

Sox2: A multitasking networker

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Abbreviations: Ascl1, Achaete-scute homolog 1; bHLH, basic helix-loop-helix; Chd7, Chromodomain helicase DNA binding protein 7; CNS, central nervous system; GABA, gamma-aminobutyric acid; HAT, histone acetyl transferase; HDAC, histone deacetylase; HMG, high-mobility-group; LINE-1, long interspersed nuclear element 1; NEP, neuroepithelial precursor; Ngn1, Neurogenin 1; NuRD, nucleosome remodelling and deacetylase; OPC, oligodendrocyte precursor cell; SMRT, silencing mediator for retinoid and thyroid receptors; NCoR, nuclear receptor corepressors; Sox, SRY-related HMG box

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Commentary to: Hoffmann SA, Hos D, Küspert M, Lang RA, Lovell-Badge R, Wegner M, Reiprich S. Stem cell factor Sox2 and its close relative Sox3 have differentiation functions in oligodendrocytes. *Development* 2014; 141:39–50; PMID: 24257626; <http://dx.doi.org/10.1242/dev.098418>

The transcription factor Sox2 is best known as a pluripotency factor in stem and precursor cells and its expression generally correlates with an undifferentiated state. Proposed modes of action include those as classical transcription factor and pre-patterning factor with influence on histone modifications and chromatin structure. Recently, we provided the first detailed analysis of Sox2 expression and function during development of oligodendrocytes, the myelin-forming cells of the CNS. Surprisingly, we found evidence for a role of Sox2 as differentiation factor and found it to act through modulation of microRNA levels. Thus, we add new facets to the functional repertoire of Sox2 and throw light on the networking activity of this multitasking developmental regulator.

A look into the PubMed database reveals that Sox2 is one of the most studied transcription factors of recent years. In 2013 alone, there are about 750 publications mentioning Sox2 in title or abstract. This outnumbers papers for other prominent competitors as Nanog and MyoD. The high number comes as no surprise. After all, Sox2 is intimately linked in the scientific consciousness to stem cells, both embryonic and neural, where it is key to induction and maintenance of pluripotency.^{1–3} It is also one of the 4 classical reprogramming factors that are necessary for the generation of induced pluripotent stem cells.⁴ Such is its name to fame.

During early mammalian development, Sox2 is originally expressed in all blastomeres, and later in all cells of the inner cell mass and the epiblast.⁵ However, around gastrulation Sox2 levels become variable. High Sox2 expression results both in the suppression of mesodermal differentiation, and the induction of a neuroectodermal fate.^{6,7} In the

early neuroectoderm, Sox2 is expressed in all NEP (neuroepithelial precursor) cells often together with its close relatives Sox1 and Sox3. These 3 related proteins constitute the SoxB1 group, and jointly ensure expansion and maintenance of NEP cells.^{2,3} Expression of Sox2 remains associated with NEP cells throughout development of the early neuroectoderm into the CNS (central nervous system), and is even maintained in the adult where Sox2 is found in adult neural stem cells in the subventricular zone of the lateral ventricle walls and in the subgranular zone of the dentate gyrus.⁸ Again, Sox2 functions by maintaining the undifferentiated pluripotent stem cell state.

One of the mechanisms by which this is achieved, is suppression of premature neurogenesis. In accord, SoxB1 proteins have been shown to counteract the activity of proneural bHLH (basic helix-loop-helix) proteins such as Ascl1 and Ngn1 in the early neural tube, and to repress neuronal gene expression.² At first glance SoxB1 proteins therefore appear to function prominently as repressors. Indeed, such functions have been described for Sox2 in studies on the transcriptional regulation of the NeuroD1 transcription factor and LINE-1 retrotransposons which both exert neurogenic activity.^{9,10} It has been postulated that Sox2 represses NeuroD1 and LINE-1 expression by direct binding to the corresponding regulatory regions and recruitment of transcriptional co-repressors such as HDAC1 (histone deacetylase1) so that Wnt/ β -catenin-dependent activation of transcription cannot take place.

While such function as a classical transcriptional repressor exists for a subset of its target genes and is thus biologically relevant, there is ample evidence that SoxB1 proteins – when acting as transcription

