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Molecules in focus

Sox2 roles in neural stem cells

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ABSTRACT

Throughout vertebrate evolution, *Sox2* marks the developing nervous system from its earliest developmental stages and, therein, the most undifferentiated precursor cells, including stem cells. Recent gene targeting studies investigated the function of *Sox2* in two neuronal systems: the developing eye and brain. These studies uncovered a requirement for *Sox2* in the maintenance of neural stem cells, as well as a downstream role in the differentiation of specific neuron sub-types. In both systems, *Sox2* action is markedly dose-dependent, and downstream-target gene studies are beginning to reveal the mechanisms of *Sox2* function.

Sox2 is a "founder member" of the *Sox* gene family: it was identified in the first screening for genes related to the sex-determining gene *Sry* by the possession of an HMG-box DNA-binding domain (Gubbay et al., 1990) and, independently, as a regulator of the *Fgf-4* gene, essential for survival of the early mouse embryo (Dailey et al., 1994).

The generation of a targeted null mutation in mouse *Sox2* revealed its fundamental function in the maintenance of the early, pluripotent stem cells of the epiblast (Avilion et al., 2003), joining a small number of other mutants (*Oct4*, *Nanog*, etc.) showing that maintenance of pluripotent cells relies on specific transcription factors.

Strikingly, acting together with three other transcription factors, *Sox2* can also re-establish pluripotency in terminally differentiated cells reprogramming them to induced pluripotent stem cells (iPS) (Takahashi and Yamanaka, 2006).

Even before the discovery of its early functions, one feature of *Sox2* that immediately attracted attention was its conserved expression in the developing central nervous system (CNS) (Collignon et al., 1996). From zebrafish to chick to human (with precedents including *Drosophila Sox2*-related *Dichaete*) *Sox2* is active in the embryonic nervous system from the earliest stages of development, predominantly in the proliferating, undifferentiated precursors (Pevny and Placzek, 2005; Wegner and Stolt, 2005).

Here, we review recent findings defining critical *Sox2* functions in neural stem cells (NSCs). In particular, we highlight how parallel studies using targeted mutagenesis to disrupt *Sox2* function in mouse eye and brain, have led independently to the discovery of roles for *Sox2* at different levels of neural stem cell function: neural stem/progenitor cell maintenance, and neuronal differentiation (Fig. 1).

1. *Sox2* is expressed in functionally defined neural stem cells

NSCs are defined functionally: they are able to self-renew and to give rise by differentiation, to neurons, astroglia and oligodendroglia within the clonal progeny of a single stem cell (i.e. they are multipotential). A *beta-geo* gene (encoding beta-galactosidase/G418 resistance), driven by *Sox2* regulatory sequences as a transgene (Zappone et al., 2000) or knock-in allele (Ferri et al., 2004), was used to show that individual G418-resistant (i.e. *Sox2*-expressing) cells cultured from embryonic and adult neurogenic regions were able to self-renew and were multipotential in clonogenic assays, demonstrating that *Sox2*-expressing cells included NSCs. Consistent with this, studies using a *Sox2* EGFP-knock-in allele (Ellis et al., 2004) demonstrated EGFP expression in proliferating precursors throughout the neuraxis *in vivo*, and in NSCs *in vitro*.

Sox2 expression is also detected in the postnatal neurogenic regions in the subventricular zone (SVZ) and hippocampus dentate gyrus (DG)(Ferri et al., 2004; Ellis et al., 2004); here, NSCs, marked by GFAP/nestin positivity, express *Sox2* (Ferri et al., 2004; Ellis et al., 2004), and recent studies demonstrated that such *Sox2*-expressing cells in the hippocampus DG indeed behave functionally as NSCs *in vivo* (Suh et al., 2007).

2. *Sox2* maintains NSC properties

The first evidence that *Sox2* can function to maintain neural precursor cell properties was obtained by gain-of-function/ectopic

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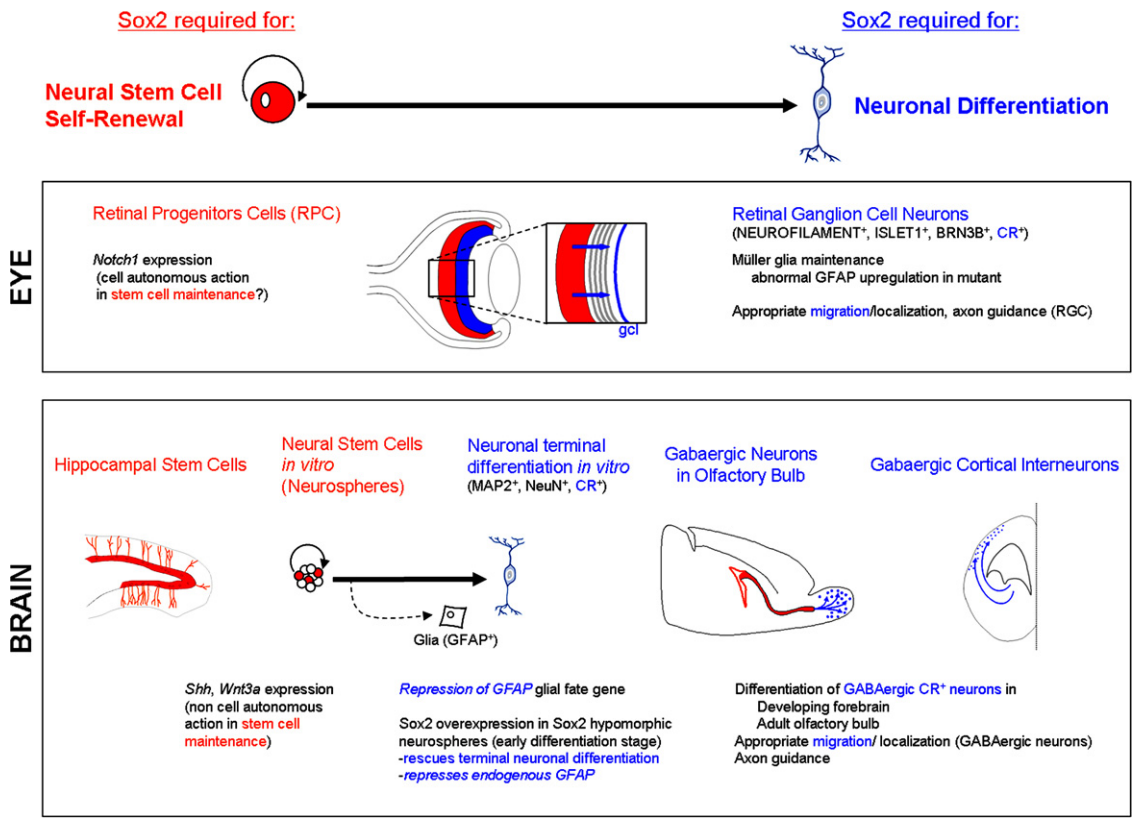

