

# Sox proteins: regulators of cell fate specification and differentiation

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## Summary

Sox transcription factors play widespread roles during development; however, their versatile functions have a relatively simple basis: the binding of a Sox protein alone to DNA does not elicit transcriptional activation or repression, but requires binding of a partner transcription factor to an adjacent site on the DNA. Thus, the activity of a Sox protein is dependent upon the identity of its partner factor and the context of the DNA sequence to which it binds. In this Primer, we provide a mechanistic overview of how Sox family proteins function, as a paradigm for transcriptional regulation of development involving multi-transcription factor complexes, and we discuss how Sox factors can thus regulate diverse processes during development.

**Key words:** HMG domain, Partner factors, Transcriptional regulation, Stem cells, Cell lineage specification

## Introduction

An emerging view of transcriptional regulation in developmental processes is that transcription factor complexes, rather than single transcription factors, play a major role (Reményi et al., 2004; Verger and Duterque-Coquillaud, 2002). This idea has been pioneered, most rigorously tested and studied in a variety of developmental contexts in the case of Sox (SRY-related HMG-box) family proteins. Sox family proteins are a conserved group of transcriptional regulators (see Box 1) defined by the presence of a highly conserved high-mobility group (HMG) domain that mediates DNA binding. This domain was first identified in Sry, a crucial factor involved in mammalian male sex determination (Gubbay et al., 1990; Sinclair et al., 1990). Multiple other Sox proteins have subsequently been identified and analysed. Vertebrate genomes contain ~20 Sox family members with highly divergent developmental functions, as was indicated by the initial analyses of Sox expression in embryos (Collignon et al., 1996; Uwanogho et al., 1995). Although Sox proteins are also conserved in invertebrate lineages and exert analogous regulatory functions (Phochanukul and Russell, 2010), we here confine our discussion to the vertebrate Sox family members. In this Primer, we first outline the structure and molecular activities of Sox proteins, before discussing their roles during vertebrate development. In particular, the action and function of Sox proteins will be overviewed as a paradigm for transcriptional regulation mediated by a family of transcription factors.

## Sox protein structure

Sox proteins can bind to ATTGTT or related sequence motifs through their HMG domain, which consists of three  $\alpha$  helices

(Badis et al., 2009; Kondoh and Kamachi, 2010). This binding is established by the interaction of the HMG domain with the minor groove of the DNA, which widens the minor groove and causes DNA to bend towards the major groove (Reményi et al., 2003). It is speculated that DNA bending itself may contribute to the regulatory functions of Sox proteins (Pevny and Lovell-Badge, 1997), but this has not been proved experimentally. Sox proteins are classified into groups A-H, depending on the amino acid sequence of the HMG domain (Fig. 1A). Outside the HMG domain, strong homology, with regards to amino acid sequence and the overall organization of protein domains, is found only within a group (Bowles et al., 2000; Schepers et al., 2002) (Fig. 1B) (see also Box 1).

The SoxA group contains only Sry, which is encoded in mammalian Y chromosomes. Although the HMG box of Sry is highly conserved between species, sequences outside this domain are highly divergent among mammalian species (Sekido, 2010).

The SoxB group is split into two sub-groups. SoxB1 comprises Sox1, Sox2 and Sox3; fish species also possess Sox19. These family members have short N-terminal sequences followed by the HMG

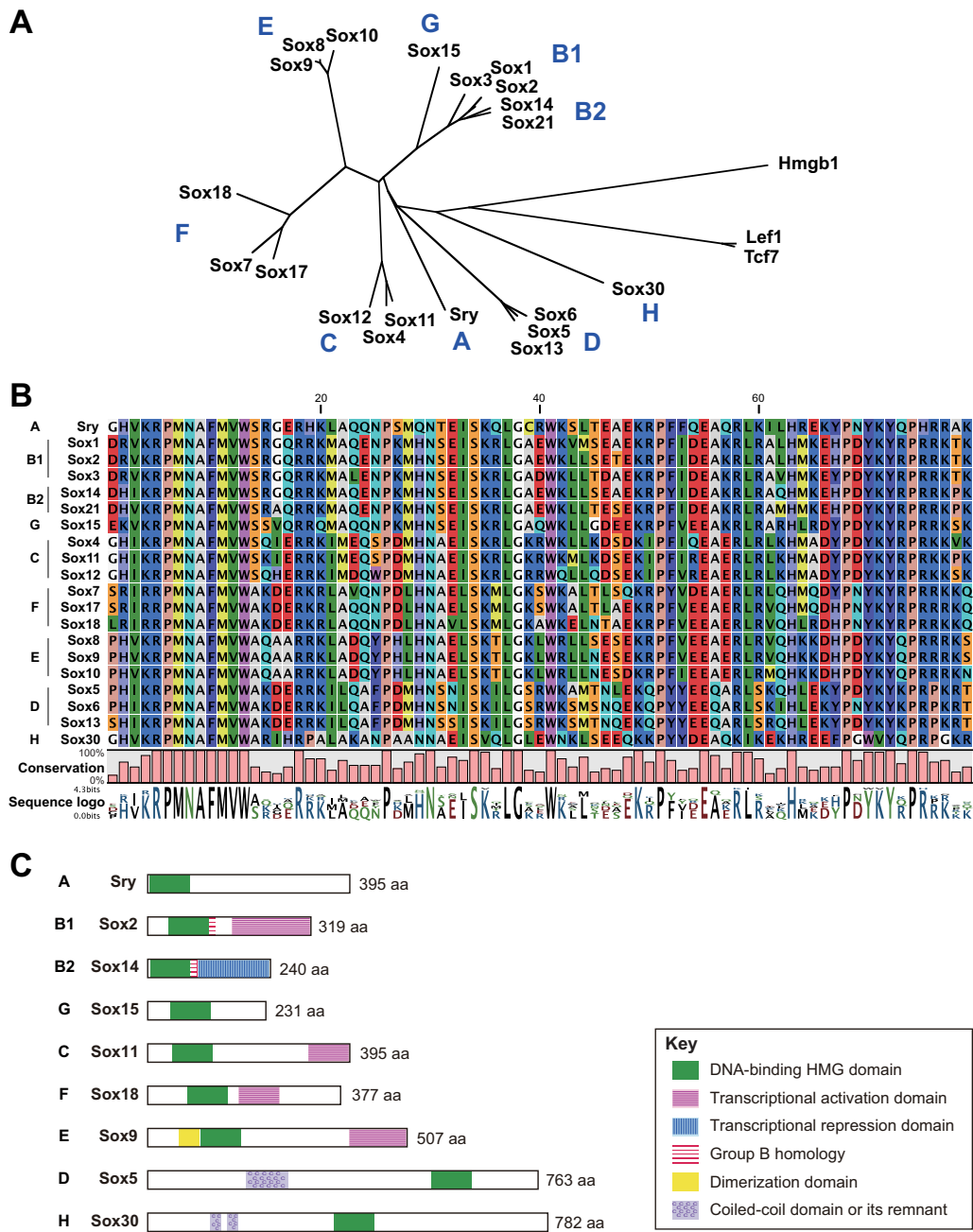
### Box 1. The evolution of Sox genes

Sox (SRY-related HMG-box) genes encoding the Sox proteins are conserved throughout the animal kingdom. The genes for Sox/Tcf type high-mobility group (HMG) domain transcription factors are already present in unicellular choanoflagellates, the animals most closely related to multicellular metazoans, although Hox and other segmentation-related transcription factor genes are absent, indicating an ancient origin of Sox factors (King et al., 2008). True Sox factors became distinguished in the most primitive metazoan *Trichoplax* (Srivastava et al., 2008). Even in invertebrates, Sox genes can be classified into B, C, D, E and F groups (Phochanukul and Russell, 2010). Sry (group A) is unique to mammalian Y chromosomes and is assumed to have been derived from Sox3 (group B1) (Foster and Graves, 1994), which is encoded on the X chromosome. The presence of multiple Sox genes in most Sox groups in vertebrates is a consequence of multiple rounds of genome duplication, which began in primitive chordates. Fish genomes have experienced three rounds of duplications, as opposed to two rounds in other higher vertebrates. This has resulted in frequent duplicated Sox genes, which are exemplified by *Sox1a* and *Sox1b* (Kamachi et al., 2009; Okuda et al., 2006).

The functional redundancy within a group of Sox genes allows individual protein members of the group to change their amino acid sequence and hence their function over evolutionary time. In mammals, *Sox15* is a singlet group G Sox, which was derived from an ancestor of *Sox19* (group B1) during vertebrate evolution, as indicated by its conserved synteny (Kamachi et al., 2009; Okuda et al., 2006). Other taxon-restricted Sox genes appear to have arisen analogously, e.g. *SoxH* (*Sox30*) from *SoxD*, as evidenced by the presence of remnant of coiled-coil domain sequence (Fig. 1C), or by tandem duplication of a genomic segment, e.g. *Sox32* in fish from duplicated *Sox17* (Kobayashi et al., 2006).