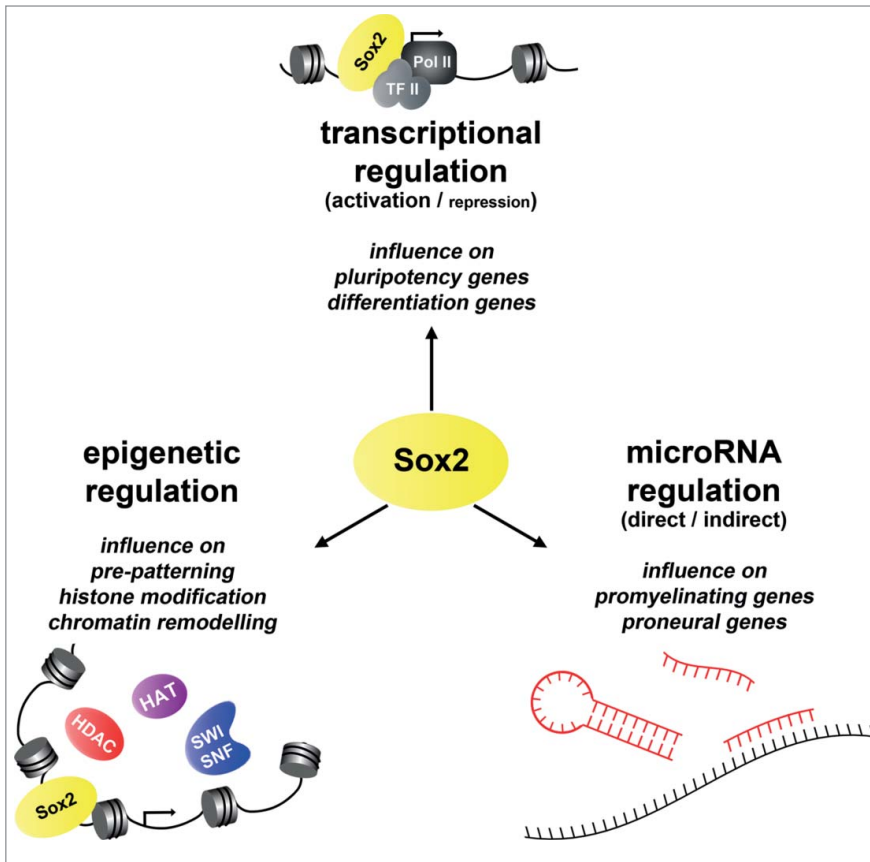


Figure 1. Sox2 – a multitasking regulator. Sox2 exerts its diverse effects on gene expression during CNS development by influencing epigenetics, transcription and microRNAs. It regulates histone modifying enzymes and chromatin remodellers as a pre-patterning factor, and at the same time acts as an activating (or repressing) transcription factor to influence transcription of pluripotency and differentiation genes. Additionally, it impacts on neural and glial gene expression through modulation of microRNAs.

factors – largely do so as activators (Fig. 1).² This is already suggested by standard *in vitro* tests for transcriptional activity, which readily identify a transactivation domain in the proteins' carboxy-terminal half, but fail to detect repressor functions. Results from *in ovo* electroporation experiments in the early chicken neural tube also point to a predominantly transactivating function. In this type of experiment, Sox2 function is mimicked by a chimeric protein in which the aminoterminal half of Sox2 (which includes its DNA-binding HMG-domain) is combined with a constitutively active heterologous transactivation domain from the herpesviral VP16 protein. Analogous combination of the aminoterminal half of Sox2 with the strong repressor domain of the *Drosophila* Engrailed protein in contrast changed properties completely and

reversed Sox2 from an anti-neurogenic into a neurogenic factor as the resulting chimeric protein forced NEP cell cycle exit and induced premature expression of neuronal markers.² This argues that suppression of neurogenesis is mostly indirect and involves the induction of factors, that then act as inhibitors of neurogenic proteins. What these factors are has not yet been analyzed in detail.

In addition to its role as transcription factor, Sox2 has epigenetic, chromatin-associated functions (Fig. 1). Genome-wide chromatin immunoprecipitation studies suggest that Sox2 functions as a pre-patterning or pioneer factor (for review see ref. 11). Such factors have the capacity to preselect and bind regulatory regions and thereby configure the local chromatin in a way that the corresponding genes are kept in a poised state. As such

they are not yet actively transcribed, but have the potential to be easily activated in ensuing developmental stages, provided certain preconditions are met. In the context of neurogenesis, this means that Sox2 not only binds to regulatory regions of genes whose products are required for realization and maintenance of NEP cell properties.¹² It is also bound to regulatory regions of many neuronal differentiation genes, and thereby ensures that these genes can later be activated. For this to happen, Sox2 is down-regulated and replaced on these regulatory regions by other factors, in particular the SoxC proteins Sox4 and Sox11, which then cooperate with proneural and other neurogenic factors to drive neurogenesis. This role as pioneer factor is supported by results from recent proteomic analyses, that find Sox2 associated with many different histone modifying and chromatin remodelling complexes and proteins including NuRD, SMRT/NCoR, SWI/SNF, Chd7, HATs and HDACs.^{10,13,14} This aspect of the overall function of Sox2 is equally important as its classical transcription factor function. Although impossible to separate, one might argue in a somewhat simplistic manner that the role as transcription factor is most obvious for maintenance of NEP cells and suppression of premature neuronal differentiation, whereas its role as pre-patterning/pioneer factor defines developmental potential and thus guarantees pluripotency.

