REVIEW

Role of SoxB1 transcription factors in development

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Abstract SoxB1 factors, which include Sox1, 2, and 3, share more than 90% amino acid identity in their DNA binding HMG box and participate in diverse developmental events. They are known to exert cell-type-specific functions in concert with other transcription factors on Sox factor-dependent regulatory enhancers. Due to the high degree of sequence similarity both within and outside the HMG box, SoxB1 members show almost identical biological activities. As a result, they exhibit strong functional redundancy in regions where SoxB1 members are coexpressed, such as neural stem/progenitor cells in the developing central nervous system.

Keywords Sox factor · SoxB1 member · ES cells · iPS cells · Neural stem cells · Human eye disorder

Introduction

The male sex determination gene *Sry* (sex-determining region of Y chromosome) was the first *Sox* (SRY-related HMG box) gene family member identified [1]. Sox proteins contain a 79-amino acid HMG box domain responsible for sequence-specific DNA binding [2, 3]. About 30 *Sox* genes have been identified, including 20 that are present in both

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the mouse and the human genome [4]. Mammalian *Sox* genes have been divided into eight groups, designated A–H, based on phylogenetic analysis of HMG box regions. The B group is further divided into two subgroups: SoxB1 proteins (Sox1, 2, 3) containing transcriptional activation domains and SoxB2 proteins (Sox14, 21) containing repressor domains [5]. SoxB1 subfamily transcription factors are predominantly expressed in the early embryo, developing testis, and nervous system, and are important for cell fate determination and cell differentiation during mouse development (Table 1), as shown by dominant negative and knockout mouse studies [6–9].

SoxB1 members control the expression of distinct sets of genes in a cell-type-specific manner by interacting with specific partners [10]. In this review, we give an overview of the functions of SoxB1 family genes in embryonic development and stem cells, focusing on the role of *Sox2*.

Cell-specific function and partnerships of SoxB1 transcription factors

Sox proteins bind to DNA in a sequence-specific manner (5′-A/TA/TCAAA/TG-3′) through their HMG domain [3, 11, 12] and either activate or repress transcription [4, 10]. The HMG domain is highly conserved among Sox factors; accordingly, in vitro DNA binding studies reveal no significant differences in recognition sequence specificity among family members. However, each Sox factor recognizes distinct sets of regulatory regions containing Sox binding sites in vivo [10]. How do Sox factors recognize their appropriate target genes and regulate their transcriptional levels in a cell-type-specific manner?

An important clue came from analysis of fibroblast growth factor-4 (*FGF-4*) expression in embryonic

Table 1 Expression and biological functions of SoxB1 members

Gene	Expressed domains	Functions
Sox1	Embryonic nerve system (CNS), lens, urogenital ridge	Lens development (induction and maintenance of gamma-crystallin gene expression) [81]
		Deletion in KO mice leads to microphthalmia, cataracts, and spontaneous seizures [81, 82]
Sox2	Inner cell mass, primitive ectoderm, trophoblast stem cells, embryonic nerve system (CNS), lens, neurogenic regions in adult brain	Deletion in KO mice is embryonic lethal at implantation stage (involved in gene expression in FGF4 [13–15], Oct4 [50, 51], Nanog [47], UTF1 [21], etc., together with Oct-3/4)
		Induction of gamma- and delta-crystallin gene expression [24, 25]
		Induction of Nestin gene expression in neural stem/progenitor cells [27, 28]
		Involved in its own expression in ES cells and neural stem cells [29–31, 34]
		Required for maintaining neural stem cell state and neurogenic potential [78–80, 86, 90]
Sox3	Epiblast, embryonic CNS, lens, urogenital ridge	Required for early embryogenesis, gonadal function, and hypothalamo-pituitary axis formation [89, 96]
		Candidate gene for human X-linked mental retardation syndromes [97]

carcinoma (EC) and embryonic stem (ES) cells [13–15]. The regulatory region controlling *FGF-4* expression in EC and ES cells contains Octamer factor and Sox binding sites, which serve as binding sites for embryonic Octamer factor 3/4 (Oct-3/4) [16–18] and Sox2, respectively (Fig. 1a). Binding of both factors to their recognition sites is essential for activating transcription, since mutation of either regulatory element results in complete loss of transcription. Subsequently, we demonstrated that the expression of undifferentiated embryonic cell transcription factor 1 (*UTF1*) gene [19, 20] in ES cells and EC cells is regulated in a similar manner [21], suggesting that the combined action of Oct-3/4 and Sox2 is a general mechanism that regulates gene expression in ES and EC cells. Indeed, a number of ES and EC cell genes, including *Oct-3/4* and *Sox2* themselves, are now known to be regulated by the same mechanism (for details, see below). However, unlike

