requirement for ZFP568 in convergent extension and morphogenesis of embryonic and extraembryonic tissues. Here we present data supporting role for TRIM28 in mediating ZFP568 function; yeast two hybrid assays identified TRIM28 as a partner of ZFP568, while positional cloning of *chatwo*, a hypomorphic allele of TRIM28, provided genetic evidence for the functional significance of a ZFP568/TRIM28 interaction. *chatwo* mutants, isolated in a forward mutagenesis screen, arrest at embryonic day 9 (E9) with defect similar to those of *chato* mutants. The phenotype of *chatwo* embryos contrasts with the early lethality of TRIM28 KO mice that die as pre-gastrula stage embryos (E5.5). Because this data suggests that the *chatwo* hypomorphic allele only affects the functions of TRIM28 required by specific KRAB domain proteins, we investigated whether the *chatwo* mutations disrupt TRIM28 stability, interactions with specific KRAB domain proteins, or recruitment of chromatin modifying enzymes. Results will be presented that support a differential requirement for TRIM28 by distinct KRAB zinc finger proteins during early embryonic development.

doi:10.1016/j.ydbio.2010.05.444

Program/Abstract # 283
A gene regulatory network that underlies the derivation of the anterior neural plate from the epiblast
Makiko Iwafuchi-Doi[a], Tatsuya Takemoto[a], Yuzo Yoshida[a], Isao Matsuo[b], Jun Aruga[c], Yusuke Kamachi[a], Masanori Uchikawa[a], Hisato Kondoh[a]
[a]*Frontier Biosciences, Osaka Univ., Osaka, Japan*
[b]*MCHRI, Osaka Prefectural Hospital Organization, Osaka, Japan*
[c]*RIKEN Brain Science Institute, Saitama, Japan*

The operation of gene regulatory networks that drive developmental processes is reflected in the regulation of core transcription factor genes such as Sox2. The expression of Sox2 in the epiblast and anterior neural plate in the mouse embryo is determined by enhancer N-2. The activation of enhancer N-2 is dependent on a phylogenetically conserved 73-bp core sequence that contains the binding sites for ZIC2, OTX2, and either POU factor OCT3/4 or OCT6. Enhancer N-2 activation requires the binding of ZIC2 and POU factors in both the epiblast and the anterior neural plate, whereas the OTX2 interaction is required only in the latter. This observation, taken together with the expression patterns of these transcription factors, suggests that the transition of major POU factors from OCT3/4 (Class V) to OCT6 (Class III) and the recruitment of OTX2 characterize a shift of the gene regulatory network that underlies the derivation of the anterior neural plate from the epiblast. The impact of POU factor class switching and OTX2 participation on the overall cellular state is currently under investigation by using in vivo and in vitro models.

doi:10.1016/j.ydbio.2010.05.445

Program/Abstract # 284
Mohawk-mediated repression of Sox6 is necessary for the expression of slow myosin heavy chain (Myh7) in differentiated satellite cells
Douglas M. Anderson[a,b], Alan Rawls[a]
[a]*School of Life Sciences, Arizona State University, Tempe, Arizona 85287-4501, USA*
[b]*Molecular and Cellular Biology Graduate Program, Arizona State University, Tempe, Arizona 85287-4501, USA*

Mohawk (Mkx) is a member of the TALE superclass of atypical homeobox genes expressed in the embryonic progenitor cell populations of skeletal muscle, tendon and cartilage. We have previously shown that Mkx functions as a potent transcriptional repressor that is capable of inhibiting skeletal muscle differentiation induced by MyoD. To further investigate the role of Mkx during skeletal muscle differentiation, we have isolated muscle satellite cells from Mkx-knockout mice and characterized them in culture under proliferating and differentiated conditions. Microarray analysis revealed that the transcription factor Sox6 is consistently upregulated in proliferating and differentiated Mkx-knockout cells. Sox6 plays a crucial role in muscle fiber type specification through direct repression of slow myosin heavy chain (Myh7). Consistent with this finding, immunostaining of differentiated satellite cells from Mkx-knockout mice revealed that Myh7 expression was reduced, whereas fast myosin heavy chain was unaffected. We have further identified an Mkx-responsive element in the Sox6 locus, suggesting that Mkx may directly repress the transcription of Sox6. Together, this data reveals a novel function for Mkx during the differentiation of skeletal muscle.

doi:10.1016/j.ydbio.2010.05.446

Program/Abstract # 285
Identification of a brain and neural tube specific enhancer associated with the expression of Emx2 during development
Brian C. Willis, Charmaine U. Pira, Shelley A. Caltharp, Kohei Kanaya, Jennifer M. Feenstra, Kerby C. Oberg
Dept. of Pathology and Human Anatomy, Loma Linda University, Loma Linda, CA, USA