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**Fig. 1. Grouping and structures of Sox proteins.** (A) An unrooted phylogenetic neighbour-joining tree of the high mobility group (HMG) domains of the mouse in comparison with outgroup HMG domains of Lef1/Tcf7 (lymphoid enhancer binding factor 1/transcription factor 7, T cell specific) and Hmgb1. (B) Alignment and classification of the Sox HMG domains. Sequences (79 amino acids) of the HMG domains from 20 mouse Sox proteins are aligned according to their relationships. The conservation levels of the amino acid sequences are shown below the alignment with sequence logos. (C) The domain structures of mouse Sox proteins, showing representatives of the groups. The basic structures are highly conserved among members of each group.

domain and long C-terminal sequences after the HMG domain. The C-terminal sequence includes a domain assigned as a transcriptional activation domain, based on an assay using fusion to heterologous DNA-binding domains, as detailed below (Kamachi et al., 1998). SoxB2 proteins, which comprise Sox14 and Sox21, not only have an HMG domain similar to SoxB1 proteins, but also share a short basic amino acid sequence, known as 'B homology', with them. Unlike Sox B1 proteins, however, SoxB2 proteins have a repression domain in the C-terminal sequence (Uchikawa et al., 1999).

SoxC, SoxE and SoxF groups have protein organizations that are analogous to SoxB1 proteins, i.e. a total length of 300-500 amino acids with HMG domains located close to the N terminus and an activation domain in the C-terminal region. However, the amino acid sequences outside the HMG domain are unique to individual groups. SoxC comprises Sox4, Sox11 and Sox12 (Dy et al., 2008); SoxE comprises Sox8, Sox9 and Sox10 (Stolt and Wegner, 2010); and SoxF comprises Sox7, Sox17 and Sox18 (Francois et al., 2010). SoxE proteins also contain a self-dimerization domain on

the N-terminal side of the HMG domain (Bernard et al., 2003; Sock et al., 2003; Stolt and Wegner, 2010).

The SoxD group, which comprises Sox5, Sox6 and Sox13, is unique because SoxD proteins have a long N-terminal sequence containing a coiled-coil domain that allows dimerization with other SoxD proteins (Lefebvre, 2010).

Finally, mammal-specific SoxG (Sox15) (Meeson et al., 2007) and SoxH (Sox30) (Osaki et al., 1999) proteins are structurally related to SoxB1 and SoxD proteins, respectively (see Box 1).

### The regulation of Sox protein expression and activity

Sox activity can be regulated at multiple levels. As discussed in detail below, Sox proteins function together with partner factors to elicit their action. However, the expression of Sox genes themselves is frequently subject to auto-regulation or control by other Sox proteins. Sox expression is also regulated post-transcriptionally by miRNAs (Peng et al., 2012; Xu et al., 2009b) (see Box 2). Sox protein function is known to be dose-dependent (Pevny and Nicolis, 2010), so modulating Sox protein levels in a cell is modulated by covalent modification or by interaction with various proteins. Notably, Sox-dependent regulations intersect with signalling systems such as the sonic hedgehog (Shh) and Wnt signalling pathways, in which Sox-Gli and Sox- $\beta$ -catenin interactions, respectively, are implicated (Bernard and Harley, 2010; Leung et al., 2011; Malki et al., 2010; Oosterveen et al., 2012; Peterson et al., 2012).

Various post-translational modifications have also been reported to modulate the activity, stability and intracellular localization of

#### Box 2. Modulation of Sox protein expression levels by microRNAs

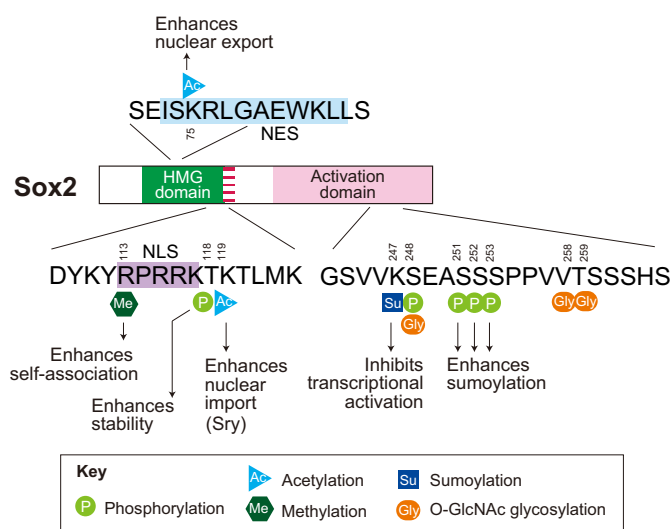
Sox (SRY-related HMG-box) protein expression levels are modulated post-transcriptionally by microRNAs (miRNAs, miRs), which repress the translation and/or promote the degradation of their target mRNAs. Several examples of such regulation are detailed below.

miR-145 expression is highly upregulated during the differentiation of human embryonic stem cells (ESCs). miR-145 directly targets 3' UTRs (untranslated regions) of the mRNAs of Sox2 and other 'core pluripotency factors', and promotes differentiation into mesoderm and ectoderm lineages (Xu et al., 2009b).

miR-200 family members are expressed at high levels in mouse ESCs and neural stem/progenitor cells. Sox2 is directly targeted by miR-200c, whereas Sox2 trans-activates the promoter of the *miR-200c/141* gene, thereby forming a negative-feedback loop (Peng et al., 2012). This miR-200-mediated negative regulation may lead to gradual reduction in Sox2 levels during neural differentiation.

During oligodendrocyte development in the CNS, SoxD proteins (Sox5/6) are expressed in precursor cells and repress oligodendrocyte differentiation, whereas SoxE proteins (Sox9/10) promote oligodendrocyte differentiation (Stolt et al., 2006). miR-219, the expression of which is induced when oligodendrocytes differentiate, directly represses Sox6 expression, which enables the rapid transition of proliferating oligodendrocyte precursors to oligodendrocyte myelination (Dugas et al., 2010; Zhao et al., 2010).

miR-124-mediated Sox9 repression is reported to be important for neurogenesis in the adult mammalian brain (Cheng et al., 2009). miR-124 is expressed at low levels in stem cell astrocytes and transit amplifying cells, whereas it is upregulated in neuroblasts in the subventricular niche and represses Sox9 expression, permitting neuronal differentiation.



**Fig. 2. Post-translational modification of Sox2.** Post-translational modification sites of Sox2 (SRY-related HMG-box 2) are shown with their functional effects. Data obtained from studies of human and mouse Sox2 are combined on the mouse amino acid sequence. The nuclear export signal (NES, blue), nuclear localization signal (NLS, purple) and Group B homology region (striped red) are highlighted.

Sox2, Sox9 and other Sox proteins. For example, studies on cultured cells have shown that Sox2 is subject to various covalent modifications such as phosphorylation (Jeong et al., 2010; Van Hoof et al., 2009), sumoylation (Tsuruzoe et al., 2006), acetylation (Baltus et al., 2009), methylation (Zhao et al., 2011) and glycosylation (Jang et al., 2012) (Fig. 2), although the biological significance of these modifications during embryogenesis remains to be confirmed. Interestingly, the regions in which Sox2 post-translational modifications can occur are located within two short amino acid stretches: one in the HMG domain and the other in the C-terminal domain, which suggests that these modifications may compete or cooperate with each other. For example, Sox2 is phosphorylated in two regions. Phosphorylation of a threonine in the HMG domain by Akt kinases enhances protein stability by inhibiting ubiquitin-mediated proteolysis (Jeong et al., 2010), whereas phosphorylation of serine residues in the C-terminal domain leads to phosphorylation-dependent sumoylation of the adjacent lysine residue (Van Hoof et al., 2009). Acetylation can also regulate Sox protein activity. Acetylation by p300 of a lysine residue close to the nuclear localization signal of Sry, and of one in the nuclear export signal region of Sox2, promotes nuclear import and nuclear export, respectively (Baltus et al., 2009; Thevenet et al., 2004). Because these two sites are highly conserved, these acetylation reactions may finely tune the subcellular localization of a broad range of Sox proteins.

In a few cases, the impact of Sox protein modifications has been demonstrated in developing embryos. Sry and Sox9 are phosphorylated by protein kinase A (PKA), which enhances their DNA-binding activity (Malki et al., 2010). This phosphorylation of Sox9 also leads to its translocation into the nucleus in cells of the testis (Malki et al., 2010), and is essential in neural crest (NC) cells for the Sox9-Snail interaction that promotes NC delamination (Liu et al., 2013). The functional relevance of sumoylation has been best demonstrated for SoxE proteins in NC development. SoxE sumoylation inhibits NC development, causing loss of pigmentation (Taylor and Labonne, 2005). Sumoylated SoxE





proteins fail to interact with the co-activators CREB-binding protein (CBP)/p300, and instead recruit the Grg4 co-repressor (Tle4, transducin-like enhancer of split 4), which leads to strong inhibition of SoxE target genes (Lee et al., 2012). By contrast, it is interesting to note that sumoylated SoxE promotes the development of non-sensory cranial placodes (Taylor and Labonne, 2005).

### Mechanism of action: Sox proteins complex with partner factors

An important characteristic of Sox proteins is that they generally exhibit their gene regulatory functions only by forming complexes with partner transcription factors (Kamachi et al., 2000; Kondoh and Kamachi, 2010). Thus, a functional Sox-binding site is accompanied by a binding site for a second partner protein, which is required for Sox-dependent transcriptional regulation, and the binding of a single Sox protein alone to a DNA site does not lead to transcriptional activation or repression (e.g. Kamachi et al., 2001; Yuan et al., 1995) (Fig. 3A).

