RNA (MIR). It is hypothesized that the secondary structure of the MIR is critical for binding a specific protein(s) required to synthesize tropomyosin and thus form organized myofibrils. Analysis of sequence data reveals a G → U point mutation in the mutant MIR. Further computational analyses, using GENESEE software to compare normal and mutant MIRs, show a significant alteration in RNA secondary structure of the point-mutated MIR. At present, we plan to use several base substitutions of the MIR that alter its secondary structure. We hypothesize that MIR acts as a noncoding RNA since its bioactivity is preserved through its unique secondary structure instead of an encoded protein. This study may facilitate identifying MIR homologs in higher mammalian systems, including humans, which might lead to regeneration of damaged heart muscle caused by infarct or other disease processes, and thus restoring normal heart function.

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Program/Abstract # 145
The transcriptional relationship between maternal Xoct60 and zygotic Xoct25 in Xenopus laevis
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Oct4, one of the POU class V transcription factor, is known as a key molecule to control the multipotency of the embryonic cell. Mammalian and Zebrafish have only one POU-V factor, whereas Xenopus has three factors, Xoct25, Xoct60 and Xoct91. It has been shown that the Xenopus POU-V factors have an ability to maintain the multipotency of the embryonic cells. Here, we examined the transcriptional relationship between maternal Xoct60 and zygotic Xoct25. At first, we constructed dominant negative form (DN) of Xoct60. Micro-injection of DN-Xoct60 mRNA caused reduction of zygotic gene expression at st. 10.5, which resulted in the cell death at st. 15. Co-injection of wild type Xoct25 completely rescued the malformation caused by DN-Xoct60. The embryo injected with Xoct60 showed the increased gene expression of Xoct25, while DN-Xoct60 caused the downregulation of Xoct25. These results suggest that Xoct25 functions in the downstream of Xoct60. To confirm the upregulation of Xoct25 by Xoct60, we isolated the upstream region of Xoct25 and carried out luciferase reporter assay. The reporter construct was actually stimulated by Xoct60. The Xoct60-induced upregulation of Xoct25 occurred in the animal cap assay. However, cycloheximide inhibited the upregulation of Xoct25, suggesting that Xoct60 activates the gene expression of Xoct25 together with the other zygotic factor. Animal cap assay using Cerberus-short (an inhibitor of Nodal signaling) showed that Nodal signaling is required for the zygotic expression of Xoct25. These results suggest that maternal Xoct60 and zygotic Nodal signaling are involved in the zygotic gene expression of Xoct25.

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Program/Abstract # 146
The regulation of SoxB1 genes during neural induction in Xenopus laevis
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The SoxB1 genes, Sox2 and Sox3 are expressed in the presumptive and developing nervous system of vertebrates, and overexpression of either gene leads to an expansion of neural tissue in Xenopus. BMP (bone morphogenetic protein) induces epidermis and inhibits the formation of neural tissue. We show that both Sox2 and Sox3 are expressed in response to BMP inhibition. Their spatio-temporal expression patterns and response to BMP make Sox2 and Sox3 strong candidates as the primary targets of neural inducers which are required for the formation of the central nervous system. By studying the regulation of Sox2 and Sox3, we can identify the molecular mechanism that drives the expression of early neural genes. Towards that aim we demonstrate that de novo protein synthesis is required for the repression of Sox3 and, in contrast, the induction of Sox2 in ectodermal explants. Additionally, gain and loss of function experiments in whole embryos demonstrate that the BMP target Xvent1, but not Msx-1, represses the expression of Sox3 and our reporter construct Sox3-GFP. Furthermore, analysis of transgenic embryos reveals the requirement of two putative Vent1 binding sites for the restriction of Sox3-GFP expression to the neural ectoderm. Future studies include the evaluation of candidate activators required for the induction and continued expression of both Sox2 and Sox3 in the absence of BMP signaling. The results from these experiments suggest that Sox2 and Sox3 have distinct modes of regulation even though they have very similar expression patterns and functions.

