evidence, ranging from early transplantation experiments in chick to gene inactivation studies in mice indicate that in amniotes, hindbrain signals are required to specify structures along the D–V axis of the inner ear. In the *kr* mutant, the cochlea is expanded and D structures such as the endolymphatic duct are absent. In this work we have studied the role of HB in axial specification of the inner ear by the means of studying the mouse *kr* mutant. We show that these embryos display an expansion of the otic neurogenic region by *neuroD* and *delta1* staining at very early stages of otic development, at expense of the non-neurogenic region. This is a result of changes in otic patterning genes such *LFng* and *Lmx1*. No differences in cell proliferation are observed in mutant ears, suggesting that this expansion is due to a cell fate change. When NT signals are explored in *kr* mutants, we observed that FGF and Wnt signals are the mainly affected, suggesting that NT signals play a key role instructing OV patterning.

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Program/Abstract # 194
El TGFβ y su papel durante el desarrollo del fenotipo TRHérgico hipotalamico
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El hipotálamo juega un papel vital en el mantenimiento de la homeosptasis, sin embargo poco se sabe del mecanismo que regula la neurogénesis en ésta región. Con el fin de caracterizar los mecanismos moleculares que regulan el establecimiento y/o mantenimiento del fenotipo TRHérgico hipotalámico recientemente, analizamos el transcriptoma de las células TRHérgicas mediante microarreglos de DNA. Encontramos incrementada la expresión del gen inducido tempranamente por TGβ (TIEG1) cuyo papel durante el desarrollo del SNC no ha sido descrito. Estudios recientes muestran que TGβpromueve la diferenciación neuronal induciendo la salida del ciclo celular de progenitores neurales. Por lo que proponemos queTGβ podría regular el establecimiento y/o mantenimiento del fenotipo TRHérgico hipotalámico. En este estudio demostramos que TGβ incrementa la expresión del RNAm de TRH en cultivos primarios de hipotálamo fetal de E17. Mostramos que la expresión del RNAm de TIEG1 y del TβR-II es regulada durante el desarrollo del hipotálamo de rata detectándose desde estadíos muy tempranos. Interesantemente, la expresión exógena de tieg1 induce los niveles del RNAm de TRH in vitro. Finalmente, ensayos de cotransfección con el gen luciferasa muestran que la expresión exógena de tieg1 en cultivos primarios hipotalámicos induce un incremento en la actividad transcripcional del promotor de TRH respecto a sus controles. Estos datos sugieren que TIEG1 en respuesta a TGβ, regula los niveles del RNAm de TRH a través de incrementar la tasa de transcripción del gen.

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Program/Abstract # 195
The homeobox gene Mohawk functions as a transcriptional repressor via three independent, evolutionarily conserved domains
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The homeobox gene *Mohawk* (*Mkx*) is a new member of the TALE superclass of atypical homeobox genes that is expressed specifically in the embryonic progenitor cell populations of a diverse set of tissues, including skeletal muscle, tendon, cartilage and the urogenital system. Using cell-based assays, we demonstrate that *Mkx* function as a potent repressor of transcription and inhibit MyoD-induced myogenic conversion of C3H10T1/2 cells. Domain mapping has revealed that *Mkx* contains three small, evolutionarily conserved repressor domains that can independently repress transcription when fused to the heterologous Gal4 DNA binding domain. In addition, one of these domains can significantly decrease the transcriptional activation activity of VP16. Further work will be presented on the mechanisms by which these unique *Mkx* repressor domains function.

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Program/Abstract # 196
Mechanisms of Hox functional specificity: The role of Hoxa10 in rib formation
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Hox genes are key regulators of embryonic development. Among other functions, they are essential for vertebral identity. Despite their binding to identical DNA sequences, Hox proteins show exquisitely specific functions, suggesting that their functional activity may reside outside of the homeodomain and involve cooperation with other proteins. A wealth of evidence supports the requirement of cofactors for Hox function but few have been described and little is known about how they modulate DNA binding. Furthermore, few Hox targets have been identified. Given the specific roles of *Hoxa10* and *Hoxa11* in the patterning of the axial skeleton, these genes provide a useful paradigm to study Hox specificity and function. In this project, we will use a combination of biochemical and genetic approaches to identify the regions that are both necessary and sufficient for *Hoxa10* functional specificity, search for possible cofactors

that might cooperatively bind to target genes and ultimately identify its downstream targets.