Emx2 is a highly conserved transcription factor expressed in the developing urogenital tract, dorsal limb girdle, and cerebral cortex. Studies of Emx2 null embryos have shown that it is required for normal neuroblast proliferation, migration and differentiation, as well as in the development of the diencephalon. Despite elucidation of some of the developmental functions of the Emx2 gene, few studies exist which characterize regulatory elements controlling the expression of Emx2. We searched the Emx2 locus *in silico*, across divergent species, for conserved noncoding regions (CNR) that might harbor sequences involved in the regulation of Emx2. We identified 34 CNR associated with the Emx2 locus. To determine whether a CNR was functioning as an enhancer element, we transfected chick embryos with a ptk-EGFP reporter construct containing the CNR upstream of a minimal HSV-TK promoter and then screened for an enhancer activity at progressive stages of development by fluorescence microscopy. We found the enhancer activity in a CNR located in the 3′ untranslated region of the Emx2 locus that coincides with the pattern of expression of Emx2 in the developing brain and neural tube. The localized enhancer activity suggests a role for this CNR in the regulation of CNS specific Emx2 expression. Further studies are in progress to confirm this hypothesis and identify specific regulatory proteins that may interact with this region.

doi:10.1016/j.ydbio.2010.05.447

Program/Abstract # 286
Structure of regulatory networks and dynamics of bio-molecules: Predicting unknown from known
Atsushi Mochizuki[a,b], Daisuke Saito[a]
[a]*Theor. Biol. Lab., RIKEN ASI, Wako, Japan*
[b]*Dept. Comput. Intelligence and Sys. Science, Tokyo Institute of Technology, Yokohama, Japan*

Regulatory relations between biological molecules constitute complex network systems, and realize diverse biological functions through the dynamics of molecular activities. However, we currently

have very little understanding of the relationship between the structure of a regulatory network and its dynamical nature. In this study we introduce a new mathematical method, named "linkage logic", to analyze dynamics of network systems. By this method, we can restrict possible steady states of a given complex network system only from the knowledge of regulatory linkages. We formalize two aspects of the linkage logic: "principle of compatibility", determines the upper limit of the degree of freedom of steady-state diversity realized by a given network. "Principle of dependency", determines the possible combinations of states of the system. By combining these two, (1) for a given network, we can identify a cluster of nodes which reflect possible steady states of the whole system, (2) we can reduce a given complex network into a simpler one without loss of the ability to generate the diversity of steady states, (3) we can examine the consistency between the structure of network and observed set of steady states, and (4) sometimes we can predict unknown states or unknown regulations only from observed set of steady states. We illustrate the method by several applications to experimentally determined regulatory networks, including gene network for early development of ascidian and the regulatory network of signal transduction pathway.

doi:10.1016/j.ydbio.2010.05.448

Program/Abstract # 287
SRY function in sex determination

Yen-Shan Chen, Michael A. Weiss
Dept. of Biochem., Case Western Reverse University, Cleveland, OH, USA

Male sex determination in mammals is regulated by SRY, a single copy gene on the Y chromosome. In inherited sex reversal families the father and the daughter share the same SRY. Here, we investigate one such inherited case, V60L. This mutation is located in the minor wing of DNA-binding HMG box. Biochemical studies demonstrated near-native DNA interaction. However, cell-culture studies of the rodent gonadal cell model indicated a partially impaired nuclear localization. The compatibility of the V60L SRY with either male (father) or female (daughter) development may reflect polymorphisms in genes affecting the efficiency of nuclear import. We next investigated the major wing of the SRY HMG box. Whereas the V60L mutation demonstrated that remodeling of the minor wing can be tolerated in nuclear import, we sought to ask if the structure of the major wing is likewise adaptable. A probe is provided by the mutations with unstructured HMG box. Upon rescue of nuclear localization signal, the double mutants exhibit substantial gene-regulating activity. These results highlight the importance of specific DNA-directed protein folding in the assembly of sex-specific transcription regulating complexes. To study further testicular differentiation, we have investigated the nuclear export of SRY. I90M, another inherited clinical father–daughter mutation, located in Nuclear Export Signal and perturbs its function. We propose that nucleocytoplasmic shuttling regulates phosphorylation of SRY, which in turn modulates specific DNA binding. Together, we generate a cascade model of SRY in male-determination, and it also shows that our model highly correlates protein characters with their roles in development biology.

doi:10.1016/j.ydbio.2010.05.449