As Sox proteins in many cases interact with the partner factors in the absence of DNA, it is presumed that the Sox-partner

**Fig. 3. Sox partner interactions.** Sox (SRY-related HMG-box) proteins function by forming complexes with partner proteins and via binding to DNA sequences, which differ from the sum of binding consensus. **(A)** Schematic representation showing DNA binding and the functions of Sox-partner complexes. **(B)** Examples of *in vivo* binding sequences for the Sox2-Pou5f1/Oct4 complex, which were identified in the enhancers of the genes indicated. The Sox2 binding sequences approximately conform to the consensus, (C/T)[A/T]TTGT(T) (the sequences in parentheses are not strong requirements) (Badis et al., 2009), whereas the Pou5f1/Oct4-binding sequences deviate from the consensus ATGCAAAT at a few base positions. This deviation from the consensus is important for discriminating them from other Sox2-Pou protein complexes; Sox2-Pou3f1/2 complexes fail to interact with some of Sox2-Pou5f1 binding sequences (as indicated on the right). Original data: *Fgf4* (Wiebe et al., 2003), *Sox2* (Tomioka et al., 2002), *Utf1* (Nishimoto et al., 1999), *nestin (Nes)* (Tanaka et al., 2004). Two different arrangements of Sox2- and Pou5f1-binding sequences have been recognized. **(C)** Functional binding sequences of Sox2-Pax6 complexes, which were identified in the enhancers indicated. The Sox2-binding sequences conform to the consensus, whereas the Pax6-binding sequences differ considerably from the Pax6-binding consensus TTCACGC[A/T][G/C]A-T[G/T][A/C]AC in either orientation. This deviation from the consensus is important for transcriptional activation by the Sox2-Pax6 protein complex: replacement of the Pax6-binding site sequence in the  $\delta$ -crystallin enhancer with the Pax6 consensus binding sequence inactivates the enhancer (Kamachi et al., 2001). **(D)** Sox9 homodimer-binding sequences found in the enhancers of collagen and the related genes indicated. In contrast to the heterodimers, the Sox9 dimer-binding sequences deviate from the Sox consensus in both sites (Bernard et al., 2003; Bridgewater et al., 2003; Dy et al., 2012; Han and Lefebvre, 2008; Sock et al., 2003). *Acan*, aggrecan; *Col9a1*, collagen, type IX,  $\alpha$ 1; *Col10a1*, collagen, type X,  $\alpha$ 1; *Col11a2*, collagen, type XI,  $\alpha$ 2; *Dppa4*, developmental pluripotency associated 4; *Fbxo15*, F-box protein 15; *Fgf4*, fibroblast growth factor 4; *Pou5f1*, POU domain, class 5, transcription factor 1; *Utf1*, undifferentiated embryonic cell transcription factor 1.

complexes can form first and then recognize target DNA sites as a complex (Fig. 3A). For SoxB1/C/F proteins, their partner factors are heterologous transcription factors belonging to protein families such as the Pou and Pax families. SoxE proteins, by contrast, exhibit two modes of partner interactions: one that employs heterologous partners and another that requires homologous dimerization. SoxD proteins also dimerize via their coiled-coil dimerization motif. Partner factors for SoxH have not yet been determined.

Classic examples of the regulatory targets of Sox-partner complexes are the *Fgf4* (fibroblast growth factor 4) enhancer, which is activated by Sox2 and Pou5f1/Oct4 (POU domain, class 5, transcription factor 1) in embryonic stem cells (ESCs) (Ambrosetti et al., 1997; Yuan et al., 1995), and the  $\delta$ -crystallin DC5 enhancer, which is activated by Sox2 and Pax6 (paired box gene 6) during lens development (Kamachi et al., 1995; Kamachi et al., 2001). In both cases, the enhancers are activated by the co-binding of Sox2 and a partner factor, and mutations in the binding site for either Sox2 or the co-factor inactivate the enhancer.

The binding site sequences of the Sox2-partner complexes are not simply the sum of the consensus binding sequences that are determined using single factors (Fig. 3B,C). For example, the replacement of the Pax6-binding site within the DC5 sequence bound by the Sox2-Pax6 complex with the Pax6 consensus binding sequence inactivates the enhancer (Kamachi et al., 2001). Similarly, the sequences bound by Sox9 dimers found in the enhancers of genes associated with chondrocyte development, e.g. those for collagens, deviate considerably from the Sox consensus binding

sequence (Bridgewater et al., 2003; Dy et al., 2012; Han and Lefebvre, 2008) (Fig. 3D). Evidence indicates that a complex of a Sox and its partner factor assumes a specific conformation to elicit transcriptional regulation upon binding to its unique target sequences (Kamachi et al., 2001).

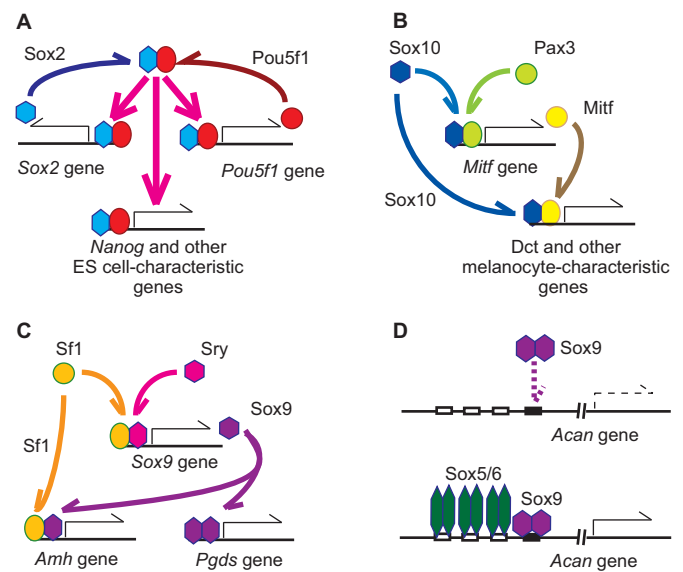
Whether a Sox-partner complex elicits transcriptional activation or repression depends on whether the complex recruits co-activator or repressor. Indeed, recent proteomics analysis identified Trrap co-activator (transformation/transcription domain-associated protein), NcoR/SMRT co-repressors (Ncor1/2, nuclear receptor co-repressors 1/2) and NuRD co-repressor complexes as strongly interacting co-factors of Sox2 (Engelen et al., 2011), suggesting that Sox2 can participate in transcriptional activation or repression depending on the co-factor recruited by the Sox2-partner factor complex.

A popular way of characterizing the transactivation/repression potential of a transcription factor is to excise regions of its non-DNA-binding domains and fuse them to the DNA-binding domain of another protein, such as that of the GAL4 protein, which binds to DNA as a dimer. If the resulting fusion protein can recruit co-activators in this context, it will activate a reporter gene, and the protein region is registered as an 'activation domain' that can recruit a co-activator under a certain molecular context. A 'repression domain' that can recruit a co-repressor is determined analogously. The various domains of Sox proteins indicated in Fig. 1 have been determined in this fashion. For example, SoxB1 proteins harbouring an activation domain participate in the transactivation process, e.g. those occurring in the *Fgf4* and  $\delta$ -crystallin enhancers (Ambrosetti et al., 1997; Kamachi et al., 2001; Yuan et al., 1995), whereas SoxB2 proteins with a repression domain participate in transrepression processes (Bylund et al., 2003; Uchikawa et al., 1999).

However, it is important to emphasize that these DNA-binding domain fusion assays do not represent the full repertoire of the regulatory potential of a transcription factor or a Sox-partner complex. For example, the interaction of Sox2 with co-repressor (Engelen et al., 2011) was not indicated by using the fusion assay (Kamachi et al., 1999; Kamachi et al., 1998). Moreover, although SoxD proteins do not possess an activation/repression domain based on the DNA-binding domain fusion assay, Sox6 is known to interact with the co-repressor CtBP (C-terminal binding protein) and to repress *Fgf3* expression during inner ear development (Murakami et al., 2001). It has also been demonstrated that Sox9 has both activation and repression modes, depending on the target site (and hence the partner factor), and in the repression mode Sox9 recruits Gli proteins as the partner factor (Leung et al., 2011). Furthermore, Sox9 interacts with Runx2 (runt related transcription factor 2) and inhibits Runx2-dependent transactivation of osteoblast-specific genes (Zhou et al., 2006). Therefore, the transcriptional regulatory activity of a Sox-partner complex needs to be assessed in individual contexts of the cells and target DNAs.

### Sox-partner exchange allows stepwise progression of developmental processes

Some Sox-partner complexes can activate the expression of their own respective genes, which stabilizes the cell state (Kondoh and Kamachi, 2010; Kondoh et al., 2004), as seen in the case of Sox2 and Pou5f1/Oct4 (Fig. 4A). However, the replacement of either component of the complex can result in large-scale changes in target genes, which in turn can contribute to developmental progression. Two representative cases of Sox-dependent developmental regulation are discussed below.