other regulatory elements containing Octamer factor and Sox binding sites, the *UTF1* regulatory region can selectively recruit Oct-3/4–Sox2 complexes and prevent the binding of similar protein complexes, such as Oct-1–Sox2 and Oct-6–Sox2 (Fig. 1b). This specificity is dictated by the unique ability of the Oct-3/4 POU-homeodomain to recognize a variant of the Octamer motif in the *UTF1* regulatory element. It is noteworthy that both *FGF-4* and *UTF1* regulatory regions are not located in the proximity of their promoter regions, but a couple of kilobases away from them. Therefore, Nowling et al. [22, 23] were interested in examining whether Oct-3/4–Sox2 complex directly interacts with pre-initiation complex on the basal *FGF-4* gene promoter or some additional factor(s) are involved in the cooperation. They found that the co-activator p300 physically associates with Sox2 and mediates the transcriptional stimulating activity of Oct-3/4–Sox2 protein complex to the promoter.

Sox2 cooperates with other transcription factors in other cell types as well [10]. For instance, transcription of δ-crystallin in lens cells is activated by the DC5 enhancer only when both Sox2 and Pax6 are bound [24, 25]. Cooperation with Pax6 appears to be restricted to SoxB1 group members, since other types of Sox factors, such as Sox9, fail to activate transcription through DC5, even though Sox9 binds to DC5 in vitro as strongly as do Sox1, 2, or 3. Moreover, when Sox2 and Pax6 were co-expressed in vivo, both proteins were found by chromatin immunoprecipitation (ChIP) to bind to DC5, whereas neither was able to bind to DC5 when expressed alone [24]. These data show that Pax6 and SoxB1 proteins are unable on their own to form stable protein–DNA complexes with the DC5 enhancer in vivo, even though they are able to do so in vitro. Thus, interactions between SoxB1 proteins and their partners are important not only for selecting target sequences, but also for stable binding to regulatory regions in vivo.

Similarly, the N-3 regulatory region, which controls *Sox2* expression in the diencephalon and optic vesicle, activates transcription by the combined action of Sox2 and Pax6 [26]. The core sequence of N-3 comprises a Sox binding sequence and a noncanonical Pax6 binding sequence arranged in the same orientation and spacing as found in the DC5 enhancer, suggesting that as for Oct-3/4 and Sox2 in ES and EC cells, functional cooperation between Sox factors and Pax6 is a widespread mechanism of gene regulation in regions where Pax6 and Sox factors are coexpressed.

The regulatory region of the *Nestin* gene provides another example of SoxB1 factors activating cell-type-specific transcription [27, 28]. Like the *UTF1* and *FGF-4* enhancers, the *Nestin* regulatory region contains Octamer and Sox binding sites but is not active in EC and ES cells.

