Atoh1 (Math1) is a basic helix–loop–helix (bHLH) transcription factor that specifies components of the proprioceptive pathway system in mammals. Despite genetic evidence (knockout mice and overexpression systems) implicating Atoh1 in defining the dorsal interneuron 1 (dI1) population of the dorsal neural tube, developing granule cells in the cerebellum, hair cells of the inner ear, and Merkel cells, precisely how Atoh1 functions in the generation of these cell types remains ill-defined due to the lack of known targets of Atoh1 transcription activity. To identify downstream targets of Atoh1, we performed microarray analyses of isolated Atoh1 populations. Comparison of the Atoh1 populations to other isolated bHLH populations revealed several genes enriched and potentially downstream of Atoh1 in the various proprioceptive lineages. These genes range in identity from small GTPases to tyrosine kinase receptors. By determining the regulation of such downstream targets by Atoh1, we expect to provide insights into how transcriptional networks instruct the generation of specific neuronal lineages.

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Program/Abstract # 150
Using zinc finger nucleases for targeted modification of the zebrafish genome
Jasmine M. McCammon, Michael Goldrich, Andrew Glazer, Sharon L. Amacher
Department of Molecular and Cell Biology, University of California, Berkeley, USA

Until recently, reverse genetic strategies in zebrafish for targeted mutagenesis have been unavailable. Publications from several groups, including our collaborative work with Sangamo Biosciences, have shown that zinc finger nucleases (ZFNs) can target specific loci for mutation in zebrafish. ZFNs are a fusion between zinc finger protein motifs, designed to recognize and bind to specific DNA sequences, and the nonspecific cleavage domain of the FokI endonuclease. The resulting ZFN binds and cleaves DNA at the target sequence, creating a double strand break (DSB) that must be repaired. One pathway for DSB repair is non-homologous end joining (NHEJ), an error-prone mechanism that introduces small insertions and deletions during repair. In this way, ZFNs have mutagenized 8 different loci in zebrafish. Another application of ZFNs is targeted gene modification utilizing the other DSB repair pathway, homology directed repair (HDR). HDR uses homologous sequence, such as the sister chromatid or an exogenously supplied donor sequence, as a template to direct repair of the DSB. We are currently using no tail (ntl) targeting ZFNs together with donors to knock-in a small molecular tag or GFP coding sequence into the ntl locus by HDR. We are also using a donor with wildtype sequence and golden targeting ZFNs to rescue the goldenb3 point mutation pigmentation defect. Because NHEJ is favored over HDR as the DSB repair pathway choice in zebrafish embryos, we are also examining how manipulation of proteins involved in these pathways may bias the choice towards HDR. We will present our results at the meeting.

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Program/Abstract # 152
Characterization of a conserved element at the telomerase promoter
Diana A. Tran, Robert A. Drewell
Biology Department, Harvey Mudd College, Claremont, CA, USA

Telomeres are regions of repeated nucleotides that cap the ends of linear eukaryotic chromosomes. They function as disposable safeguards to prevent the loss of genetic information from end to end fusions, degradation, and instability. Telomerase (TERT) is the enzyme that adds new telomeric repeats to the ends of chromosomes. During embryogenesis, TERT is active and is critical for telomere elongation. In normal human somatic cells, the TERT gene is suppressed after embryogenesis to control cell differentiation and to limit the proliferative capacity of cells. We are investigating the molecular mechanisms which regulate expression of the TERT gene. We analyzed the non-coding genomic region around the human TERT gene using bioinformatic analysis and identified a TERT ultra-conserved (TUC) sequence. This 308 bp region is over 75% conserved between distantly related mammalian species and over 91% conserved among primate species. We tested the cis-regulatory potential of the TUC region in a dual luciferase reporter gene assay. Transient transfection into HeLa and lung fibroblast cells demonstrated transcriptional enhancer activity from the TUC region, while transient transfection into mouse embryonic stem cells revealed little regulatory activity, suggesting that different mechanisms may govern the expression of TERT in these cells.

