factor binding sites and we are now testing the ability of this element to drive reporter gene expression consistent with the expression of *Meis*2.

doi:10.1016/j.ydbio.2009.05.162

Program/Abstract # 141
The regulation of mouse Hoxb9
Tara B. Alexander, Youngwook Ahn, Robb Krumlauf
Stowers Institute of Medical Research, Kansas City, MO, USA

Hox proteins play a fundamental role in assigning anterior–posterior positional identity to segments, such as the rhombomeres and somites, during vertebrate development. Since segmental identity is affected by even small changes in spatial or temporal aspects of *Hox* gene expression, *Hox* expression must be precisely regulated and coordinated with the processes of segmentation. In this study, we focus on the regulation of *Hoxb9* in the mouse with the ultimate aim of identifying the trans-acting factors responsible for different phases of its expression. A large plasmid reporter recapitulates early *Hoxb9* expression in the neural tube and somites, but neural expression is not maintained at later stages. We have identified three independent enhancers within the larger plasmid capable of driving some aspects of *Hoxb9* expression. One of these enhancers lies within the first coding exon of *Hoxb9* and is highly conserved between zebrafish and mouse. This enhancer contains consensus binding sites for YY1, Cdx2, and TCF/Lef. These are known to play a role in the regulation of some *Hox* genes. Mutation or deletion of these sites from this enhancer implicates their involvement in the regulation of *Hoxb9*. Moreover, removal of this region from a large reporter plasmid leads to expanded mesodermal expression at early stages and expanded neural expression at later stages suggesting that this region may play a repressive role in *Hoxb9* expression. Further characterization of these enhancers should lead to additional insight into the mechanisms regulating *Hoxb9* expression during early vertebrate development.

doi:10.1016/j.ydbio.2009.05.163

Program/Abstract # 142
Counting Hox transcript numbers within single cells in fixed *Drosophila* embryos: Evidence for a stochastic mode of gene expression
Adam Pare[a], Derek Lemons[a], Dave Kosman[a], William Beaver[b], William McGinnis[a]
[a]*Department of Cell and Developmental Biology, UC San Diego, USA*
[b]*Department of Computer Science, UC San Diego, USA*

Determining mRNA concentrations within a population of cells has been a difficult problem for molecular biologists. Traditionally the problem has been addressed using Northern Blot analyses, RT-PCR, and most recently microarrays. While relatively simple to carry out these methods all have their shortcomings. Most importantly they are limited by poor temporal and spatial resolution, especially when examining complex tissues, because they necessitate the destruction of the sample. We demonstrate that FISH (fluorescent *in situ* hybridization) is capable of detecting and resolving, at extremely high efficiency, single mRNA molecules in fixed *Drosophila* embryos. We also briefly report a method for the segmentation and automated quantification of mRNA signals. The method is used to characterize and quantify the expression pattern of the Hox gene *Scr* at several stages during development. Importantly, we provide evidence for a stochastic mode of transcription, and show that levels of nascent transcription in the nucleus do not correlate well with cellular mRNA levels.

doi:10.1016/j.ydbio.2009.05.164

Program/Abstract # 143
Quantitative RT-PCR analysis of Dll-B knockdown in the ascidian *Ciona intestinalis*
Matthew D. Blanchette, Steven Q. Irvine
Department of Biological Sciences, Univ. of Rhode Island, Kingston, RI, USA

The Dll-B homeobox transcription factor in the simple chordate *Ciona intestinalis* is expressed in the entire animal hemisphere, fated to produce ectoderm, in the blastula and gastrula stages. We used transgenic siRNA and dominant negative strategies to knock down wild type CiDll-B expression. We are investigating the effects of these knockdown constructs through quantitative RT-PCR analysis of reported CiDll-B regulatory targets.

doi:10.1016/j.ydbio.2009.05.165

Program/Abstract # 144
Identification of cis-regulatory elements controlling Six6 expression
Kelley L. Crouse, Meaghan McCarthy, Andrea S. Viczian, Matthew Theisen, Michael E. Zuber
Department of Ophthalmology, SUNY Upstate Medical Univ., Syracuse, NY, USA

The homeobox-containing transcription factor, Six6, regulates eye size by stimulating retinoblast proliferation. In order to identify cis-regulatory elements controlling its expression we first identified conserved sequences in the flanking regions of the *Six6* gene. Three evolutionarily conserved regions (ECRs) were identified. ECR1 and 2 are located in the 5′ flanking region within 1.5 kb of exon 1, while ECR3 is approximately 2 kb 3′ of Six6 exon 2. We compared endogenous Six6 expression to that of eGFP in transgenic *Xenopus laevis* generated using constructs containing all three conserved regions (ECR1/2/3) and regions 1 and 2 (ECR1/2). GFP expression in ECR1/2/3 transgenics mimicked that of Six6 as it was first detected in the eye field at stage 15. By stage 24/25, eGFP was uniformly expressed throughout the developing eyes. By comparison eGFP mRNA expression was first detected in the developing eyes of ECR1/2 transgenics at st. 33/34. At stage 33/34 the expression patterns in ECR1/2/3 and ECR1/2 transgenics were similar. At later developmental stages eGFP expression remained strong in the eyes of both sets of transgenics and could be observed in the axons of retinal ganglion cells leading to their tectal targets. These results suggest ECR3 is required for early (neural plate stage) expression, while ECR1 and 2 are sufficient for the late retinal expression of Six6. Further analysis is needed to better define the role of ECR1–3 in Six6 expression. Bioinformatics and mutational analysis of these cis-regulatory regions will help identify transactivating factors controlling Six6 expression and ultimately eye size.

doi:10.1016/j.ydbio.2009.05.166

Program/Abstract # 145
The role and regulation of FoxN2/3 in the skeletogenic cells during sea urchin development
Ho Kyung Rho, David R. McClay
Department of Biol., Duke Univ., Durham, NC, USA