Fig. 1 Combinatorial action of Sox2 and its partner proteins on regulatory enhancers.
a Sequences of Sox-dependent regulatory regions of *FGF-4* and *δ-crystallin* genes.
b Selective recruitment of the Oct-3/4–Sox2 complex by the UTF1 regulatory region. The *UTF1* regulatory enhancer serves as a specific binding site for Oct-3/4–Sox2 complex, but prevents similar protein complexes, such as Oct-1–Sox2, from binding to the variant Octamer sequence (for details, see reference [21])
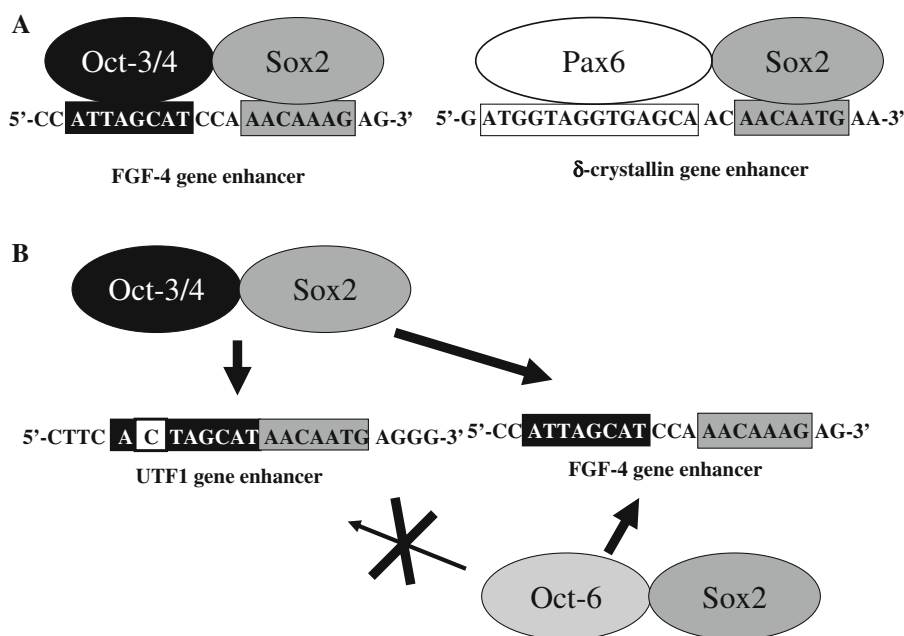
Rather, it functions specifically in neural stem/progenitor cells, in which Sox2 cooperates with Octamer factors Brn 1/2/4 or Oct-6 to activate transcription. Unlike other regulatory regions containing Octamer factor and Sox binding sites, the *Sox2* regulatory region 2 (SRR2) is able to support transcription in both ES cells and neural stem/progenitor cells [29, 30]. Oct-3/4 binds to this site to stimulate transcription in ES cells, whereas Brn1/2/4 and Oct-6 cooperate with Sox2 at SRR2 to drive expression of genes in neural stem/progenitor cells.

Thus, regulatory regions containing Octamer factor and Sox binding sites can be divided into three groups based on their specificity: those that function in ES cells, those that function in neural stem cells, and those that function in both. However, apart from the *UTF1* regulatory region, which contains an Octamer binding sequence variant that is only recognized by the Oct-3/4 and Sox2 complex (Fig. 1b) [21], it is not clear at present why regulatory regions with similar organizations display such distinct cell-type specificity.

Sox2 regulatory region 2 shows strong regional specificity in CNS

SRR2 and the *Nestin* enhancer have distinct regional specificities in neural stem/progenitor cells of the developing central nervous system (CNS), as shown by transgenic analyses [31]. The *Nestin* enhancer is active in neural stem/progenitor cells throughout the CNS [27, 28], whereas SRR2 functions only in the ventricular and subventricular zone of restricted telencephalic areas (Fig. 2).

In the rostral telencephalon, SRR2 is active in the ventricular and subventricular zones of the ganglionic eminence and lateral cortex, but not the medial cortex. In the adult mouse brain, SRR2 activity was detected in the subventricular zone of the lateral ventricle and along the rostral migrating stream, where actively dividing cells reside. At least some of these cells display the hallmark properties of neural stem cells, as shown by an in vitro clonogenic assay. However, the molecular mechanisms governing the regional activity of SRR2 are not known at present, since Brn1, Brn2, and Sox2 are expressed in the entire CNS. A likely possibility is that the chromatin structure around SRR2 allows access to Brn-Sox2 complexes specifically in the lateral cortex and ganglionic eminence, but not in the medial telencephalon and other regions of the CNS. This hypothesis is supported by our ChIP analysis demonstrating that Sox and Brn factors interact with SRR2 specifically in the telencephalon [31]. Thus, the specific recruitment of these proteins to SRR2 in the telencephalon appears to define the spatiotemporal activity of the enhancer in the developing nervous system.