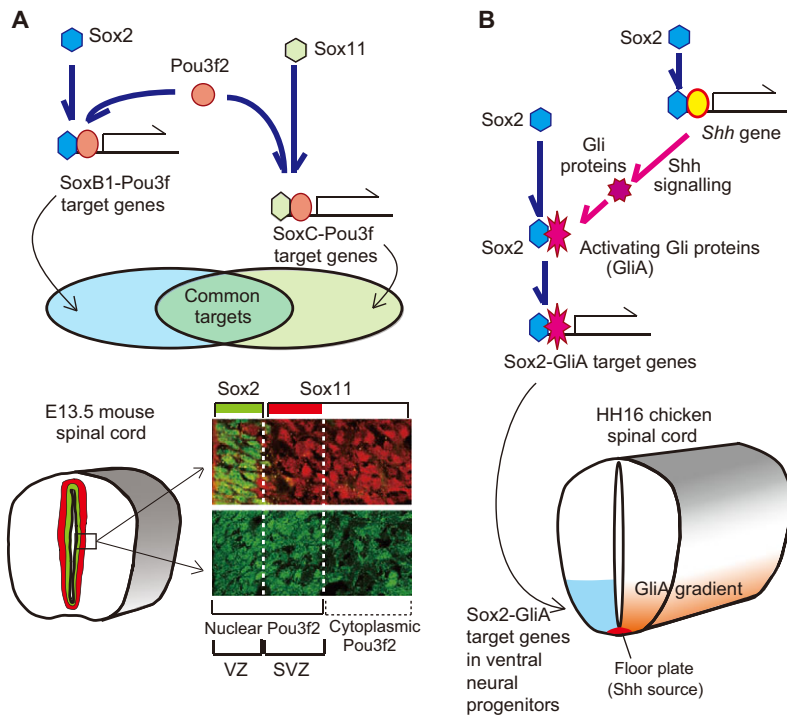


**Fig. 4. Paradigms of developmental regulation by Sox-partner complexes.**

(A) Sox-partner complexes can activate cell-specific genes and their own genes, thereby stabilizing the cell state. Sox2-Pou5f1/Oct4 in ESCs is shown. Sox2- and Pax6-dependent lens cell development (not shown) is also reliant on Sox2-Pax6 co-regulatory loops. (B) Sox-partner complexes can also activate the gene for the partner protein required during the next developmental step, thereby promoting a domino-like progression of the developmental process. Sox10-Pax3 interactions, which then activate Mitf expression and Sox10-Mitf complex formation, during melanocyte development from the neural crest are shown. Schwann cell development (not shown) is also a representative of this paradigm, as discussed in the main text. (C) Sox-partner complexes can activate a second Sox gene that acts in the following step employing the same partner factor, thereby promoting the developmental process. The case of Sox9 induction by Sry-Sf1 during male gonad development is shown. (D) 'Sox trio' cases. Although Sox9 often employs itself as its own partner and dimerizes, Sox9 dimers further require Sox5/6 dimers that bind to nearby DNA sites for their activity in many occasions of chondrocyte development. An example of *Acan* enhancer regulation is shown. The nature of Sox9-Sox5/6 interaction has not been clarified. Amh, anti-Müllerian hormone; Dct, dopachrome tautomerase; Mitf, microphthalmia-associated transcription factor; Pax3, paired box gene 3; Pgds, prostaglandin D synthase; Pou5f1, POU domain, class 5, transcription factor 1; Sf1, steroidogenic factor 1; Sox, SRY-related HMG-box.

In the first case, a Sox-partner pair activates the gene for another transcription factor, which then functions as a new Sox partner (Fig. 4B). For example, during melanocyte development from the NC, a Sox10-Pax3 pair activates expression of the transcription factor Mitf (microphthalmia-associated transcription factor), which then acts as a Sox10 partner to activate the *Dct* (dopachrome tautomerase) and *Tyr* (tyrosinase) genes involved in melanocyte differentiation (Bondurand et al., 2000; Ludwig et al., 2004; Murisier et al., 2007). In the second case, a Sox factor changes while the same partner factor is shared, as exemplified by the Sry-Sox9 switch during gonadal development (Fig. 4C).

Another example of such a developmental switch is seen during neural tube development (Fig. 5A). In embryonic neural tubes, neural progenitor cells in the ventricular zone express SoxB1 proteins, while the postmitotic neuronal precursors in subventricular zone express SoxC proteins (Bergslund et al., 2011; Tanaka et al., 2004; Uwanogho et al., 1995). The mechanism



**Fig. 5. Developmental regulation by Sox-partner complexes during neural tube development and patterning.** (A) An example in which one component of the Sox-partner complex is replaced with another from a related protein family, thereby causing a step-wise but gradual change in the developmental state because of the sharing of a fraction of target genes between the two complexes. The example shown is the transition in the developing spinal cord from SoxB1-dependent neural progenitor cells in the ventricular zone (VZ) to SoxC-dependent postmitotic neural progenitors in the subventricular zone (SVZ). These Sox proteins share Pou3f1/2, which is expressed and localized in the nucleus in these two zones, and activate nestin (*Nes*) expression in VZ and SVZ as their common regulatory targets. Immunofluorescence data are reproduced, with permission, from Tanaka et al. (Tanaka et al., 2004). (B) An example in which a partner of a Sox protein is activated by an extracellular signalling input. In the ventral forebrain, *Shh* is activated in a Sox2-dependent fashion. During the activation of a group of ventral CNS-characteristic transcription factor genes, Sox2 cooperates with Shh signal-dependent activated form of Gli proteins (although physical interactions between Sox and Gli proteins are yet to be demonstrated). Pou3f2, POU domain, class 3, transcription factor 2; Shh, sonic hedgehog; Sox, SRY-related HMG-box.

underlying this SoxB1-SoxC switch has not been characterized. SoxB1 and SoxC proteins both form complexes with Pou3f factors (e.g. Pou3f2/Brn2), which are expressed and nuclear localized in the ventricular and subventricular zones. The switching of Sox expression changes the gene expression profile of the cells from the SoxB1-Pou3f target group to the SoxC-Pou3f target group. These sets of target genes show some overlap, e.g. *Nes* (nestin) (Tanaka et al., 2004), resulting in graded, rather than abrupt, changes between cells.

### Developmental functions of Sox group proteins

The aforementioned modes of Sox protein-dependent regulation are found in various developmental processes, and there are strong associations between individual Sox groups and specific cell lineages. It is often observed that the members of the same Sox group, sharing equivalent functions, are expressed in the same developing tissues (e.g. SoxB1 proteins in the CNS, SoxE proteins in the NC and SoxF proteins in the vascular system), although their precise spatial and temporal tissue expression patterns differ slightly. This situation creates functional redundancy between the group members that co-regulate the same targets, which safeguards the developmental processes against genetic variations between individual animals. However, the contribution of individual members of a Sox group to a developmental process is not always equivalent, with regard to the timing of expression, expression levels and the activity of a protein, as exemplified by the more minor contribution by Sox8 among SoxE proteins in the development of NC-derived tissues (Kellerer et al., 2006; Stolt et al., 2005). Documented cases of Sox-dependent gene regulation in various developmental contexts are listed in Table 1, highlighting Sox-partner interactions. It is interesting that the members of the same Sox group tend to interact with the same partner factor, even in different lineages, e.g. Sox10-Mef2c in NC versus Sox9-Mef2c in chondrogenesis. Mutant and tissue-specific knockout mouse phenotypes, as well as the congenital disorders in humans associated with Sox protein function, are also indicated.

Representative cases of cell lineage-specific regulation by particular Sox groups are summarized below.

### The Sox2-Pou5f1/Oct4 complex in embryonic stem cells and the epiblast

Many ESC-expressed genes, such as *Fgf4*, are regulated by the Sox2-Pou5f1/Oct4 complex (Boyer et al., 2005; Chen et al., 2008; Kondoh and Kamachi, 2010). As these genes include *Sox2* and *Pou5f1* themselves, it appears that ESC states are stabilized by an autoregulatory loop. In addition, Sox2 and Pou5f1/Oct4 are two of the essential four transcription factors initially identified for induced pluripotent stem cell (iPSC) production (Takahashi and Yamanaka, 2006), indicating their pivotal contribution to the ESC state. Sox2-Pou5f1/Oct4 complex function is also essential for the formation of the inner cell mass of pre-implantation embryos, from which ESCs are derived (Avilion et al., 2003), and of the epiblast of the post-implantation embryo. However, this complex is downregulated in embryos after somatic differentiation is initiated (Iwafuchi-Doi et al., 2012). In zebrafish embryos, the SoxB1-Pou5f1 complex also regulates early developmental processes, including embryo patterning (Okuda et al., 2010; Onichtchouk et al., 2010; Shih et al., 2010).

