting paradigm for transcriptional control, and the results lead to a model explaining the dose-dependent effects of this important transcription factor.

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Program/Abstract # 464
Dissecting the gene regulatory network regulated by activin/nodal signaling during *Xenopus* germ layer specification
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Activin/nodal (the TGF-β superfamily member) signaling pathway has a conserved and critical role in vertebrate mesoderm development. Fast1, a forkhead domain containing transcription factor also known as FoxH1, has been implicated to be a key "common" mediator of this signaling pathway. However, the phenotypes of FoxH1 deficient mice and zebrafish FoxH1 mutations are significantly milder than those of Nodal deficient embryos. The results taken together with the identification in *Xenopus* of other transcription factors such as FoxH1.2/Fast3, GTF2I, and GTF2Ird1 in mediating activin/nodal signaling suggest an altered model of activin/nodal signaling. Instead of FoxH1 playing an essential role in mediating all activin/nodal mediated mesoderm formation, there are other transcription factors that also participate in this process. In order to better understand the activin/nodal signaling gene regulatory network, we have generated polyclonal antibodies against FoxH1 to facilitate the identification of in vivo target genes. Additionally, we have annotated and identified 140 transcription factors that appear to be involved in early germ layer specification. Using the Nanostring nCounterTM analysis system, we have begun to dissect the gene regulatory network regulated by activin/nodal signaling in *Xenopus* germ layer specification.

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Program/Abstract # 465
The HoxB complex: Is there a beginning?
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Hox genes code for transcription factors that specify positional identity along the anterior–posterior (A–P) axis during early embryogenesis. In mammals, there are 39 *Hox* genes organized into four complexes (*HoxA*, *HoxB*, *HoxC*, *HoxD*) each located on a different chromosome. Their linear arrangement within each complex mirrors their sequential activation. This phenomenon is known as colinearity and it establishes different combinations of Hox proteins along the A–P axis. In order to develop a working model of colinearity, we have attempted to determine the regulatory beginning of the *HoxB* complex using a transposon-lacZ reporter system in BAC transgenesis. We found that the region preceding the *HoxB* complex has enhancer activity. When this area was further subdivided into a series of 10 kb constructs, four regions were shown to have cis-acting activity. However, further reduction of these 10 kb constructs to define smaller regulatory fragments abolished expression in most cases. 3C (chromosome conformational capture) analysis shows that these regions are brought near the *Hoxb1* promoter suggesting that they play a role in modulating *Hox* gene expression in the trunk. Together these data suggest that synergistic interactions over a large region preceding the *HoxB* complex has input into the regulatory behavior of the complex, in addition to the known regulatory elements that reside within the complex itself. We are currently testing these synergistic interactions and extending our 3C analysis to other *HoxB* members as well as the next non-*Hox* neighbor, *Scap1*, which precedes the *Hoxb1* gene and has an expression pattern different than that of the *Hox* genes themselves.

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Program/Abstract # 466
Hox genes in axial patterning
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Hox genes play a critical role in the patterning of the axial skeleton. This has been clearly demonstrated in mice mutant for the entire *Hox10* or *Hox11* paralogous group. *Hox10* triple mutants demonstrate an anterior homeotic transformation of all lumbar vertebrae toward a thoracic fate with rib processes protruding from each vertebral segment through the lumbar and sacral regions. *Hox11* triple mutants display normal development of the thoracic and lumbar region; however, no sacral vertebrae are formed. The vertebral elements in the sacral region are transformed to a lumbar fate. Although genetic analyses have provided important insight regarding *Hox* gene patterning of the axial skeleton, the molecular mechanisms involved in this process are not understood. In order to elucidate the genes and pathways that are regulated by Hox in axial patterning, I have performed microarray analysis on isolated sclerotomal cells, the precursors of vertebrae, from wild type and *Hox11* triple paralogous mutants at several developmental stages to identify genes that are differentially expressed in these animals. This analysis has uncovered differential expression of several genes, including several BMP pathway members. *In situ* hybridization analyses have shown that *Bmp2* expression is significantly reduced in the developing sacral region of *Hox11* mutants. Together, our data suggest that regional *Hox* expression might control localized expression of *Bmps* during morphogenesis of the axial skeleton.