However, this brings up another question: how is it that *Sox2* is expressed in neural stem/progenitor cells along the entire CNS, when SRR2 only functions in a restricted area of the telencephalon? The *Sox2* locus contains an additional regulatory element that drives expression in the dorsal telencephalon [32, 33], in a pattern largely complementary to that of SRR2, suggesting that endogenous *Sox2* expression in the telencephalon reflects the combined actions of these two regulatory elements. Moreover, regulatory regions controlling expression in other areas of the CNS, such as the midbrain and spinal cord, have been
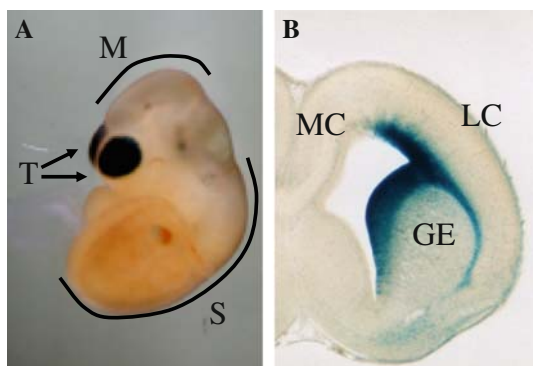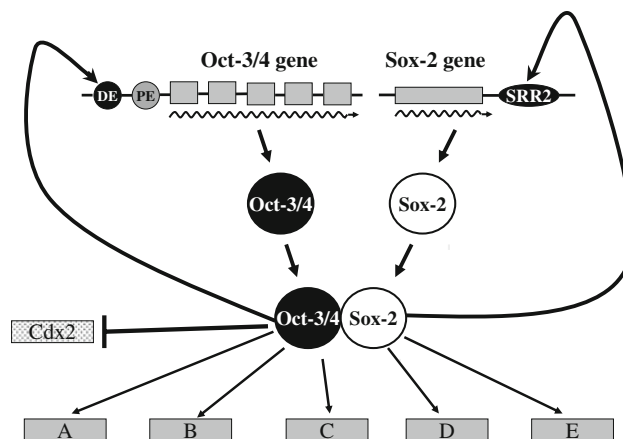
Fig. 2 Strong regional specificity of *Sox2* regulatory region 2 (SRR2) in midgestation mouse embryos. **a** β-galactosidase activity of SRR2-β-geo transgenic mouse embryo at 10.5 days postcoitum (dpc). **b** Section of β-gal-stained embryonic brain at 16.5 dpc. These data were originally published in [30]. *T* Telencephalon, *M* midbrain, *S* spinal cord, *GE* ganglionic eminence, *LC* lateral cortex, *MC* medial cortex

Fig. 3 Autoregulatory circuit of *Oct-3/4* and *Sox2* gene expression in ES cells. Expression of *Oct-3/4* and *Sox2* are controlled by the Oct-3/4-Sox2 complex through the DE and SRR2 enhancers, respectively. *A–E* represent downstream genes, including *Nanog* and *UTF1,* that are under the control of this pluripotency transcription factor complex. Oct-3/4 also functions to repress *Cdx2* gene expression in which Sox2 is probably not involved. Although not depicted in the figure, Cdx2 blocks Oct-3/4 gene expression and commits differentiation to trophoblast cell lineage. Thus, reciprocal repression between Oct-3/4 and Cdx2 appears to be involved in cell fate determination between inner cell mass cells, which are the in vivo equivalent of ES cells, and trophectoderm within the blastocyst. *DE* Distal enhancer, *PE* proximal enhancer

identified [34–36]. Thus, the endogenous Sox2 expression pattern results from a piecemeal compilation of regional expression domains controlled by different regulatory elements. The expression patterns of a number of other genes, including *Otx2* and *Pax6*, are similarly patched together by numerous regulatory regions that each control expression within a specific subregion [37–41]. This is likely the best way to ensure expression within similar but molecularly distinct populations throughout the CNS.

Sox2 is a key regulator of ES cell pluripotency

At early embryonic stages, *Sox2* expression is mainly restricted to cells with stem cell characteristics, such as the inner cell mass, epiblast, trophoblast stem cells, and germ cells [8, 42, 43], and is downregulated in cells with restricted developmental potential. *Sox2* null embryos die immediately after implantation, and knockdown or conditional knockout of *Sox2* in mouse ES cells leads to spontaneous differentiation [8, 44, 45], underscoring its importance in the maintenance of pluripotency. As described above, Sox2 forms a complex with Oct-3/4 to activate transcription of numerous genes in ES and EC cells whose regulatory regions contain Octamer factor and Sox binding sites. In addition to *FGF-4* and *UTF1*, *Fbxo15* [46], *Nanog* [47, 48], and *Lefty1* [49] are transcriptionally regulated by the Oct-3/4–Sox2 complex. Interestingly, the *Oct-3/4* and *Sox2* loci themselves possess enhancers that can be activated by the Oct-3/4–Sox2 complex in a stem-cell-specific manner [29, 50, 51], creating an autoregulatory transcriptional loop that maintains pluripotency of ES cells (Fig. 3). Impairment of this regulatory circuit likely leads to ES cell differentiation; indeed, Cdx2 abrogates the