### SoxB1 proteins in neural development

The neural plate (the embryonic neural progenitor tissue) expresses Sox2 (Kishi et al., 2000; Takemoto et al., 2011; Uwanogho et al., 1995), and this expression persists in all neural progenitor/stem cells throughout life (Pevny and Placzek, 2005; Pevny and Nicolis, 2010; Suh et al., 2007). Presumably, the same Sox2 protein recruits different partner proteins during development that fulfil context-dependent regulatory functions, in addition to Pou3f proteins, which are common Sox2 partners during neural development (Tanaka et al., 2004). SoxB1 proteins maintain the neural stem/progenitor state of cells by preventing the transition to a more advanced developmental stage that is marked by SoxC expression (Graham et al., 2003). In addition, it is possible that SoxB1 proteins

**Table 1. Examples of Sox factor participation in the developmental processes**

Sox group	Tissues	Participating Sox	Known partner factors*	Known target genes*	Phenotypes of Sox KO/tissue-specific KO/hypomorphs	Associated congenital human disease	References
A	Gonad	Sry	Sf1	<i>Sox9</i>	Male gonad defect	XY gonad dysgenesis	(Sekido and Lovell-Badge, 2008)
B1	ICM, ESCs, iPSCs, epiblast	Sox2	Pou5f1 (Oct4)	<i>Sox2, Pou5f1, Nanog, Fgf4, Utf1, Fbxo15, Lefty1, Mycn, Tcf3, Dppa4, Trp53, Rest, Zfp42</i>	Failure in implantation (KO)		(Boyer et al., 2005; Chen et al., 2008; Kondoh and Kamachi, 2010)
B1	Embryonic neural tube	Sox2/3/1	Pou3fs	<i>Nes, Shh, Sox2</i>	Neural hypoplasia, abnormal thalamus, retinal defects	Septo-optic dysplasia	(Favaro et al., 2009; Miyagi et al., 2006; Tanaka et al., 2004; Zhao et al., 2012)
B1	Ventral neural tube	Sox2/3/1	Activating form Gli proteins	<i>Nkx2.2, Olig2, Nkx6.1/6.2</i>		Septo-optic dysplasia	(Oosterveen et al., 2012; Peterson et al., 2012)
B1	Neural progenitors in adult brain	Sox2	Pou3fs	<i>Shh</i>	Loss of neural progenitor/stem cells		(Favaro et al., 2009; Zhao et al., 2012)
B1	Neural stem cells derived from ESCs	Sox2	Chd7	<i>Jag1, Tulp3, Hes5, Mycn</i>		AEG (anophthalmia-esophageal-genital) syndrome	(Engelen et al., 2011; Williamson et al., 2006)
B1	Pituitary	Sox2/3			Hypogonadism caused by pituitary defect ( <i>Sox3</i> )	Hypogonadism caused by pituitary defect ( <i>SOX2</i> )	(Kelberman et al., 2006; Rizzoti et al., 2004; Sato et al., 2007)
B1	Lens	Sox2/1	Pax6, Pou2f1 (previously Oct1), Maf1	<i>Sox2, Pax6, crystallins, N-cadherin</i>	Lens defects	Anophthalmia, cataractus lens	(Aota et al., 2003; Donner et al., 2007; Inoue et al., 2007; Kamachi et al., 2001; Matsumata et al., 2005; Rajaram and Kerppola, 2004; Smith et al., 2009)
B1	Otic placode	Sox2/3		N-cadherin	Inner ear defects	Inner ear defects	(Kiernan et al., 2005; Matsumata et al., 2005)
B1	Inner ear sensory patches	Sox2	Six1-Eya1 complex	<i>Atoh1</i>	Sensory tissue loss in the inner ear		(Ahmed et al., 2012; Kiernan et al., 2005)
B1	Dermal papilla of hair follicle	Sox2		<i>Sostdc1</i>	Hair growth reduction		(Clavel et al., 2012; Driskell et al., 2009)
B2	Hair bulge	Sox21			Cyclic alopecia		(Kiso et al., 2009)
C	Early embryonic cells	Sox4/11		<i>Tead2</i>	Organ hypoplasia		(Bhattaram et al., 2010; Schilham et al., 1996; Sock et al., 2004)
C	Developing neurons	Sox4/11		<i>Tubb3</i>			(Bergsland et al., 2006)
C	Kidney	Sox11	Wt1	<i>Wnt4</i>			(Murugan et al., 2012)
D	Neocortex neurons	Sox5/6	Sox5/6		Aberrant histogenesis of neocortex		(Azim et al., 2009; Kwan et al., 2008; Lai et al., 2008)
D	Oligodendrocytes	Sox5/6	Sox5/6	Myelin basic protein	Precocious oligodendrocyte differentiation		(Stolt et al., 2002; Stolt et al., 2006)
D	Chondrocytes	Sox5/6	Sox5/6, Sox9	Collagens, <i>Acan</i>	Chondrogenesis defects		(Lefebvre et al., 1998; Smits et al., 2001)
E	Hair bulge	Sox9			Alopecia	Hypertrichosis ( <i>Sox9</i> dysregulation)	(Fantauzzo et al., 2012; Vidal et al., 2005)
E	Otic placode	Sox9			Otic placode invagination defect		(Barrionuevo et al., 2008)
E	Neural stem cells	Sox9			Failure of neural stem maintenance		(Cheng et al., 2009; Scott et al., 2010; Stolt et al., 2003)
E	Glial progenitors	Sox9	Nf1a		Impaired gliogenesis		(Kang et al., 2012; Stolt et al., 2003)

Table 1. Continued on next page



Table 1. Continued

Sox group	Tissues	Participating Sox	Known partner factors*	Known target genes*	Phenotypes of Sox KO/tissue-specific KO/hypomorphs	Associated congenital human disease	References
E	Oligodendrocyte progenitors/oligodendrocytes	Sox9/10		Suppressor of fused, myelin basic protein	Loss of oligodendrocytes		(Pozniak et al., 2010; Stolt et al., 2003; Stolt et al., 2002; Stolt et al., 2006)
E	Neural crest	Sox9	Snail2	<i>Snail2</i>	Loss of neural crest delamination		(Liu et al., 2013; Sakai et al., 2006)
E	Neural crest	Sox9	Sox9, Ets1, Myb	<i>Sox10</i>			(Betancur et al., 2010; Cheung and Briscoe, 2003)
E	Cranial neural crest	Sox10	Mef2c			Waardenburg-Hirschsprungs disease	(Agarwal et al., 2011)
E	Neural crest (melanocytes)	Sox10	Pax3, Mitf	<i>Mitf, Ret, Dct, Tyr</i>	Albino	Waardenburg-Hirschsprungs disease	(Bondurand et al., 2000; Ludwig et al., 2004; Murisier et al., 2007)
E	Neural crest (Schwann cells)	Sox10	Pou3f2 (previously Brn2), Egr2	<i>Egr2, connexin 32, myelin protein zero</i>		Waardenburg-Hirschsprungs disease	(Ghislain and Charnay, 2006; Reiprich et al., 2010)
E	Neural crest (Schwann cells)	Sox8				Waardenburg-Hirschsprungs disease (modifier)	(Maka et al., 2005)
E	Gonad	Sox9	Sf1, Sox9	<i>Sox9, Amh, Pgds</i>	Male gonad defect	XY gonad dysgenesis	(De Santa Barbara et al., 1998; Sekido and Lovell-Badge, 2008; Wilhelm et al., 2007)
E	Chondrocytes	Sox9	Sox9, Sox5/6	Collagen genes, <i>Acan</i>	Chondrogenesis defects	Campomelic dysplasia	(Bell et al., 1997; Bi et al., 2001; Han and Lefebvre, 2008; Lefebvre et al., 1997; Leung et al., 2011; Zhao et al., 1997)
E	Chondrocytes	Sox9	Mef2c	<i>Col10a1</i> (activation)	Chondrogenesis defects	Campomelic dysplasia	(Dy et al., 2012)
E	Chondrocytes	Sox9	Gli2/3	<i>Col10a1</i> (repression)	Chondrogenesis defects	Campomelic dysplasia	(Leung et al., 2011)
F	Endoderm	Sox17/7	Pou5f1 (previously Oct4)		Gut defects		(Aksoy et al., 2013; Kanai-Azuma et al., 2002)
F	Vascular endothelium	Sox18/17	Mef2c	<i>Vcam1, N-cadherin, Mmp7</i>	Vascular defects	Hypotrichosis-lymphoedema-telangiectasia syndrome	(Downes et al., 2009; Hoeth et al., 2012; Hosking et al., 2001; Hosking et al., 2004)
F	Lymphatic endothelium	Sox18		<i>Prox1</i>	Lymphatic defects	Hypotrichosis-lymphoedema-telangiectasia syndrome	(Francois et al., 2008)
F	Dermal papilla of hair follicle	Sox18			Reduction of zigzag hair (null), alopecia (dominant negative)	Hypotrichosis-lymphoedema-telangiectasia syndrome	(Pennisi et al., 2000a; Pennisi et al., 2000b)
G	Muscle satellite cells	Sox15	Fhl3	<i>Foxk1</i>	Muscle regeneration defect		(Meeson et al., 2007)
H	Male germ cells	Sox30					(Osaki et al., 1999)

\*The partner factors and the target genes do not necessarily correspond. In some cases, interacting partners are known without defined target genes, whereas in other cases target genes have been identified without the knowledge of partner factors.

