Patterning and Transcription Factors

Program/Abstract # 128
Rescue-potential of chimeric Pou5f1 transcription factors
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Oct4, a Pou5f1 homeodomain transcription factor of the POU family, is critically involved in the self-renewal of embryonic stem cells, and initially active as a maternal factor in the oocyte. Maternal and zygotic (MZ) Pou5f1 mutant zebrafish provide an experimental vertebrate system to study Pou5f1 function. Both mouse Oct4 and zebrafish Pou5f1 are able to phenotypically rescue zebrafish MZPou5f1 mutants. This rescue is not reciprocal: zebrafish Pou5f1 cannot efficiently rescue induced Oct4 knockout in murine ES cells. Attempts to identify the basis for this difference in DNA binding or linker domains of Pou5f1 proteins have so far not yielded conclusive results. We hypothesize that species-specific differences in Oct4 homologues are rather due to properties of the less conserved N- and C-terminal non-DNA binding domains, than different DNA recognition specificity. For that purpose, we created two chimeric constructs: an AZB fusion protein that consists of the zebrafish homeobox domain (Z) and the N- and C-terminal domains of murine Oct4 (A and B), and the corresponding counterpart CMD, consisting of the mouse DNA binding domain (M) flanked by N- and C-terminal domains of zebrafish (C and D). We expect both AZB and CMD chimeras to be able to rescue the zebrafish mutant MZPou5f1. Experiments are in progress to determine whether a rescue of an induced Oct4 knockout in murine embryonic stem cells would answer the question which domains are crucial for conferring species specificity in Oct4 function.

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Program/Abstract # 129
Identification of direct targets of the Caenorhabditis elegans global sexual regulator TRA-1 by chromatin immunoprecipitation
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The nematode Caenorhabditis elegans naturally occurs as two highly dimorphic sexes, the XX hermaphrodite and the XO male. Sex is determined by a genetic pathway culminating in the transcription factor TRA-1, the worm homologue of vertebrate GLI proteins. Null mutations in tra-1 result in hermaphrodite-to-male sex reversal, indicating that TRA-1 and its downstream targets are responsible for generating all or nearly all sexual dimorphism in the worm. However only a few direct TRA-1 targets have been described, and additional biologically important targets likely remain to be identified. To identify TRA-1 target genes throughout the C. elegans genome, we are performing chromatin immunoprecipitations using an affinity-purified rabbit polyclonal TRA-1 antibody followed by deep sequencing (ChIP-seq). We have successfully identified several previously described TRA-1 binding sites with this approach, including those in the regulatory regions of mab-3 (Yi et al., 2000), fog-3 (Chen and Ellis, 2000), and xol-1 (Hargitai et. al., 2009) and have also identified many novel TRA-1 binding sites. To examine what role putative TRA-1 targets may play in sexual development, we have generated translational fusion reporters by fosmid recombineering, and are examining how TRA-1 binding site ablation affects the expression patterns of these reporters and whether this altered expression results in sexual differentiation phenotypes.

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Program/Abstract # 130
Oocyte polarity and the patterning of zygotic gene expression are regulated by maternal PCP genes
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In Xenopus and zebrafish, the transmembrane planar cell polarity proteins, Strabismus (Stbm/Ltap/Vangl2) and Flamingo (Fmi/Stan/Celsr2) are known to be required for convergence extension movements during gastrulation and neurulation. Real-time RT-PCR analysis shows that full-grown Xenopus oocytes express mRNAs for these PCP proteins as well as for other “core planar polarity components”, frizzled, prickle and dishevelled. Antisense oligonucleotide-mediated depletion of maternal Stbm and Fmi results in a complex phenotype with reduction of the canonical Wnt signaling activity and induction of the ectodermal patterning gene, Foxi1e. Since, in epithelial sheets, PCP genes are used for establishing intercellular polarity, we examined here the effects of Stbm or Fmi depletion on oocyte polarity by analyzing apkc, (a core protein of apical/basal polarity) localization. We show that depleting Stbm or Fmi interferes with the animal enrichment of apKC and GFP-Crumb during oocyte maturation. Furthermore, The alteration of maternal PCP proteins and their target protein apKC leads to the mis-localization and changes of expression levels of zygotic genes including Sox17a, Wnt11 and Xnr3 which are important for normal patterning and morphogenesis of the embryo. These findings suggest a novel regulatory role for PCP components, that, by controlling the localization of the