circuit by forming a reciprocal inhibitory loop with Oct-3/4, thereby promoting trophectodermal differentiation [52]. Although inducible *Sox2* null ES cells fail to maintain pluripotency and differentiate primarily into trophectoderm-like cells, Sox2 is unexpectedly not required for activation of most regulatory enhancers containing Octamer factor and Sox binding sites (Utf1, Fgf4, Pou5f1, Sox2, Nanog, and Lefty1) [45]. This is likely due to functional redundancy, since Sox4 [5], Sox11 [53], and Sox15 [54] are also present in ES cells. Nonetheless, DNA microarray analysis with *Sox2* null ES cells revealed that expression of *Nr5a2* [55], a positive regulator of Oct-3/4 expression, was downregulated. Conversely, *Nr2f2* [56], a negative regulator of Oct-3/4 expression, was upregulated, suggesting that Sox2 is required for ES cell pluripotency because it maintains the requisite level of *Oct-3/4* expression. Consistent with this hypothesis, forced expression of *Oct-3/4* renders *Sox2* expression dispensable for the maintenance of ES cell pluripotency [45]. Although Sox2 is indispensable for the maintenance of ES cell pluripotency, it has been shown that a moderate increase (twofold or less) in the level of Sox2 expression in ES cells has been shown to reduce their self-renewal activity and promote differentiation into neuroectoderm, mesoderm, and trophectoderm, but not endoderm, while a high level of Sox2 expression affects the ES cell viability [57, 58]. Thus, like Oct-3/4 [59], Sox2 also functions as a molecular rheostat to control the self-renewal and differentiation of ES cells.

A recent ChIP-on-Chip analysis revealed that more than half of the promoter regions occupied by Oct-3/4 are also bound by Sox2 in human ES cells [60]. Interestingly, the transcription factor Nanog, which also plays a crucial role in maintaining ES cell pluripotency, binds to most of these same promoters in ES cells, suggesting that these three transcription factors form a core transcriptional regulatory unit. Moreover, a substantial number of transcriptionally inactive genes in ES cells are also co-occupied by these pluripotency regulators. Notably, many of these encode transcription factors, such as HoxB1, Pax6, and Myf5, that regulate various developmental processes. Thus, together with Oct3/4 and Nanog, Sox2 maintains the stem cell state in ES cells by repressing expression of genes encoding key developmental regulators and by activating genes involved in self-renewal and pluripotency. It is not clear how these three pluripotency factors can activate some genes and repress others, but polycomb repressive complexes are known to bind to a substantial subset of inactive genes occupied by pluripotency regulators [61].

iPS cells

Induced pluripotent stem (iPS) cells have similar pluripotency to ES cells but can be generated from somatic cells, such as mouse embryonic fibroblast cells or human skin cells. This can be accomplished by forced expression of four transcription factors, Oct-3/4, Sox2, Klf4, and c-Myc [62, 63], though c-Myc does not appear to be essential for iPS cell generation [64]. Either Sox1 or Sox3 can substitute for Sox2 to produce iPS cells, providing further evidence of the functional equivalence among SoxB1 members. Recently, forced expression of Sox2 was shown to be dispensable for generation of iPS cells when neural stem/progenitor cells are used as the starting somatic cells [65–67]. Obviously, since neural stem/progenitor cells endogenously express *Sox2*, these results do not mean that *Sox2* is not required for generation of iPS cells. However, iPS cells have successfully been generated from somatic cells that do not express any of the genes deemed essential for reprogramming by treating Oct-3/4-Klf4-transfected mouse embryonic fibroblast cells with BIX-01294, a G9a histone methyltransferase inhibitor, and BayK8644, an L-channel calcium agonist [68]. It is likely that BIX-01294 facilitates shifting of the epigenetic balance from a silenced to an active transcriptional state at the pluripotency genes necessary for reprogramming. However, it is unclear how and why BayK8644 impacts the reprogramming process, since activation of L-type calcium channels had never been linked to reprogramming before. Elucidating the molecular mechanisms by which these small molecules exert their function will uncover the real role of Sox2 in iPS cell production.

