10 independent DNA injections, 11 different lines carrying the transgene (Bi-noggin) were generated and bred. To generate mice that express noggin in the SVZ zone, we crossed Bi-noggin mice with nestin-rtTA mice and double transgenic animals (F1 to F3) selected for further studies. The nestin-rtTA transgenic mouse contains the tetracycline transactivator (rtTA) under the control of the CNS specific second intronic enhancer of the nestin gene fused to the β-galactosidase coding sequence. Double transgenic mice were treated with doxycycline to specifically induce noggin in nestin expressing cells in the SVZ. The goals our studies are to determine the role of noggin in: maintaining a neurogenic environment in the SVZ stem cell niche, the effects of noggin overexpression during embryonic development, and its role in repair following injury. Supported by NIH grants NS-048187.

doi:10.1016/j.ydbio.2006.04.097

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Neurogenin 1 expression in mouse embryonic stem cells produces growth factor-sensitive neural progenitors

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Delineating the cascades of growth and transcription factor expression that shape the developing nervous system will improve our understanding of its molecular histogenesis and should also suggest strategies for cell replacement therapies and approaches to control the ectopic neurogenesis that contribute to neuropathology following injury. Despite the relative complete understanding of the basic histogenesis of the nervous system, the precise cascades of growth factors and transcription factors required in neural cell fate determination are largely unknown. In the current investigation, we have exploited the essential pluripotentiality of embryonic stem cells (ESC) to determine the ability of pulsed expression of the neurogenic gene, Ngn1, to drive neuronal differentiation. Transient Ngn1 expression in ESC was sufficient to produce widespread neuronal differentiation – even in the presence of LIF and inhibitors present in serum? – and yielded both peripheral and central nervous system phenotypes. Somewhat surprisingly, the induced cells were sensitive to A-P and D-V patterning molecules including retinoic acid, BMP4, noggin and Shh. Consistent with current theories regarding neural induction, FGF signaling was required in this process. The results of these experiments establish inducible transgene expression in ES cells as a powerful model to understand the role of bHLH factors in neurogenesis, to tease out growth factor effects in progenitor cell differentiation and potentially also provides a source of primitive cells that could respond to local signals to integrate successfully following injury. Supported by NIH-NS 39438, DE-07057.

doi:10.1016/j.ydbio.2006.04.098

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Response of glial precursors to penetrating embryonic brain injury

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Understanding embryonic brain response to injury has become increasingly more important since abnormal brain development has been linked to many neurological disorders. We have analyzed the response of glial progenitor cells in a novel embryonic chick brain injury model. A unilateral stab wound injury was made in embryonic day 11 or 15 optic tecta and the response was followed by analyzing the mRNA expression of several specific markers. Two days after injury histological defects such as lateral ventricular dilatation, necrotic foci, periventricular cyst and intraventricular hemorrhage were observed at distance from the stab wound but associated with the injured tectum. Expression of neuronal and oligodendrocyte precursors markers are down-regulated in the injured tectum, even far removed from the stab wound site. In contrast, up-regulation of the mature astrocyte marker, glial fibrillary acidic protein (GFAP), was observed at the wound site, around necrotic areas and cysts, as well as in normal embryonic expression areas for this marker at this stage. The expression and distribution of GFAP in the uninjured site maintained the normal pattern. Also, distribution and levels of radial glial markers such as BLBP and GLAST were increased at the wound site and in the ventricular zone of the injured tectum. By Northern blot, up-regulation of cyclooxygenase-2 mRNA in the injured tectum was also observed. In sum, these results indicate unilateral up-regulation of ventricular zone precursors and an inflammatory response occurs soon after injury in this model.

doi:10.1016/j.ydbio.2006.04.099

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Wnt signaling controls proliferation and differentiation of retinal stem cells in zebrafish

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The zebrafish eye contains stem cells at the retinal margin that allow continual growth of the retina throughout the life of the fish. The mechanism by which stem cells are reserved in this niche during development and how proliferation and differentiation are controlled is unknown. In other epithelial stem cell niches, such as intestine, Wnt signaling maintains cells within the niche in a proliferative state, and the absence of Wnt signaling outside the niche permits differentiation. We test the hypothesis that Wnt signaling promotes proliferation of stem cells within the retina. Pharmacologic inhibition of GSK3â, mimicking activation of the Wnt cascade, when