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Program/Abstract # 153
Mapping Dlk1 regulatory elements
Eric D. Rogers, Jennifer V. Schmidt
Department of Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL, USA

The transcription factor c-Myb plays an essential role in stem/progenitor cell proliferation and differentiation in the blood, the brain and the intestine. It is also known to be expressed in other tissues, incl. the retina and the olfactory placode. Little is known about the transcriptional regulation of the c-myb gene. This is why we are investigating the transcriptional regulation of c-myb in zebrafish embryos. In zebrafish embryos, c-myb expression is detected in the retina, the intestine, the olfactory placode, the branchial arches, the brain and in haematopoietic tissues. To find regulatory elements controlling c-myb expression in zebrafish we have used three different approaches. Firstly, we have tested sequences upstream of the zebrafish c-myb gene. These were amplified by PCR and cloned in front of a gfp reporter gene in a Tol2 transposon vector. The resulting constructs were injected into zebrafish embryos to generate transgenic lines. Secondly, we have searched for regulatory elements outside the promoter proximal region. Assuming that such elements may be evolutionally conserved we searched for conserved noncoding elements in interspecies genomic comparisons using the sequence alignment programme MLAGEN. These elements were individually cloned in front of a basal promoter of c-myb in the Tol2 reporter vector and tested in transgenic zebrafish. Finally, we have modified a BAC which contains 176 kb of genomic sequence around the c-myb locus by introducing an rfp reporter gene into the c-myb gene. The modified BAC has been injected into zebrafish embryos. At the meeting, we will report on the progress we have made.

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Genomic imprinting is the process by which two alleles of an autosomal gene are differentially expressed depending on their parent of origin. Our lab investigates the imprinting and expression of the reciprocal imprinted genes, \( \text{Dlk1} \) (Delta-like 1) and \( \text{Gtl2} \) (Gen trap locus 2). The purpose of this study is to identify and characterize the regulatory elements, particularly the enhancers, controlling expression at the \( \text{Dlk1} \)–\( \text{Gtl2} \) locus. This information will provide a more complete understanding of the mechanisms by which \( \text{Dlk1} \) and \( \text{Gtl2} \) control growth and development, and is required to produce testable models of imprinted gene regulation on mouse distal chromosome 12. Previous studies by our laboratory localized a subset of \( \text{Dlk1} \)–\( \text{Gtl2} \) regulatory elements to two regions, one upstream of \( \text{Dlk1} \) and one downstream of \( \text{Gtl2} \), using BAC (Bacterial Artificial Chromosome) transgenic mice. Cross-species sequence conservation was used to identify putative regulatory elements upstream of \( \text{Dlk1} \) that were examined for enhancer activity using a cell culture-based luciferase expression assay. A subset of the sequences analyzed show enhancer activity in the cell culture assay. Putative enhancer elements were further analyzed for in vivo activity by characterizing their ability to direct expression of a \( \text{lacZ} \) transgene in the mouse. Analysis of multiple transgenic mice at embryonic day 13.5 reveals a pattern of \( \text{lacZ} \) expression that partially recapitulates endogenous \( \text{Dlk1} \) expression in the developing skeletal muscle, vertebrae, trigeminal ganglia, dorsal root ganglia, and pituitary gland. These findings indicate that the DNA sequences tested upstream of \( \text{Dlk1} \) can function as enhancers of \( \text{Dlk1} \) expression.

Program/Abstract # 154
Context-dependent regulation of SoxE activity
Caroline E. Haldin, Carole LaBonne
Department of Biochem., Mol. Biol. and Cell Biol. Northwestern Univ, Evanston, IL, USA