Fig. 1. Sox2 is required for the maintenance of neural stem cells (left; red) and for their differentiation into neurons (right, blue), in eye (top) and brain (bottom). Neural stem cell and specific neuronal types which are impaired in Sox2-targeted mutants are shown in red and blue, respectively; blue arrows indicate altered neuronal migration/localization in mutants (mutants are hypomorphic or conditional null, in eye or brain; see text for details). Gcl: ganglion cell layer (differentiating neurons/RGC).

expression and by dominant interfering studies in *Xenopus* and chick (reviewed in Wegner and Stolt, 2005; Pevny and Placzek, 2005). In *Xenopus*, expression of dominant-negative forms of *Sox2* in early embryos indicated that *Sox2* is required for neuroectoderm cells to maintain their neural identity (Kishi et al., 2000). In *Xenopus* retina, blockage of the *frizzled5* (*Xfz5*) receptor, or of *Sox2*, inhibited neural progenitor cell proliferation and neurogenesis, and biased progenitors' fate towards Muller glia at the expense of (neuronal) retinal ganglion cells; rescue experiments indicated that *Sox2* is downstream to *Xfz5* (Van Raay et al., 2005).

In chick, electroporation of SoxB1 genes (Sox2, Sox1 or Sox3) maintained neural progenitor identity, whereas expression of SoxB1 mutants acting as chimeric transcriptional repressors led to cell cycle exit and the onset of differentiation (Graham et al., 2003; Bylund et al., 2003). Ectopic expression of *Sox2* or *Sox1* in mouse ES cells biased their differentiation towards a neuroectoderm fate, at the expense of mesodermal or endodermal fate (Zhao et al., 2004). Important evidence also came from studies of *in vitro* reprogramming of optic nerve oligodendrocytes (O-2A cells) to self-renewing, multipotent neural stem-like cells, by *in vitro* PDGF treatment. It was concluded that the de-differentiation of O-2A cells is dependent on *Sox2* reactivation following recruitment of *Brm* chromatin-remodelling factors onto its 5′ enhancer (Kondo and Raff, 2004).

3. *Sox2* is required to maintain eye and brain NSC

Loss-of-function experiments in mice to investigate *Sox2* function in the developing CNS made use of gene targeting to generate hypomorphic and conditional/null knock-out mutations.

The targeted deletion of a 5′ telencephalic enhancer of *Sox2* generated a hypomorphic allele expressing 25–30% of wild type *Sox2* in brain (*deltaEnh*) (Ferri et al., 2004; Cavallaro et al., 2008). Mice homozygous for the *deltaEnh* mutation, or heterozygous for a null mutation, did not show overt abnormalities; however, compound null/hypomorphic mutants showed significant brain defects including reduced cortex volume, thalamo-striatal parenchymal loss, epilepsy, and motor/neurological problems. At the cellular level, a marked loss of GFAP/nestin-positive NSC and precursor cells in the hippocampus DG, and of neurogenesis in both DG and SVZ, was found (Ferri et al., 2004).

Conditional neural-specific *Sox2* deletion with a *nestinCre* transgene resulted in minor impairment of embryonic brain development and neurogenesis, yet was lethal at birth (Miyagi et al., 2008).

Using an independently generated conditional *Sox2* mutant allele (Favaro et al., 2009) that results in complete panneural deletion from E12.5, mutant mice were born with minor brain defects and survived for few weeks after birth; however, within 1 week (P7), NSCs and neurogenesis were completely lost in the hippocampus, leading to DG hypoplasia resembling that caused by late Sonic Hedgehog – *Shh* – loss. Loss of *Shh* expression in the *Sox2*-mutant developing hippocampus was observed and stimulation of the *Shh* pathway *in vivo* partially rescued NSCs, neurogenesis, and DG growth. The possibility that *Shh* is a direct target of Sox2 is being examined by chromatin immunoprecipitation (ChIP), site-directed mutagenesis and transgenic assays. Interestingly, recent data indicate that Sox2 itself is regulated by *Gli2*, a *Shh* effector (Takanaga et al., 2008), suggesting a loop by which the *Shh* pathway, activated by *Sox2*, might in turn stimulate Sox2 expression.