During development, NEP cells switch from generation of neurons to generation of glial cells. Oligodendroglia develop through the OPC (oligodendrocyte precursor cell) stage into mature oligodendrocytes, the myelinating glia of the CNS. We noted that expression of Sox2 and Sox3 is maintained after NEP cell specification into OPC, and is downregulated in the oligodendroglial lineage not earlier than during terminal differentiation to mature myelinating oligodendrocytes.¹⁵ By analogy to neuronal development we initially assumed that SoxB1 proteins would ensure OPC maintenance and suppress differentiation into oligodendrocytes. Surprisingly, this was not the case. Neither deletion of Sox2 alone nor in combination with Sox3 interfered with proper proliferation or generation of OPC

in normal numbers. Instead, consequences of SoxB1 deficiency became obvious during terminal differentiation. Substantially fewer OPC underwent timely differentiation and myelin gene expression was strongly reduced. Thus, the role of Sox2 and the related Sox3 during oligodendrogenesis differs dramatically from the one during neurogenesis with SoxB1 proteins being involved in oligodendroglial differentiation events rather than the maintenance of progenitor characteristics. Despite this clear-cut overall difference, it should be noted that differentiation functions have also been detected or proposed in late developmental phases of certain subtypes of retinal or GABAergic telencephalic neurons.^{16,17} This argues that it is not a complete black-and-white between neurons and glia.

Our mechanistic studies indicated that Sox2 is bound to regulatory elements of myelin genes and that SoxB1 proteins can act as moderate transactivators of glial differentiation genes (Fig. 1).¹⁵ However, activation potential appears much less than that of other activators of myelin gene expression such as Sox10 and Olig2.¹⁸⁻²⁰ Considering that expression of the latter is essentially unaltered in the combined absence of Sox2 and Sox3, it appears unlikely that the differentiation defect observed in SoxB1-deficient oligodendroglia is attributable to the lack of direct activation of differentiation genes by Sox2 and Sox3. Rather we found evidence for an alternative mode of action that involved a microRNA (Fig. 1). During oligodendrocyte development, Sox2 counteracts expression of miR145, which targets pro-differentiation factors such as the transcription factor Myrf (myelin gene regulatory factor) or the mediator subunit Med-12 that is equally required for terminal differentiation of oligodendrocytes.^{21,22} In the absence of Sox2, terminal differentiation of oligodendrocytes and hence myelination are reduced at least in part because miR145 is depressed and inhibits expression of Myrf and other factors that favor myelination. Our studies thus identify Sox2-dependent modulation of microRNA expression as another important facet of its action. Intriguingly, we also found evidence for a repressive influence of miR145 on Sox2

expression, similar to what has been described in glioblastoma cells.²³ These findings argue that a reciprocal negative feedback-loop may exist in developing oligodendroglia between the 2 regulators.

The overall impression from current studies therefore is that – in addition to its role as a transcriptional activator – Sox2 primarily functions as a pre-patterning/pioneer factor during neurogenesis and as a microRNA regulator during oligodendrogenesis (Fig. 1). Thus, it is legitimate to ask whether Sox2 function during the 2 processes is indeed mechanistically as different as the evoked effect. Although there is no definite answer to this question, we like to suggest that this is not the case. We rather assume that Sox2 relies on all its different modes of action in both processes.

As already mentioned, we have found Sox2 on regulatory regions of myelin genes in our study. While this is compatible with a role of Sox2 as a weak activator of myelin gene expression, it could equally be interpreted as the result of a pre-patterning function in which Sox2 preselects those regulatory regions in OPC that have to be activated later on in differentiating oligodendrocytes following the exchange of Sox2 on these regions by Sox10. Thus, it seems perfectly plausible that Sox2 also functions as a pre-patterning/pioneer factor in oligodendroglial differentiation.

On the other side, a first functional link between Sox2 and a microRNA was recently detected during *in vitro* neurogenesis. Sox2 was found to activate expression of Lin28, which is a potent inhibitor of expression of the let-7 microRNA family.¹⁴ Let-7i in turn inhibits neuronal differentiation, by targeting among others the 2 proneural genes *Ascl1* and *Ngn1*. These findings provide support for the assumption that at least some of the long known repressive effects of Sox2 in NEP cells on neurogenesis may be mediated by microRNAs that are under direct or indirect control of Sox2.

In our opinion, Sox2 is a true central regulator of gene expression because it acts not only at the transcriptional level, but also pretranscriptionally as a chromatin-associated pioneer factor and posttranscriptionally as a microRNA modulator. Its mechanistic versatility is not only basis

for its central role in many regulatory networks, but also endows it with a tremendous flexibility that allows it to acquire different tasks in diverse developmental setups including maintenance of the precursor state, suppression of neurogenesis and promotion of glial differentiation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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