Amh, anti-Mullerian hormone; Atoh1, atonal homologue 1; Chd7, chromodomain helicase DNA binding protein 7; Col10a1, collagen, type X,  $\alpha 1$ ; Dct, dopachrome tautomerase; Dppa4, developmental pluripotency associated 4; Egr2, early growth response 2; ESCs, embryonic stem cells; Ets1, E26 avian leukemia oncogene 1, 5' domain; Eya1, eyes absent 1 homologue; Fbxo15, F-box protein 15; Fgf4, fibroblast growth factor 4; Fhl3, four and a half LIM domains 3; Foxk1, forkhead box K1; Hes5, hairy and enhancer of split 5; ICM, inner cell mass; iPSCs, induced pluripotent stem cells; Jag1, jagged 1; KO, knockout; Lefty1, left right determination factor 1; Maf1, MAF1 homologue; Mef2c, myocyte enhancer factor 2C; Mitf, microphthalmia-associated transcription factor; Mmp7, matrix metalloproteinase 7; Myb, myeloblastosis oncogene; Mycn, v-myc myelocytomatosis viral related oncogene, neuroblastoma derived; Nf1a, nuclear factor 1A. Nkx, NK1 transcription factor related; Olig2, oligodendrocyte transcription factor 2; Pax6, paired box gene 6; Pgds, prostaglandin D synthase; Pou2f1, POU domain, class 2, transcription factor 1; Pou3fs, POU domain, class 3, transcription factors (which comprise Pou3f1 (Oct6), Pou3f2 (Brn2), Pou3f3 (Brn1) and Pou3f4 (Brn4)); Pou5f1, POU domain, class 5, transcription factor 1; Prox1, prospero-related homeobox 1; Rest, RE1-silencing transcription factor; Ret, Ret proto-oncogene; Sf1, steroidogenic factor 1; Shh, sonic hedgehog; Six1, sine oculis-related homeobox 1; Sostdc1, sclerostin domain containing 1; Sox, SRY-related HMG-box; Tcf3, transcription factor 3; Tead2, TEA domain family member 2; Trp53, transformation related protein 53; Tubb3, tubulin  $\beta 3$ , class III; Tulp3, tubby-like protein 3; Tyr, tyrosinase; Utf1, undifferentiated embryonic cell transcription factor 1; Vcam1, vascular cell-adhesion molecule 1; Wnt4, wntless-related MMTV integration site 4; Wt1, Wilms' tumour 1; Zfp42, zinc-finger protein 42.



also support the survival of neural stem/progenitor cells, as a large decrease in SoxB1 activity results in the massive neural tissue degeneration (Ferri et al., 2004).

An important regulatory target of Sox2 in the brain is the *Shh* gene, the activity of which is essential for stem cell maintenance in the hippocampus and for hypothalamus development (Favaro et al., 2009; Zhao et al., 2012). The downstream effectors of the hedgehog (Hh) pathway, the Gli proteins, in turn act as Sox2 partners during the Hh signal-dependent activation of transcription factor genes, such as *Nkx2.2* (NK2 homeobox 2), *Olig2* (oligodendrocyte transcription factor 2), *Nkx6.1* and *Nkx6.2*, which are characteristic of ventral spinal cord neural progenitors (Oosterveen et al., 2012; Peterson et al., 2012). This represents a paradigm for signal-dependent activation of Sox-partner complexes (Fig. 5B).

The expression of Sox3, another SoxB1 protein, largely overlaps with that of Sox2 in neural and sensory tissues (Dee et al., 2008; Nishimura et al., 2012; Okuda et al., 2006; Uchikawa et al., 2011; Wood and Episkopou, 1999). Sox3 itself is dispensable in mice (Rizzoti et al., 2004; Weiss et al., 2003), but it safeguards Sox2 functions by functional redundancy (Iwafuchi-Doi et al., 2011; Rizzoti and Lovell-Badge, 2007). Sox1, the third SoxB1, is first activated weakly in a part of the developing anterior neural plate, then strongly in the developing spinal cord, and finally throughout the neural tube in mouse and chicken embryos (Cajal et al., 2012; Uchikawa et al., 2011; Wood and Episkopou, 1999). The functions of Sox1 are largely shared with those of Sox2 and Sox3, although it has a unique function during the migration of neuronal precursors in the ganglionic eminence (Economou et al., 2005) and in the lens (Nishiguchi et al., 1998).

### Other Sox proteins in the nervous system

Sox21, a SoxB2 protein with transcriptional repression activity, is often co-expressed with Sox2 in the neural, sensory and endodermal tissues (Uchikawa et al., 1999). The repressive activity of *Sox21* modifies the balance between progenitor cell maintenance and the progression to postmitotic neural development, during which time SoxC activity is involved (Graham et al., 2003; Sandberg et al., 2005). In the adult hippocampus, Sox21 represses the expression of the transcriptional repressor *Hes5* to promote neurogenesis (Matsuda et al., 2012). Sox9 is also expressed in the developing CNS, and is essential for the establishment and maintenance of multipotent neural stem cells (Cheng et al., 2009; Scott et al., 2010). During the late stages of neural development, Sox9 regulates gliogenesis in neural stem cells (Stolt et al., 2003) using NFIA as its partner protein (Kang et al., 2012). Within the glial cells, oligodendrocyte development is redundantly supported by co-expressed Sox9 and Sox10 (Stolt et al., 2003), which repress suppressor of fused (*Sufu*) expression (Pozniak et al., 2010).

### SoxE proteins during neural crest development

Neural crest cells delaminate from the dorsal border of closing neural tube and give rise to the peripheral nervous system and melanocytes, and (in the cephalic region) to muscle and bone precursors as well. The SoxE family member Sox9 is expressed initially in the NC-competent cell population present in the dorsal neural tube (Cheung and Briscoe, 2003). As these cells migrate from the neural tube, the expression of Sox10, the major NC regulator, is activated in a Sox9-dependent manner (Betancur et al., 2010). Sox9 cooperates with Ets1 and cMyb to activate *Sox10* in the cranial NC (Betancur et al., 2010). Sox10 first interacts with the following partner factors: Mef2c for the cranial NC (Agarwal

et al., 2011); Pax3 for melanocytes (Bondurand et al., 2000); and Pou3f1/2 for Schwann cell development (Kuhlbrodt et al., 1998). These Sox10-partner complexes then activate a second set of Sox10 partner genes, such as *Mitf* for melanocytes (Bondurand et al., 2000) and *Egr2* for Schwann cells (Ghislain and Charnay, 2006; Reiprich et al., 2010), thereby activating the genes characteristic of their respective terminally differentiated cells, e.g. connexin 32 (*Gjb1*) and myelin protein zero during Schwann cell development (Bondurand et al., 2001; LeBlanc et al., 2007).

### Sox2 in the sensory placodes

Sensory placodes (i.e. the nasal, lens, otic and taste bud placodes, as well as the hypophyseal placode), which are the precursors of sensory tissues, develop from the cephalic ectoderm and express Sox2 as a key regulator (Okubo et al., 2006; Schlosser et al., 2008; Uchikawa et al., 2011; Wood and Episkopou, 1999). All of these placodes express N-cadherin (cadherin 2, *Cdh2*) in a Sox2-dependent fashion during their morphogenesis (Matsumata et al., 2005). Pax6 and Pou2f1 (Oct1) act as Sox2 partners during nasal and lens placode development (Donner et al., 2007; Kamachi et al., 1998; Kamachi et al., 2001). The hypophyseal placode also expresses Sox3, and Sox3 deficiency causes pituitary defects in mice, consistent with an important role for Sox3 in this tissue (Rizzoti et al., 2004).

The otic placodes express Sox9 and Sox2 (Mak et al., 2009), both of which play specific roles during inner ear development. Sox9 is involved during early otocyst development (Saint-Germain et al., 2004), and its mutational disruption in mouse leads to the failure of otic placode invagination (Barrionuevo et al., 2008). Sox2 is involved in the production and maintenance of the neural progenitors in the otocysts, i.e. the sensory patches. Inner ear-specific loss of Sox2 expression in mutant mice results in the loss of neural components: hair cells and supporting cells (Kiernan et al., 2005). During hair cell development, Sox2 cooperates with the Six1-Eya1 complex to activate the bHLH protein gene *Atoh1* (Ahmed et al., 2012; Neves et al., 2011; Neves et al., 2012). Sox2 and Atoh1 then cooperate to prime cells for hair cell development, which is initiated upon Sox2 downregulation (Dabdoub et al., 2008). An analogous scenario occurs during the development of vestibulo-cochlear ganglion neurons, where the bHLH proteins are neurogenin 1 and Neurod1 (Evsen et al., 2013; Puligilla et al., 2010).

### SoxG (Sox15) in skeletal muscle regeneration

*Sox15*-null mice are grossly normal, but show defects in skeletal muscle regeneration from satellite cells, which are muscle stem cells for regeneration (Lee et al., 2004; Maruyama et al., 2005). It has been shown that Sox15 recruits Fhl3 (four and a half LIM domains 3) as a partner factor to regulate the *Foxk1* gene (forkhead box protein K1), which is essential for the cell cycle progression of myogenic progenitor cells (Meese et al., 2007).

### The Sox trio (Sox9 and Sox5/6) in chondrogenesis during skeletal development

The crucial role of Sox9 during skeletal development was first indicated when heterozygous human *SOX9* mutations were identified as the cause of campomelic dysplasia, a skeletal dysmorphology syndrome associated with male-to-female sex reversal (Foster et al., 1994; Wagner et al., 1994). *Sox9*<sup>+/-</sup> heterozygous mouse mutants die perinatally because of skeletal abnormalities (Bi et al., 2001). Sox9 is highly expressed throughout the chondrocyte lineage, from mesenchymal chondroprogenitors to

hypertrophic chondrocytes, exhibiting an expression profile similar to that of the type II collagen gene *Col2a1* (Ng et al., 1997; Zhao et al., 1997), and it is essential throughout these developmental stages (Dy et al., 2012). Sox9 binding to the *Col2a1* intronic enhancer is required for the activation of the *Col2a1* gene (Bell et al., 1997; Lefebvre et al., 1997), but strong activation of the 48 bp *Col2a1* minimal enhancer occurs only when Sox9 and the SoxD proteins Sox5 and Sox6 (Sox5/6) together bind to several sites in the enhancer (Lefebvre et al., 1998). The same combination of Sox9 and Sox5/6, known as the ‘Sox trio’ is involved in the activation of many extracellular matrix genes, e.g. aggrecan (*Acan*) and *Col11a2*, which are secreted by the chondrocytes (Han and Lefebvre, 2008). This ‘Sox trio’ primarily functions as combination of a Sox9 homodimer and multiple Sox5/6 dimers that bind to adjacent DNA sites (Fig. 4D). Indeed, individuals with mutations in the *SOX9* dimerization domain develop campomelic dysplasia without causing sex reversal (Bernard et al., 2003; Sock et al., 2003). In line with this, single knockouts of *Sox5* or *Sox6* in mice lead to mild skeletal abnormalities, whereas a *Sox5/6* double knockout leads to a lack of chondrogenesis despite normal Sox9 expression (Smits et al., 2001), thereby confirming the functional cooperation of SoxD and SoxE proteins during chondrogenesis. The molecular mechanism underlying the SoxD-SoxE cooperation has not been clarified.