Role of SoxB1 members in CNS and lens development

Besides being expressed in early embryos, *Sox2* is also found in the developing CNS [9, 32, 42, 69–73]. *Sox2* is first uniformly expressed in the neural plate, in which most cells are multipotent. However, once the columnar epithelium of the neural plate acquires a more complex, stratified structure, expression becomes restricted to the germinal layer, where multipotent neural stem cells are enriched. Expression of other SoxB1 group members, such as *Sox1* and *Sox3,* overlaps extensively with that of *Sox2* in the CNS [9, 42, 69, 74]. Since SoxB1 factors are functionally redundant [7, 9, 75–79], loss of any individual SoxB1 gene may lead to defects in the specific regions where the mutated gene is uniquely expressed. For example, loss of *Sox2* expression in mouse retina causes the complete loss of proliferative and differentiation capacity of neural retinal progenitor cells, because although other SoxB1 members are initially expressed in the anterior neural plate and invaginating optic vesicle, only *Sox2* expression is maintained throughout neural retinal cell development [80]. Likewise, *Sox1* mutant mice are viable and show no overt CNS abnormalities, probably due to the expression of other SoxB1 members [81, 82]. Interestingly, however, these mutant mice show defects in lens cells. This is presumably because *Sox3* is never expressed in the lens, while *Sox2* expression is restricted to an early stage of lens cell development. As *Sox2* is abruptly downregulated in the lens around 12.5 days post coitum (dpc), *Sox1* gene expression takes over and persists thereafter.

However, retinal and lens cells are rather exceptional cases, since expression of SoxB1 members overlaps in most regions of the CNS [9, 42, 69, 74]. This makes it difficult to assess the function of individual SoxB1 factors by gene-targeting analysis. Inhibiting SoxB1 function with the dominant negative form of Sox2 causes delamination of neural stem/progenitor cells from the ventricular zone and exit from the cell cycle [9]. Conversely, constitutive expression of *Sox2* inhibits neuronal differentiation, resulting in the maintenance of neural stem/progenitor character and retention in the ventricular zone. Moreover, the defects caused by the dominant negative form of Sox2 can be rescued by expression of *Sox1*, further underscoring the functional redundancy among SoxB1 group members.

SoxB1 members play a central role in maintaining the undifferentiated state of neural stem/progenitor cells by counteracting the activity of proneural basic helix-loop-helix (bHLH) proteins, such as Ngn2 [83]. In turn, the capacity of proneural bHLH proteins to promote neuronal differentiation depends on their ability to suppress the expression of SoxB1 members. However, SoxB1

members do not directly inhibit expression of bHLH genes; instead, Notch signaling, which is also involved in maintaining neural stem/progenitor cell identity [80, 84–86], is thought to directly repress expression of bHLH genes [87]. Interestingly, forced expression of SoxB1 factors allows cells to maintain stem/progenitor character even in the absence of Notch signaling. How then do the SoxB1 proteins counteract the neurogenic activity of proneural bHLH proteins, such as Ngn2 and E47, to maintain neural stem/progenitor cell identity? One possibility is that they directly block E47/Ngn2 complexes. Alternatively, SoxB1 members may produce a molecular environment in which proneural bHLH protein complexes are unable to promote neuronal differentiation. Notably, SoxB1 members fail to inhibit transcriptional activation by the E47/Ngn2 protein complex at the E box in the *NeuroD* promoter. Moreover, high levels of Ngn2 and E47 do not abrogate the neural stem/progenitor cell-promoting activity of SoxB1 members. These findings support the latter model [87], but more work is necessary to elucidate the mechanism by which SoxB1 proteins function.

Interestingly, in vitro differentiation of embryonic neural stem/progenitor cells into mature neurons, but not into astroglia, is profoundly affected in *Sox2* hypomorphic mutant neurospheres [88]. Neurospheres in which *Sox2* was knocked down are able to differentiate into β-tubulin-positive immature neurons with abnormally poor arborization but fail to progress into more mature MAP-2-positive neurons. Moreover, overexpression of Sox2 in neural cells at an early but not late stage of differentiation can rescue the neuronal maturation defect. Some of the β-tubulin-positive immature neurons from Sox2-deficient neural stem/progenitor cells coexpress a glial marker (GFAP), suggesting that Sox2 functions to suppress *GFAP* gene expression in wild-type neuronal cells. Forced expression of Sox2 in astroglia also suppresses *GFAP* expression but does not affect astroglial morphology or expression of other glial marker genes, such as S100 and connexin-43. These data suggest that Sox2 acts as a direct transcriptional repressor of GFAP. Indeed, the genomic region upstream of the *GFAP* promoter contains potential Sox2 binding sites. This regulatory region has been shown to amplify the level of GFAP transcription, and its ability to activate transcription is impaired by the forced expression of Sox2. In addition, Sox2 deficiency results in a decrease in the number of GABAergic neurons in the newborn mouse cortex and adult olfactory bulb, with some showing abnormal morphology and migration. This loss of GABAergic neurons may be the primary cause of the epilepsy occasionally observed in Sox2 knockdown mice and Sox2-deficient individuals.