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Program/Abstract # 147
A novel β-catenin-associated histone methyltransferase activity and its role in dorsoventral patterning
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The Wnt/β-catenin signaling pathway directs multiple cell fate decisions during embryogenesis. In early Xenopus laevis embryos, the Wnt/β-catenin pathway specifies dorsal cell fates by activating genes that will give rise to Spemann’s organizer. Previously, we have shown that Wnt/β-catenin functions during a discrete window of time prior to the midblastula transition (MBT) to activate gene expression. We have also reported β-catenin/TCF-dependent transcription before the MBT despite large-scale transcriptional repression. This indicates that β-catenin functions during pre-MBT development to regulate gene expression and that this information is heritable through multiple cell divisions. How is activation of
β-catenin target genes transduced through the pre-MBT period to yield differential gene expression after MBT? We hypothesize that β-catenin recruits chromatin remodeling factors to promoters during the pre-MBT period to deposit a heritable mark of transcriptional activation. Promoters bearing this mark would thus be poised to begin transcription. Supporting this hypothesis, we demonstrate pre-MBT interaction of β-catenin with organizer gene promoters and β-catenin-dependent histone H3 K4 trimethylation, a histone modification associated with gene activation. We have also identified an endogenous histone methyltransferase (HMT) activity associated with β-catenin by IP/HMT assays in eight-cell embryos. Remarkably, this endogenous HMT methylates histone H3 mutated at K4 and therefore we are testing other candidates for targets. We are currently focusing on identifying this β-catenin-associated HMT and assessing its role in dorsoventral patterning.

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Program/Abstract # 148
Molecular cloning of zebrafish tortuga: Insights into cyclic transcript regulation
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Segmentation is an essential organizing process in embryonic development that, by dividing the body into morphologically similar units along the rostral–caudal axis, lays down a basic pattern for later elaboration of the body plan. In vertebrates, this process begins with somitogenesis: the sequential formation of bilateral pairs of epithelial spheres of paraxial mesoderm flanking the notochord. Cyclic transcription of several genes is involved in pre-patterning the unsegmented paraxial mesoderm, or presomitic mesoderm (PSM). The tortuga gene was identified in a mutagenesis screen looking for disruptions of transcript patterning of the PSM cyclic gene her1. In tortuga mutants, her1 stripes persist when they should be in the “off” cycle of transcription in the PSM. Using an intron-specific probe for her1, it was shown that Tortuga regulates her1 at the post-transcriptional level. We are in the process of molecularly identifying tortuga. With little known about post-transcriptional regulation of the splicing, translation, stability and degradation of cyclic transcripts, characterizing Tortuga will likely identify a novel regulatory component involved in somitogenesis. Using a positional cloning approach, we have mapped tortuga to a small interval on linkage group 16.

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Program/Abstract # 149
T-box transcription factors in Zebrafish mesoderm development
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T-box proteins are a large family of transcription factors important in many aspects of embryonic patterning. These proteins are expressed in a wide variety of tissues and regulate a diverse set of target genes, but the source of this diversity is not clear since those tested have very similar DNA binding preferences. In zebrafish, the T-box genes no tail (ntl)/Brachyury, spadetail (spt)/tbx16 and tbx6 are expressed in partially overlapping patterns in the presumptive mesoderm, and mutants lacking spt, ntl or both gene functions have abnormalities in the development of mesodermal structures. We are studying how all three factors recognize the proper cis-regulatory elements and activate transcription of the appropriate target genes. We have performed in vitro DNA binding selection assays (SELEX assays) with bacterially-produced Ntl, Spt and Tbx6 proteins and have found that the three factors bind similar, but not identical, sequences. We are using these binding site models along with comparative genomics to identify potential regulatory regions in non-coding DNA near genes we have identified as putative direct T-box targets through microarray analysis. We are testing the ability of these sequences to drive transcription using reporter assays in both wild type fish and fish depleted of spt and/or ntl activity. Preliminary analyses of these enhancers suggest that these T-box factors interact with transcription factors in the Wnt and BMP families of signaling pathways to activate transcription.

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Program/Abstract # 150
Regulation of odd-skipped related 1 (osr1) in the chicken
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Osr1 (odd skipped related 1) is a member of the odd gene family defined by a highly conserved set of zinc finger domains. The founding member of this family, the odd-skipped gene was identified during a genetic screen in Drosophila. This pair-rule gene plays a vital role in segmentation of the fly, and is thought to function as a repressor. Homologues of the odd family have been identified in C. elegans, chicken, mouse and human. In the chicken embryo osr1 is the earliest known marker for the intermediate mesoderm (IM). The IM is situated between the somites and the lateral plate mesoderm in the embryo and gives rise to all vertebrate kidney tissues. As development progresses...