#### Sry and Sox9 in sex determination and testis development

Following the discovery of SRY/Sry (Gubbay et al., 1990; Sinclair et al., 1990), the involvement of Sox9 in male sex determination was recognized on the basis of genetic analysis of human campomelic dysplasia, as mentioned above, in which the majority of XY individuals with heterozygous *SOX9* mutations exhibit male-to-female sex reversal (Foster et al., 1994; Wagner et al., 1994). In mouse embryos, *Sry* is expressed transiently between E10.5 and E12.5 in the somatic supporting cells of the undetermined gonads, which triggers the differentiation of these cells into Sertoli cells during subsequent development. *Sry* directly activates *Sox9* synergistically with steroidogenic factor 1 (Sf1, Nr5a1) via the testis-specific enhancer of *Sox9* (Tes) (Sekido and Lovell-Badge, 2008). After Sox9 expression has been activated beyond a crucial level, its expression is maintained in Sertoli cells via positive-feedback loops, including autoregulation, whereby Sox9 activates the Tes enhancer cooperatively with Sf1. Sox9 then activates other genes that play crucial roles during testis development, such as *Amh* (anti-Müllerian hormone) and *Pgds* (prostaglandin D synthase) (Fig. 4C); *Amh* is activated by the cooperative action of Sox9 and Sf1 (De Santa Barbara et al., 1998), while *Pgds* is activated by the Sox9 dimer (Wilhelm et al., 2007). Another SoxE gene, *Sox8*, is also activated in the Sertoli cells following Sox9 expression, and plays essential roles in the maintenance of testicular functions at later stages (Barrionuevo et al., 2009).

#### SoxF proteins in the initiation of endoderm development

The involvement of SoxF proteins in endoderm development was first indicated in *Xenopus* (Hudson et al., 1997). In early mouse embryos, the SoxF protein Sox7 is expressed in the primitive endoderm, whereas another SoxF protein, Sox17, is expressed primarily in the definitive endoderm. During the derivation of the endoderm from the epiblast, a Sox2-Pou5f1/Oct4 complex switches to a Sox17-Pou5f1/Oct4 pair (Aksoy et al., 2013), analogous to the case shown in Fig. 5A. Sox7/17-expressing cells intercalate with each other when the definitive endoderm is formed (Kwon et al.,

2008). *Sox17* knockout mouse embryos develop severe defects in gut tube formation (Kanai-Azuma et al., 2002). In fish species, endodermal *Sox17* is under the regulation of Sox32 (also known as ‘casanova’), a fish-specific SoxF (Dickmeis et al., 2001; Kikuchi et al., 2001). During later endodermal development, Sox2 also participates in the regulation of foregut and trachea in mice (Que et al., 2009; Que et al., 2007).

#### SoxF proteins in vascular/lymphatic development

The SoxF proteins Sox7/17/18 are all expressed in endothelial cells during mouse vascular development. The first insight into SoxF functions during vascular development was provided by the spontaneous mouse mutation *Ragged*, which results in the synthesis of Sox18 with a truncated C-terminal domain (Pennisi et al., 2000b). Homozygous *Ragged* embryos exhibit severe cardiovascular defects, as well as hair follicle defects (discussed below), presumably owing to dominant-negative effects over other SoxF and related Sox proteins (Pennisi et al., 2000b). By contrast, *Sox18*-null knockout mice are viable and exhibit only minor coat defects (Pennisi et al., 2000a). *Sox17* knockout mouse embryos exhibit cardiovascular defects, whereas *Sox17/18* double knockout embryos have more severe defects than *Sox17* single knockout in regions where *Sox7* is only weakly expressed. *Sox7*-null embryos die around E14.5 due to cardiovascular defects (Wat et al., 2012). These results support the overlapping functions of Sox7/17/18 (Sakamoto et al., 2007). It is thought that these SoxF proteins play important roles during the establishment and maintenance of the integrity of blood vessels (Downes et al., 2009), employing Mef2c (myocyte enhancer factor 2C) as a partner factor (Hosking et al., 2001) and by regulating *Vcam1* (vascular cell-adhesion molecule 1) (Hosking et al., 2004), N-cadherin and *Mmp7* (metalloprotease 7) expression (Hoeth et al., 2012).

Sox18 is also involved in lymphatic development. In humans, *SOX18* mutations cause hypotrichosis-lymphedema-telangiectasia syndrome, which involves lymphatic dysfunction. In mice, Sox18 is expressed by a subset of cardinal vein cells and directly activates *Prox1* (prospero-related homeobox 1) gene transcription by binding to its proximal promoter; *Prox1* expression then causes these cells to differentiate into lymphatic endothelial progenitor cells (François et al., 2008).

#### Sox proteins and hair follicle development

The hair follicle, the site of hair growth, comprises two tissue components, the dermal papilla of condensed mesenchyme and the epithelial hair bulb cells that serve as the stem cells of hair shaft, which interact with each other to control overall hair growth. Sox2 and Sox18 play major regulatory roles in the dermal papilla and Sox9 in the hair bulb (Clavel et al., 2012; Driskell et al., 2009; Pennisi et al., 2000b). In the skin on the back of the mouse, four different hair types exist, which are produced in three waves during embryogenesis: guard hairs during the first wave (~E14.5), awl and auchene hairs during the second wave (~E16.5), and zigzag hairs during the third wave (E18.5). Sox2 is expressed in the developing papillae in the first and second waves, and in the papillae of guard/awl/auchene hairs throughout life, but not in the papillae of zigzag hairs (Driskell et al., 2009). Hair follicle-specific knockout of *Sox2* results in growth impairment of the non-zigzag hairs (Clavel et al., 2012). This effect of *Sox2* inactivation is partly attributed to loss of the expression of a BMP antagonist sclerostin domain-containing protein 1 (Sostdc1), a direct regulatory target of Sox2 (Clavel et al., 2012). All embryonic dermal papillae express Sox18, but *Sox18*-null mutant mice show only a reduction of the

zigzag hair type (Pennisi et al., 2000a). This difference in Sox2 dependence and Sox18 dependence distinguishes non-zigzag and zigzag hairs. However, expression of the dominant-negative form of Sox18 (*Ragged*) in the heterozygous condition causes the loss of auchene hairs in addition to zigzag hairs, and in the homozygous condition leads to the entire loss of hairs (Pennisi et al., 2000b). This observation suggests inhibition of the function of related proteins, possibly Sox2, by dominant-negative Sox18. In the hair bulb compartment, Sox9 plays a major role in hair development, and skin-specific knockout of *Sox9* results in the loss of hair shaft stem cells, causing alopecia (Vidal et al., 2005), whereas loss of the *Sox9* repressor *Trps1* (a GATA-type transcription factor) causes hypertrichosis in humans (Fantauzzo et al., 2012). Additionally, Sox21 expressed in the hair bulb plays a role in regulating the hair cycle (Kiso et al., 2009).

### SoxC proteins have versatile functions

The SoxC proteins Sox4/11/12 are expressed widely in embryonic tissues, with the highest expression levels found in neural and mesenchymal progenitor cells. *Sox4* and *Sox11* knockout mice develop multiple organ defects (Schilham et al., 1996; Sock et al., 2004), whereas *Sox12* knockout mice are grossly normal (Hoser et al., 2008). However, various combinations of homo- and heterozygous SoxC mutations indicate that continued and cumulative activities of these three genes are required for widespread organ development; increasingly severe organ hypoplasia phenotypes were found with decreasing wild-type SoxC alleles (Bhattaram et al., 2010). Partially SoxC-deficient embryos (*Sox11*<sup>-/-</sup>, *Sox4*<sup>+/-</sup>*Sox11*<sup>+/-</sup>, etc.) also display malformation of various organs. For example, *Sox4*<sup>-/-</sup>*Sox11*<sup>-/-</sup> embryos are minute at E9.5 and show highly severe organ defects and extensive cell death. Nevertheless, these embryos appropriately express genes involved in cell lineages and embryo patterning, such as *Pax1/7*, *Otx2* (orthodenticle homolog 2), *Shh* and *Bmp4* (bone morphogenetic protein 4) (Bhattaram et al., 2010). This suggests that SoxC proteins play important roles during early embryogenesis in sustaining neural and mesenchymal progenitor cells, rather than in cell lineage specification. In support of this, a reported target of SoxC is *Tead2* (TEA domain family member 2), which is a transcriptional mediator of the Hippo signalling pathway that is crucial for organ size regulation (Bhattaram et al., 2010).