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Program/Abstract # 467
Hox genes control the timing of somite precursor cells ingression during gastrulation in the chicken embryo
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A striking characteristic feature of the spine is the subdivision of groups of vertebrae into anatomical domains such as the cervical, thoracic, lumbar, sacral and caudal regions. This axial regionalization is controlled by a set of transcription factors called Hox genes. These genes are arranged along chromosomal domains, which are linearly deployed during embryonic development – a property termed colinearity. This striking genomic organization is translated into the colinear Hox expression domains during gastrulation. Recently, it has been shown that the genes from the *Hoxb* cluster are activated in the somite precursors of the epiblast in a temporal sequence that reflects their colinear arrangement and subsequently controls the progressive ingression of somite precursors into the nascent paraxial mesoderm (Iimura et al., 2006). Because the *Hoxa*, *c* and *d* clusters are expressed also in the epiblast during gastrulation, we explored the hypothesis of a conserved role of Hox paralogs during this process by overexpressing various Hox genes from the four clusters using the successive electroporation technique in the chicken embryo. We show that all of the paralogs we tested control the timing of epiblast cells ingression into the primitive streak in a colinear fashion. In parallel, we are currently using a microarray-based approach to identify the Hox target genes responsible for the progressive ingression of the somite precursors.

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Program/Abstract # 468
Role of Hox11 genes in anteroposterior patterning of nephrogenic mesenchyme
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Hox genes are critically important for anteroposterior (AP) patterning in a wide variety of organisms. Specific spatial and temporal expression of *Hox* genes along the AP body axis is necessary for proper embryonic development. In the mammalian kidney, it has been shown that *Hox11* paralogous genes are essential for ureteric bud induction, one of the first steps in kidney organogenesis. Further work has demonstrated that Hox11 proteins directly regulate *Six2* and *Gdnf* to control these early processes. Embryos in which five of the possible six alleles are mutated do not exhibit loss of induction, but demonstrate severe AP patterning defects in the nephrogenic mesenchyme. In five allele mutants, the mesonephros persists at later developmental stages and the mesonephric mesenchyme does not separate from the metanephric mesenchyme. These animals die perinatally due to hydronephrosis at the ureteric pelvic junction. Here, we begin to analyze the structural and molecular phenotype of the nephrogenic mesenchyme in these mutants. We believe that these studies will lead to an understanding of how *Hox* genes pattern the nephrogenic mesenchyme along the AP axis.

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Program/Abstract # 469
Functional relevance of Hox-specified positional identities in adult vasculature
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Hoxa3 and *Hoxc11* are expressed in vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) in regionally restricted patterns that closely resemble their respective embryonic expression domains (Pruett et al., 2008). To investigate whether this regionalized expression plays a role in determining the physiological diversification of vessel segments we explored the functional relevance of *Hoxc11* in VSMCs both in vitro and in vivo. Primary cultures of VSMCs established from explanted hindlimb vessel segments of *Hoxc11* reporter mice revealed persistent reporter transgene expression in distinct VSMC subpopulations facilitating phenotypic characterization of *Hoxc11*-positive versus -negative VSMCs. In vitro wound healing and serum-response assays provide evidence that *Hoxc11* expression promotes differentiation towards a contractile SMC phenotype. These results were supported by subsequent functional assays involving *Hoxc11*-transfected mouse vascular cells (MOVAS). These in vitro functional data suggest an important role for *Hoxc11* in the regulation of the phenotypic properties of VSMCs.To study the functional relevance of *Hoxc11* expression in vivo we adopted an innovative murine, doxycycline (Dox)-inducible transgene system, which results in the systemic over-expression of *Hoxc11* in VSMCs using VSMC-specific control elements of the Transgelin (SM22-alphaA) promoter. Together these in vitro and in vitro analyses demonstrate a significant role for Hox code-specified positional identities in the vascular network.

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Program/Abstract # 470
Identification and characterization of Six1 enhancers
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Six1, one of the members of Six homeobox family genes, is expressed in sensory organs and ganglia such as olfactory epithelium,