Notwithstanding the functional redundancy issues, each SoxB1 member has been knocked out and examined. As described above, *Sox1* null mutant mice are viable and show no obvious CNS defects [81, 82], although they usually have spontaneous seizures between 4 and 6 weeks of age. Loss of *Sox3* results in early lethality in chimeras due to a gastrulation defect. When *Sox3* was deleted using a conditional knockout strategy, X-linked *Sox3* null mutant males had a variable phenotype, with one-third appearing normal and most dying before weaning stage [89]. Sox3 is required in the presumptive hypothalamus and infundibulum for proper morphogenesis of Rathke's pouch and later for hypothalamo-pituitary axis formation. Since *Sox2* null mice also show early embryonic lethality [8], we have disrupted *Sox2* specifically in neural stem/progenitor cells in the developing brain using the Nestin-Cre deleter strain [90]. These mice have enlarged lateral ventricles, a phenotype that was not observed in either *Sox1* or *Sox3* mutant embryos (Fig. 4a). Moreover, mutant mice possess significantly fewer neurosphere-forming cells in the developing mouse brain (Fig. 4b). Interestingly, Sox2-deficient primary neurospheres are able to produce secondary and tertiary neurospheres as efficiently as wild-type neurosphere cells. However, although Sox2 null neurospheres are able to differentiate into both neurons and glia, the efficiency of neuronal but not glial production is reduced (Fig. 4c, left panel). Impaired neurogenic potential of the mutant was evident also when neuronal differentiation was induced with forskolin and retinoic acid for 3 days [91] to neural stem/progenitor cells cultured as adherent cells according to Nakashima et al. [92] (Fig. 4c, right panel). These results indicate that *Sox2* null neural stem cells are able to self-renew quite well, probably with the help of *Sox1* and *Sox3* expression, but fail to maintain normal neurogenic potential. Notably, the level of *Sox3* expression was elevated in the mutant embryos, suggesting functional compensation by Sox3 upon loss of Sox2. We and others have previously demonstrated that SoxB1 autoregulatory loops direct the expression of *Sox1* and *Sox2* in neural stem/progenitor cells [9, 30]. However, our data suggest that expression of *Sox3* is controlled at least in part by a SoxB1-independent mechanism, since *Sox3* expression was upregulated in Sox2-deficient cells. Although the molecular basis of this upregulation is not known at present, one intriguing possibility is that *Sox3* is regulated differently from other SoxB1 members, so that its expression is assured even when SoxB1 factor-dependent *Sox* expression is impaired. Though disruption of different SoxB1 members has variable effects on CNS development, these differences likely do not reflect functional differences among SoxB1 members, but rather differences in expression level, since many lines of evidence indicate

Fig. 4 Morphological and functional analyses of mouse embryonic brains in which *Sox2* has been conditionally knocked out. **a** Coronal sections of 19.5 dpc mutant (Sox2$^{Fl/FL}$; Nestin-Cre) and littermate control (Sox2$^{WT/FL}$; Nestin-Cre) embryos. **b** Primary, secondary, and tertiary neurosphere assay of 14.5 dpc mutant (Sox2$^{FL/FL}$; Nestin-Cre) and littermate control (Sox2$^{WT/FL}$) brains. **c** Attenuated neurogenic potential of Sox2-deficient neurosphere cells. Neurospheres are induced to differentiate using fetal bovine serum and retinoic acid (*left panel*). Impaired neurogenic potential is also evident in neural stem/progenitor cells cultured as adherent cells. Two panels in **a** and *left panel* in **c** were originally published in [90]. *Right panel* in **c** represents original data of S.M. and A.O.
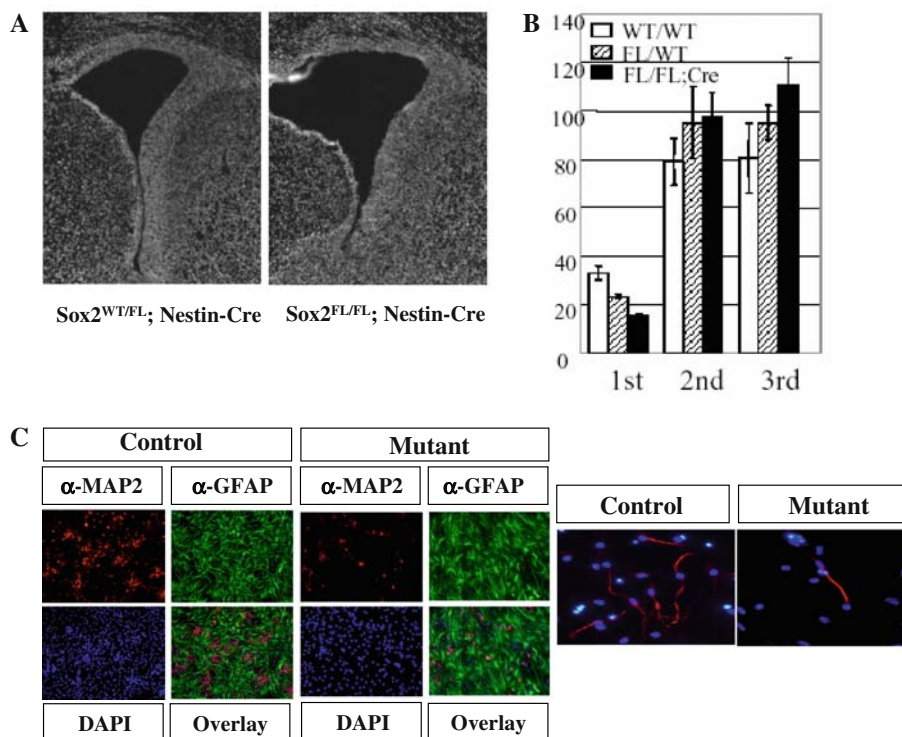
that SoxB1 members exert equivalent biological functions in neural stem/progenitor cells [9, 80, 83].

