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Program/Abstract # 236
Lin28 controls cell fate in mammalian neurogenesis

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Lin28, the mammalian homolog of the *C. elegans* heterochronic gene *lin-28*, is a factor in stem-cell identity and pluripotency and is in diverse developing tissues in the embryo and adult, including muscle, neural crest, and epithelia. It is a cytoplasmic protein with two RNA-binding domains. In *C. elegans*, lin-28 positively regulates genes involved in developmental timing that are repressed by miRNAs. Mammalian Lin28 was found to help induce pluripotency, but its natural role is not known. We addressed whether Lin28 regulates cell fates in mammals using EC cells undergoing neuro/gliogenesis, where the birth of neurons precedes that of glial cells. Lin28 is abundant in EC cells, and is rapidly down-regulated prior to the formation of neurons. When constitutively expressed, Lin28 increases the number of neurons and blocks formation of glial cells (GFAP-positive). Neuron differentiation does not start earlier than usual, based on the accumulation of nestin and neuronal tubulin. In addition, we do not detect a change in proliferation rate of undifferentiated cells nor an increase in overall cell number by the time of bulk neuron differentiation. These observations suggest that Lin28 activity controls cell fate, possibly acting in transit amplifying cells. Its effect is analogous to the heterochronic phenotype of lin-28 mis-expression *C. elegans*, suggesting that mammalian Lin28 has a similar function. Finally, it has been reported that Lin28 blocks the processing of miRNAs in the nucleus in EC and ES cells. Our data are consistent with Lin28 acting as a cytoplasmic mRNA-binding protein: Lin28 localizes primarily in the cytoplasm in EC cells to polyribosomes and P-bodies.

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Program/Abstract # 237
An essential role for Frizzled5 in neuronal survival in the parafascicular nucleus of the thalamus

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Frizzled5 (Fz5), a putative Wnt receptor, is expressed in the retina, hypothalamus, and the parafascicular nucleus (PFN) of the thalamus. By constructing *Fz5* alleles in which beta-galactosidase replaces Fz5 or in which Cre-mediated recombination replaces Fz5 with alkaline phosphatase, we observe that Fz5 is required continuously and in a cell autonomous manner for the survival of adult PFN neurons but is not required for proliferation, migration, or axonal growth and targeting of developing PFN neurons. A motor phenotype associated with loss of Fz5 establishes a role for the PFN in sensorimotor coordination. Transcripts coding for Wnt9b, the likely Fz5 ligand *in vivo*, and beta-catenin, a mediator of canonical Wnt signaling, are both down-regulated in the $Fz5^{-/-}$ PFN, implying a positive feedback mechanism in which Wnt signaling is required to maintain the expression of Wnt signaling components. These data suggest that defects in Wnt-Frizzled signaling could be the cause of neuronal loss in degenerative CNS diseases.

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Program/Abstract # 238
***Pitx2* is critical for the survival and specification of extraocular muscles**

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Pitx2 is the only known genetic regulator of extraocular muscle (EOM) development, but little is understood about the mechanisms underlying EOM development. The purpose of the current experiments was to determine which cell type *Pitx2* is required in and test potential mechanisms of *Pitx2* function in EOM development. Cre-Lox technology was used to determine that *Pitx2* is required in the mesoderm-derived myoblasts, but not the neural crest-derived fascia for EOM formation. In wildtype mice, PITX2 is expressed in EOM primordia two days prior to the expression of PAX7, MYOD and MYOG. $Pitx2^{null/null}$ embryos completely lack EOMs and expression of *Pax7* and the muscle regulatory factors (MRFs) *Myf5*, *MyoD* and *Myog*. Conversely, embryos lacking *Pax7* function have EOMs with normal expression of PITX2, indicating that *Pitx2* is genetically upstream of *Pax7*. Predicted conserved PITX2 binding sites in the MRF promoters and enhancers were identified and tested for functionality *in vitro*. PITX2 is able to bind and activate the *MyoD* promoter in C2C12 muscle precursor cells. Additionally, EOM precursor cells are greatly reduced in $Pitx2^{null/null}$ embryos at e11.5. A massive increase in cell death was observed at e10.5 in these embryos, while no changes in cell proliferation were seen. These results indicate that *Pitx2* is genetically upstream of *Pax7*, *Myf5*, *MyoD*, and *Myog* in EOM development, making it the earliest known activator of EOM specification. *Pitx2* is required for the early survival of the EOM precursors and later to specify them as myoblasts by activating *MyoD*, thus *Pitx2* plays multiple roles in EOM development.

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Program/Abstract # 239
Evidence for functional conservation of myogenic regulatory factors: Electric fish MyoD and myogenin induce mammalian skeletal muscle differentiation

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The myogenic regulatory factors (MRFs) are pivotal in the commitment of stem cells to the skeletal muscle lineage and the transcriptional activation of muscle-specific genes during differentiation in vivo. In vitro, each MRF (MyoD, myogenin, myf5, MRF4) can induce the myogenic program in non-myogenic cells. However, the role of MRFs in maintaining the muscle phenotype is less well understood. The muscle-derived electric organ (EO) of the electric fish *S. macrurus* is an excellent model system to address this question because of its partial muscle phenotype. In addition, EO cells transcribe all four MRF genes and sequence analysis reveals conservation of the functional domains in *S. macrurus* MRFs. In the present study, we tested the capacity of *S. macrurus* MyoD and myogenin to convert non-myogenic C3H10T1/2 and NIH3T3 mouse embryonic cells into muscle cells. Transfection of *S. macrurus* MyoD or myogenin into these cells resulted in the expression of sarcomeric myosin heavy chain (MHC) mRNA and protein. Our expression studies also showed that endogenous mammalian MyoD and myogenin genes