SoxE family transcription factors are required for the formation of neural crest precursor cells as well as the differentiation of a subset of neural crest derivatives including cartilage, melanocytes and glia. An important question about widely deployed factors such as SoxE proteins is how their activities are modulated in order to ensure that they direct the correct developmental outcome. Using Xenopus as a model, we are investigating two mechanisms via which context-dependent regulation of SoxE factors might be achieved: 1) Promoter-specific regulation of SoxE function by a subunit of the mediator complex, TRAP230 and 2) Context-dependent regulation of SoxE function by SoxD factors (Sox5/6). Here we report that, as in zebrafish, Xenopus Trap230 (\( \text{kohtalo} \)) is expressed in both premigratory and migratory neural crest cells. We show that morpholino-mediated depletion of XTRAP230 results in perturbation of neural crest formation, migration and differentiation, and we examine the extent to which this is due to effects on SoxE activity, using both endogenous neural crest induction and SoxE-dependent luciferase reporter assays. We further show that Sox5 can modulate the activity of Sox9 in a context-dependent manner in Xenopus: while these two factors cooperate to activate the \( \text{Col2A1} \) promoter, Sox5 inhibits both normal Sox10 expression and the up-regulation of Sox10 mediated by Sox9 overexpression. Sox5 is expressed in neural crest precursors at a time consistent with the modulation of SoxE function and its expression is regulated by neural crest regulatory factors. Together these findings lend important insights into the post-translational mechanisms used to regulate the diverse activities of SoxE proteins.

Program/Abstract # 155
SoxE factors can function as SUMO-dependent transcriptional repressors by recruiting transcriptional co-repressor Grg4
Pei-Chih Lee, Kimberly Taylor, Carole LaBonne
Department of Biochemistry, Molecular Biology and Cell Biology; and Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Evanston, IL 60208, USA

A growing number of transcriptional regulatory proteins have been found to be modified by the small ubiquitin like modifier, SUMO. Post translational modification by SUMO may be one means by which transcriptional regulatory factors that play context-dependent roles in multiple processes can be regulated such as they direct the appropriate cellular and developmental outcomes. In early vertebrate embryos, SUMOylation of transcription factors of the SOXE family has a dramatic affect on their function, inhibiting their neural crest inducing activity and promoting ear formation. Here we provide mechanistic insight into how SUMO modification affects SoxE function. We show that SUMO has a dramatic affect on the ability of SoxE transcription factors to recruit transcriptional co-regulator factors, displacing the binding of CBP and p300 while promoting the recruitment of a co-repressor, Grg4. These data demonstrate that SoxE transcription factors can function as transcriptional repressors in a SUMO-dependent fashion. They further suggest a novel multi-valent mechanism for SUMO-mediated recruitment of transcriptional co-regulatory factors.

Program/Abstract # 156
The endoderm specification, a view from the \( \text{foxa} \) cis-regulatory modules
Smadar Ben-Tabou de-Leon, Eric H. Davidson
Division of Biology, California Institute of Technology, Pasadena, CA, USA

The transcription factor \( \text{foxa} \) is expressed in the developing endoderm of many bilaterians and also in cnidarians, indicating strong conservation of its function and regulation. In the sea urchin embryo \( \text{foxa} \) is essential for the gut formation and for exclusion of mesodermal fate in the endoderm. The early expression pattern of \( \text{foxa} \) is very broad, however in about 12 h it resolves to specific endodermal sub-domain. We utilize the advanced state of the sea urchin endomesoderm gene regulatory network to decode the genomic regulation of \( \text{foxa} \). We find that several cis-regulatory modules interact with each other and switch their dominance in controlling \( \text{foxa} \) expression at different times. This enables different factors to regulate \( \text{foxa} \) expression in different territories and regulatory times. \( \text{foxa} \) is downstream of both \( \text{Wnt} \)-\text{beta catenin} and Delta–Notch signaling pathways and its complex and dynamic expression is regulated by a combination of excluding repressors, permissive switches and localized activators. This study illuminates the regulatory process that leads to the endodermal sub-domains specification.

Program/Abstract # 157
Promoter–enhancer tethering is critical for long-range regulatory interactions in the bithorax complex of \( \text{Drosophila} \)
Margaret C. Ho, Benjamin J. Schiller, Omar S. Akbari, Esther Bae, Robert A. Drewell
Biology Department, Harvey Mudd College, Claremont, CA