Human eye disorders with Sox2 mutations

Bilateral anaphthalmia/microphthalmia is the most severe type of eye malformation. It is known that in humans, 3% of unilateral and ~10% of bilateral eye disorders are linked to haploid insufficiency of *Sox2* [93, 94]. Most mutations in the *Sox2* gene identified to date are nonsense mutations leading to truncations of the Sox2 protein. No individuals diagnosed with a *Sox2*-related eye disorder had a parent with the same mutation in the *Sox2* gene, suggesting that in most cases, this genetic disorder occurs as the result of a fully penetrant de novo mutation. Therefore, the risk of this disease to siblings is usually very small. However, in one case, a phenotypically normal mother was found to have germline mosaicism of a loss-of-function mutation. In such cases, the risk of *Sox2*-related eye disorders to siblings may be up to 50% [95]. *Sox2*-related eye disorders are often associated with other abnormalities, such as myopathy, esophageal atresia, learning disabilities, and defects in male genital organs, although it is not clear how these phenotypes are associated with Sox2 function. It is also of note that heterozygous *Sox2* mice do not usually exhibit obvious phenotypes, apart from male fertility defects. However, such differences in haploinsufficiency between mice and humans are not rare.

Concluding remarks

Like other Sox factors, SoxB1 proteins function mainly by regulating transcription. Their functions are context-dependent, as they are modified by the action of partner proteins that interact with SoxB1 members. For example, Oct-3/4 works together with Sox2 to support ES and EC cell-specific gene expression. Numerous studies have demonstrated the importance of SoxB1 members in initial specification and maintenance of stem cell state in neural stem/progenitor cells and in the maintenance of ES cell pluripotency. It was undoubtedly a bit of a surprise for researchers in the field of neural development, especially those studying *Xenopus*, to find that one of their favorite molecules, Sox2, was important not only for neural stem cells but also for embryonic stem cells in mice. It is still somewhat of an enigma that among all the SoxB1 family members, only Sox2 was assigned this task. Understanding the molecular mechanisms governing the partner switch from Oct3/4 in ES cells to other partners in neural stem/progenitor cells remains a compelling challenge. Interestingly, from a molecular phylogenetic point of view, the Sox2 proteins of egg-laying animals (e.g., fish and birds, in which Sox2 is not expressed in early embryonic cells) segregate sharply from the orthologs of placental mammals, in which Sox2 is expressed at early stages. Perhaps as a consequence of this difference, Sox2 expression in the mouse and probably in other mammals marks the neural stem/progenitor lineage throughout its entire developmental

time. As mentioned earlier, this may facilitate the reprogramming of neural stem/progenitor cells into pluripotent stem cells. It is tempting to speculate that the developmental default pathway of ES cells follows the neural differentiation program under the direction of Sox2. This idea could be tested by Sox2 ChIP-on-Chip experiments with ES cells undergoing neural differentiation.

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