the expression of osr1 is maintained in undifferentiated IM but is downregulated in differentiating kidney tissues. In addition osr1 expression is observed in the developing limbs, heart and palate, and mice deficient for osr1 lack kidneys and die in utero. Despite its importance in vertebrate development very little is known about the transcriptional regulation of osr1 or its function beyond its putative role as a transcription factor. The aim of our study is to identify and characterize the regulatory elements that control the expression of osr1 in the IM of the chicken. Our method is to clone various fragments of the genomic DNA surrounding the osr1 gene into a reporter vector containing GFP and a minimal promoter, electroporate these constructs into the IM in the chicken embryo, and analyze each construct for regulatory activity.

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Program/Abstract # 151
Hoxa13 regulation and function during embryonic development
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During embryogenesis, the Hox family of transcription factors is involved in setting up the body architecture. Mammalian genomes contain 39 Hox genes, organized in four clusters, referred to as HoxA to HoxD, and the gene order within a given cluster reflects the sequence of their spatial and temporal activation. Briefly, genes transcribed early on are expressed anteriorly, while expression of genes activated later on is progressively restricted to posterior domains of the developing embryo. Hox functions have been investigated using both gain of function and targeted gene inactivation. Unlike other Hox genes, inactivation of Hoxa13 leads to embryonic lethality. Homozygote mutants die between 11.5 dpc and 15.5 dpc. Even though stenosis of umbilical arteries has been observed in 30% of Hoxa13−/−embryos, it remains unclear why Hoxa13 inactivation is detrimental to the survival of the embryo. In a study aimed to identify Hoxa13 cis-regulatory elements, we have generated a targeted deletion of non-coding sequences that mimics the lethality associated to Hoxa13 inactivation. Using a transgenic-based approach, we are currently dissecting the enhancer activities of this DNA fragment to perform rescue assays. In turn, the rescue of Hoxa13−/−lethality will be a useful tool to fully characterize Hoxa13 functions during embryonic development. This transgenic approach combined to the analysis of targeted deletions that we have engineered will provide a better understanding of Hoxa13 regulation and function during embryogenesis.

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Program/Abstract # 152
Regulatory enhancers in the RALDH2 gene
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Retinoic acid (RA) signaling is essential for embryogenesis and organogenesis. RA is synthesized from vitamin A through a reversible reaction catalyzed by alcohol dehydrogenases (ADHs), and an irreversible reaction catalyzed by retinaldehyde dehydrogenases (RALDHs). The high sequence conservation of RALDH2, its similar expression patterns in vertebrates and its several roles in development indicate the importance of this gene. In contrast to other RALDHs, RALDH2 is the only gene to display deep vertebrate evolutionary conservation outside coding regions. To identify regulatory modules of the RALDH2 gene we aligned several vertebrate genes and identified evolutionary conserved regions (ECRs). Through transient and stable transgenesis in mice, we identified three major enhancers present in 5′ regulatory region, intron 1 (RALDH2.2) and 3′ region. RALDH2.2 is a neural tube enhancer with a dynamic expression pattern that varies according to maturation stage and axial level of the neural tube. RALDH2.2 also drives expression in the epicardium of the developing mouse heart. In situ hybridization experiments showed a strong correlation between mRNA localization of the RALDH2 gene and Lac-Z expression in the roof plate. The identification of several enhancers in the RALDH2 gene, as well as the dynamic expression and involvement of this gene in the patterning of several organs during development is consistent with the idea that RALDH2 expression is governed by multiple, tissue-specific, stage-dependent regulatory modules.

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Program/Abstract # 153
Identification of a conserved roof plate enhancer in the RALDH2 gene
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Retinoic acid signaling is crucial for correct embryonic development. RALDH2 is the major enzyme involved in retinoic acid synthesis during early development and its expression pattern is dynamic. Using bioinformatics we detected multiple evolutionary conserved regions (ECRs) among vertebrate RALDH2 genes. These sequences are distributed from the 5′ region to the 3′ region, including intron 1, where there is an
ECR from amphibians to human (RALDH2.2). The ECR RALDH2.2 (1600 bp) was amplified from the Gallus gallus genome and electroporated into the chick neural tube. RALDH2.2 is a neural enhancer that drives strong expression in the roof plate and interneurons of the chicken and mouse developing spinal cord. Deletion mutants of RALDH2 revealed a roof plate enhancer between 1078 bp and 799 bp that can be useful for targeted expression or Cre-driven recombination, while between 1600 bp and the 1078 bp there is a repressor of neural tube expression. Characterization of a roof plate enhancer in the RALDH2 gene is consistent with a highly modular regulation of RALDH2 expression and confirms that the evolutionary approach is an efficient tool to identify control mechanisms of the RALDH2 gene.

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Program/Abstract # 154
Genomic regulation of the Dll-B gene in the ascidian Ciona intestinalis
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Three members of the Dlx homeobox family have been found in the ascidian Ciona intestinalis. Two of these, Dll-A and Dll-B, are arranged in a cluster, with convergent transcriptional orientations like those seen in the multiple vertebrate Dlx clusters. We are examining the genomic regulation of these genes, as a simple case of the common genomic arrangement of developmental genes in clusters conserved over long evolutionary time spans. A small non-coding region within 1 kb upstream of CiDll-B drives most of the early expression pattern of CiDll-B in a reporter transgene. Other conserved non-coding elements appear to be responsible for refining the expression pattern. Further analysis of other regions flanking each gene of the CiDll-A/B cluster may show whether there are interactions, such as sharing, between enhancer elements and both of the genes.

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Program/Abstract # 155
Gene regulation in the ancestral notochord: Insights from a collection of cis-regulatory elements from the ascidian Ciona intestinalis
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A survey of ∼19 Megabases (∼12%) of the C. intestinalis genome, has led to the identification of 300 novel enhancer elements directing expression in one or more of the six main tissues that compose the larval body. Here we focus on the characterization of 25 of these enhancers, all directing expression in the notochord. This first collection of notochord enhancers has provided us with a training set of sequences, which have been scanned using as a reference the consensus binding sites for transcription factors known to play roles in notochord gene regulation. In addition to that, using a combination of bioinformatic tools, we have identified novel, additional sequence motifs that are frequently found within the notochord enhancers. Both classes of sequence motifs are often found in clustered arrangements. Some of the clusters are able to function as minimal enhancers, thus recapitulating the activity of the “full-length” elements. We have used the combinations of functional motifs to gain insights on architectural constraints required for the enhancers’ function, such as number, assortment, spacing and orientation of the motifs as well as to scan the Ciona genome and to predict novel notochord enhancers.

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Program/Abstract # 156
Dissecting Pax6 regulation in a basal chordate Ciona intestinalis
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Comparative sequence analysis between the Pax6 genomic sequences of Ciona intestinalis and Ciona savignyi reveals conserved non-coding sequences between the two species. Conserved non-coding elements were found in two large introns and in upstream from the first exon. This pattern is reminiscent of the locations of cis-regulatory elements in the Pax6 gene of vertebrates. We have constructed reporter transgenes, incorporating these conserved non-coding regions of the CiPax6 clone, which were tested by electroporation into C. intestinalis one-cell embryos. We found that a transgene including 2.5 kb of upstream sequence, along with the first intron (CiP6-2.5U11) was sufficient to drive CNS expression similar to the endogenous expression seen in the ISH experiments. This investigation of cis-regulation via comparative sequence analysis demonstrates the strength of comparative sequence analysis between the two Ciona species, in combination with functional transgenics, for examining gene regulation. The analysis will be extended to other ascidians to track the functional evolution of regulatory DNA in this gene.

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