Subsequently, microRNA-9 enhances Nr2e1 and Pax6 expressions to promote progenitor proliferation in the ventricular and subventricular zones. MicroRNA-9 targets Nr2e1 mRNA to enhance its protein expression in cooperation with Elav1 and Msil, and it suppresses the expression of Meis2 that inhibits Pax6 expression. Concomitantly, each cortical layer is reduced and the tangential migration of interneurons into the pallium is impaired in the double mutants. In the subhippocampal, microRNA-9 suppresses Gsh2 and Fox1 expression to negatively control progenitor proliferation for the production of the striatum neurons; microRNA-9 also regulates the formation of the pallial/subpallial boundary and ventral pallium through its regulation of Pax6, Nr2e1 and Gsh2 expressions. Furthermore, the globus pallidus is missing, the coridor is malformed and thalamoscortical axons and corticofugal axons are miss-routed in the double mutants.

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Program/Abstract # 279

The same enhancer regulates the earliest Emx2 expression in caudal forebrain primordium, subsequent expression in dorsal telencephalon and later expression in cortical ventricular zone

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We have analyzed Emx2 enhancers to dissect Emx2 functions during forebrain development. The FB enhancer we identified immediately 3′ downstream of the last coding exon is well conserved among tetrapods and unexpectedly directed all the Emx2 expression in the forebrain: caudal forebrain primordium at E8.5, dorsal telencephalon at E9.5–10.5 and cortical ventricular zone later than E12.5. Otx, Tcf and two unknown transcriptional factor binding sites were essential to all these activities. The mutant that lacked this enhancer demonstrated that the Emx2 expression under the enhancer is essential to diencephalon development and contributes to dorsal telencephalon development and corticogenesis. However, the FB enhancer did not have activities in cortical hem or Cajal–Retzius cells; nor was its activity in the cortex graded. The Emx2 expression was greatly reduced, but persisted in the telencephalon of the enhancer mutant. There exists another enhancer for the Emx2 expression unique to mammalian telencephalon.

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Program/Abstract # 280

Characterization of the novel interaction between muskelin and TBX20, a critical cardiogenic transcription factor

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The genetic regulation necessary for cardiogenesis is tightly regulated by transcription factors like TBX20, a member of the T-box (Tbx) transcription factor family. TBX20 is expressed in the heart throughout development and missense mutations in TBX20 have been found in patients with congenital heart defects (CHD). Characterization of modifiers of TBX20 will help elucidate the genetic mechanisms of heart development and CHD. A yeast two-hybrid screen using an embryonic mouse heart cDNA library and TBX20b as bait was used to identify potential modifiers of the TBX20 activity and identified an interaction with muskelin (Mkln), a primarily cytoplasmic protein with potential roles in signal transduction machinery scaffolding and nucleocytoplasmic protein shuttling. The hypothesis of this project is that TBX20 is regulated by muskelin during mouse cardiogenesis. To determine how muskelin regulates the TBX20 activity, the protein interaction, expression patterns, and functional significance of the TBX20b–Mkln interaction will be characterized. In cellular studies, I have shown that muskelin directly binds to the T-box domain of only the TBX20b isoform by its kelch repeats domain. Immunostaining of a myocardium cell line, transfected with tagged TBX20b and muskelin, revealed colocalization in the cytoplasm. Preliminary immunohistochemistry staining on embryonic mouse hearts indicate coexpression in the endocardial valvular and myocardial interventricular cells. Functional significance will be explored using embryonic mouse cardiomyocytes.

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Program/Abstract # 281

Prrx1 expression in mouse nociceptive neurons is controlled by alternative promoters

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The transcription factor Prrx1 has a crucial role in the differentiation/survival of nociceptive neurons of dorsal root ganglion (DRG), spinal cord dorsal horn and functionally equivalent supraspinal areas. Nevertheless, our understanding of the transcriptional mechanisms that control the proper spatiotemporal expression of the Prrx1 gene remains limited. To approach this issue, the 5′–flanking region of Prrx1 translation start point was analysed by luciferase reporter assays using a DRG-derived neuronal cell line (ND7/23). Three regions displaying promoter activity were identified which are suggestive of alternative promoter usage as a mechanism of control of Prrx1 expression. Moreover, 5′ RACE analysis led us to the identification of Prrx1 mRNA variants containing distinct 5′UTR regions on Exon 1. These alternative first exons have no consequences in the Prrx1 open reading frame and therefore are likely involved in differential mRNA stability. In addition, a detailed analysis of the distal promoter revealed the presence of a bona fide TATA box that was validated by EMSA and site-directed mutagenesis. Further analysis of this sequence revealed the presence of two adjacent regulatory elements, one presenting a capability to strongly reduce the combined activity of the three promoters and another one with the potential to inhibit the repressive trait of the former. Altogether, the present results led to the identification of Prrx1 alternative promoters and some regulatory motifs likely implicated in the modulation of the Prrx1 expression.

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Program/Abstract # 282

The transcriptional co-repressor TRIM28 is differentially required by KRAB zinc finger proteins during early mammalian embryogenesis

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TRIM28 (aka TIF1β and KAP-1) is a transcriptional co-repressor that functions by inducing heterochromatin formation. The target specificity of TRIM28 is believed to reside in its ability to bind different KRAB domain proteins. Although KRAB zinc finger proteins represent the largest family of transcriptional regulators in mammals, the functions of individual members of this family are largely unknown. Our previous work on chotu, a mutation in the KRAB zinc finger protein 568, revealed a