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Program/Abstract # 197
Contribution of *Hoxb1* to the development of the vertebrate skeleton

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Hoxb1-null mice exhibit early patterning defects in the hindbrain, including the failure of rhombomere 4 to maintain its unique identity (Studer et al., 1996). As a result, the facial muscles are not innervated, and these embryos (98%) die soon after birth, unable to suckle. *Hoxb1* expression, however, is not limited to the developing hindbrain. During early somitogenesis, *Hoxb1* expression in the paraxial mesoderm extends into the cervical somites (Studer et al., 1998). Although skeletal phenotypes have been reported in other *Hox* mutants, no skeletal phenotypes have been described in the *Hoxb1* knock-out line. To investigate whether *Hoxb1* is involved in vertebral development, we use Alizarin Red/Alcian Blue staining to characterize the skeletons of 18.5 dpc *Hoxb1* mutant embryos. Our analysis reveals that the loss of *Hoxb1* function leads to multiple homeotic transformations along the vertebral column. In the cervical region, the second cervical vertebra (C2, axis) exhibits morphological changes characteristic of the first cervical vertebra (C1, atlas), including an expanded neural arch and a duplication of the anterior arch of the atlas. In contrast, the neural arch of C1 is reduced in size, similar to C2. The ribs of the first thoracic vertebra often fuse with those of the second thoracic vertebra. Finally, the ribs of the eighth thoracic vertebra often form unilateral or bilateral ectopic sternocostal junctions in *Hoxb1* mutants. Interestingly, the defects observed in these mutants occur at the boundaries between morphologically distinct vertebral domains. In conclusion, these data show that *Hoxb1* plays a critical role in patterning the paraxial mesoderm.

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Program/Abstract # 198
Third helix of murine Hoxc8 homeoprotein facilitates protein transduction in PPFF cells

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The protein transduction domain (PTD) of Antp or HIV-1 TAT has been extensively documented with regard to its membrane transduction potential. Hoxc8, a subgroup of homeobox genes plays a crucial role as a transcription factor in pattern formation by expressing at a specific time and position during early embryogenesis. Hoxc8 contains a 60-aa homeodomain composed of three α-helixes, and the third helix possesses 94% homology with that of Antp. Here, we demonstrate that the third helix of Hoxc8 homeodomain has membrane transduction potential like that of Antp. For high level prokaryotic expression, oligonucleotides encoding the 3rd helix of Hoxc8 homeodomain was designed and synthesized considering the bacterial codon usage. To analyze the entrance of PTD into cells using the fluorescence microscope, PTD was designed to be fused with enhanced green fluorescent protein (EGFP), which is incapable of entering mammalian cells by itself. In addition, the PTD was also designed to be expressed as a thioredoxin- and his-tag fused form, to increase the solubility and purify easily. After purification, the transduction efficiency of PTD was analyzed in PPFF cells: the penetrating activity was increased in dose-dependent manner; as PTD number was increased in tandem, the more EGFP signal was observed. These results altogether imply that the third helix of Hoxc8 homeodomain acts as a PTD, and could be used as a vehicle to transport cargo molecules in the future.

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Program/Abstract # 199
Hoxc8 directly regulates the expression of glucose-regulated protein 78, an ER chaperon

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The Hox proteins are key regulators for the regional pattern formation along the antero-posterior (AP) and other embryonic axes. Despite the fact that homeobox genes are DNA-binding protein, it is still not well understood what Hox regulates. In an attempt to analyze Hoxc8 downstream target genes, a stable cell line over-expressing Hoxc8 was established using F9 murine teratocarcinoma cells, proteome samples were analyzed by 2-DE, and compared with the control. Out of 14 putative downstream target genes analyzed, glucose-regulated protein (Grp) 78 was chosen and analyzed further after cloning a 2.4-kb upstream region of murine Grp78 into the pGL2 promoter vector (pGL2-ugrp78) harboring a luciferase gene. We found that the expression of Grp78 was up-regulated by Hoxc8 *in vitro* and this up-regulation was down-regulated by the siRNA against Hoxc8. Deletion analysis revealed that the 11 putative Hoxc8 binding sites along this region were responsible for Hoxc8-mediated regulation of Grp78 transcription. When *in vitro* EMSA analysis was performed with the recombinant Hoxc8 protein, several areas along this region turned out to bind with Hoxc8 protein, although one promoter proximal region bind rather weakly. This binding was also confirmed *in vivo* through ChIP analysis. These results altogether strongly indicate that Hoxc8 directly regulates the expression of Grp78, implying that Grp78 is one of the putative Hoxc8 downstream target genes.