However, during more advanced developmental stages, SoxC proteins regulate cell differentiation and tissue patterning. For example, following the initiation of neurogenesis in the CNS, SoxC protein expression becomes confined to post-mitotic differentiating neurons, which complements SoxB1 protein expression in neural progenitor cells (Tanaka et al., 2004). During these stages, a SoxC target is the neuron-specific tubulin gene *Tubb3* (tubulin  $\beta$ 3, class 3) (Bergsland et al., 2006). During *Xenopus* pronephros development, Sox11 and *Wt1* (Wilms tumor 1 homolog) combine to regulate *Wnt4*, which is essential for nephric tissue development (Murugan et al., 2012).

### Genome-wide approaches to studying Sox proteins: new interacting partners, interacting sites and an emerging pioneer factor function

The recent development of high-throughput proteomic/genomic approaches has enabled the systematic identification of Sox2-interacting proteins and Sox2-bound genomic sites. For example, Engelen et al. (Engelen et al., 2011), using neural stem cell lines, identified a series of Sox2-interacting proteins, including 19 transcription factors as candidate Sox2 partners, in addition to the

Sox2-interacting co-activator and co-repressors discussed above. Among these transcription factors, chromodomain helicase DNA-binding protein 7 (*Chd7*) was investigated and was shown to bind close to genomic positions occupied by Sox2 in the vicinity of many genes. Moreover, knockdown of *Sox2* reduced the occupancy of both Sox2 and *Chd7*, and reduced expression of the potential target genes in a way analogous to *Chd7* knockdown, indicating that *Chd7* functions as an important partner of Sox2 in neural stem cells. In support of this notion, *Chd7* often colocalizes with Sox2 in ESC genomes (Schnetz et al., 2010). Furthermore, using a proteomics approach, Sox21 was found to be associated with Sox2 during ESC differentiation (Mallanna et al., 2010).

Analyses of the genomic regions bound by Sox2 and other factors (Boyer et al., 2005; Chen et al., 2008) not only confirmed that Sox2 and *Pou5f1* are functional partners in ESC regulation, but also indicated that the same genomic sites are frequently co-bound by other transcription factors, such as *Nanog*. These observations suggest that the partnering between a Sox protein and its co-DNA-binding protein serves as a core of higher-order transcription factor complex.

It must be noted that, although ESCs and other cells cultured *in vitro* offer an abundant source of material for such high-throughput studies, these *in vitro* cultured cells may differ from real embryonic cells. For example, oestrogen receptor-related b (*Esrrb*) has been identified as a Sox2 partner in ESCs (Hutchins et al., 2013) and was shown to be essential for ESC maintenance in serum-free culture media (Festuccia et al., 2012; Martello et al., 2012), but in normal embryogenesis *Esrrb* is not expressed in the embryo proper but is required only for placental development (Mitsunaga et al., 2004; Pettersson et al., 1996). In the future, the analysis of cells isolated from embryonic tissue will be needed to validate the *in vivo* relevance of these interactions.

The recently identified function of transcription factors as pioneer factors (Liber et al., 2010; Xu et al., 2009a) may also apply to Sox proteins. The binding of a pioneer factor to a regulatory region does not elicit gene activation, but places the gene in a state poised for transcription. The subsequent recruitment of additional transcriptional factors to the site, or replacement of the pioneer factor with an actuating factor, then activates the genes that are characteristic of differentiated cells (Bergsland et al., 2011). Sox2-bound genomic sites in ESCs are often associated with such genes, which are not expressed in ESCs but are activated in, for example, neural progenitor cells. Moreover, many SoxB1-bound genomic sites present in neural progenitor cells are also involved in the regulation of neuron-specific genes that will be activated later when SoxB1 is replaced with SoxC (Bergsland et al., 2011), suggesting that Sox proteins exhibit general pioneer factor functions. If these SoxB1 factors do indeed function as pioneers, their knockdown will affect occupancy by the second factor, thereby inhibiting developmental progression. However, many interesting issues remain unresolved, such as whether the pioneer factor function of Sox proteins still need partners.

### Conclusions and perspective

As exemplified by the cases documented in this Primer, Sox proteins do not exert their activating or repressive regulatory functions when they bind to DNA on their own, but instead bind specific DNA sequences in combination with partner proteins to exert their effects. The combinatorial code of target specificity produced by Sox proteins and their partner proteins (the 'Sox-partner code') (Kamachi et al., 2000; Kondoh and Kamachi, 2010) ensures the stringent selection of target genes in the genome. In



addition, changes in one component of the Sox-partner complex facilitate the stepwise progression of developmental processes, whereas the formation of co-activating regulatory loops between Sox and its partner proteins stabilizes the state of cell differentiation (Kondoh and Kamachi, 2010; Kondoh et al., 2004). These basic principles will broadly apply to other transcription factor families that function as transcription factor complexes.

The recent advancement of genome-wide, high-throughput approaches holds promise to gaining an overall view of the gene regulatory networks that operate in the spatiotemporal dynamics of embryogenesis. Availability of more microscale analysis will be of great help in the analysis of embryonic tissues. To inquire into and to experimentally validate the regulatory networks in developing embryos, the refinement of gene knockout/knock-in technologies, such as inducible systems and CRISPR/Cas technology-based tailor-made gene manipulations (e.g. Cong et al., 2013) will make invaluable contributions. Such approaches will allow highly time-resolved and tissue-restricted analysis of the impact of changes in the activity of Sox or other transcription factors. Such molecular manipulations could include mutations in the Sox-partner molecular interface. In this respect, the information that lags behind the above-mentioned advances is the structural characterization of Sox-partner complexes. The structural data available to date are only those of DNA-bound Sox HMG domains (e.g. Reményi et al., 2003), rather than real complexes of full-length Sox and partner factors. Solving the three-dimensional structures of representative Sox-partner complexes on target DNA will contribute greatly to the elucidation of Sox partner-dependent regulations at the molecular level.

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The authors declare no competing financial interests.

#### References

- Agarwal, P., Verzi, M. P., Nguyen, T., Hu, J., Ehlers, M. L., McCulley, D. J., Xu, S. M., Dodou, E., Anderson, J. P., Wei, M. L. et al. (2011). The MADS box transcription factor MEF2C regulates melanocyte development and is a direct transcriptional target and partner of SOX10. *Development* **138**, 2555-2565.
- Ahmed, M., Wong, E. Y., Sun, J., Xu, J., Wang, F. and Xu, P. X. (2012). Eya1-Six1 interaction is sufficient to induce hair cell fate in the cochlea by activating Atoh1 expression in cooperation with Sox2. *Dev. Cell* **22**, 377-390.
- Aksoy, I., Jauch, R., Chen, J., Dyla, M., Divakar, U., Bogu, G. K., Teo, R., Leng Ng, C. K., Herath, W., Lili, S. et al. (2013). Oct4 switches partnering from Sox2 to Sox17 to reinterpret the enhancer code and specify endoderm. *EMBO J.* **32**, 938-953.
- Ambrosetti, D. C., Basilico, C. and Dailey, L. (1997). Synergistic activation of the fibroblast growth factor 4 enhancer by Sox2 and Oct-3 depends on protein-protein interactions facilitated by a specific spatial arrangement of factor binding sites. *Mol. Cell. Biol.* **17**, 6321-6329.
- Aota, S., Nakajima, N., Sakamoto, R., Watanabe, S., Ibaraki, N. and Okazaki, K. (2003). Pax6 autoregulation mediated by direct interaction of Pax6 protein with the head surface ectoderm-specific enhancer of the mouse Pax6 gene. *Dev. Biol.* **257**, 1-13.
- Avilion, A. A., Nicolis, S. K., Pevny, L. H., Perez, L., Vivian, N. and Lovell-Badge, R. (2003). Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* **17**, 126-140.
- Azim, E., Jabaudon, D., Fame, R. M. and Macklis, J. D. (2009). SOX6 controls dorsal progenitor identity and interneuron diversity during neocortical development. *Nat. Neurosci.* **12**, 1238-1247.
- Badis, G., Berger, M. F., Philippakis, A. A., Talukder, S., Gehrke, A. R., Jaeger, S. A., Chan, E. T., Metzler, G., Vedenko, A., Chen, X. et al. (2009). Diversity and complexity in DNA recognition by transcription factors. *Science* **324**, 1720-1723.
- Baltus, G. A., Kowalski, M. P., Zhai, H., Tutter, A. V., Quinn, D., Wall, D. and Kadam, S. (2009). Acetylation of sox2 induces its nuclear export in embryonic stem cells. *Stem Cells* **27**, 2175-2184.
- Barriounevo, F., Naumann, A., Bagheri-Fam, S., Speth, V., Taketo, M. M., Scherer, G. and Neubüser, A. (2008). Sox9 is required for invagination of the otic placode in mice. *Dev. Biol.* **317**, 213-224.
- Barriounevo, F., Georg, I., Scherthan, H., Lécureuil, C., Guillou, F., Wegner, M. and Scherer, G. (2009). Testis cord differentiation after the sex determination stage is independent of Sox9 but fails in the combined absence of Sox9 and Sox8. *Dev. Biol.* **327**, 301-312.
- Bell, D. M., Leung, K. K., Wheatley, S. C., Ng, L. J., Zhou, S., Ling, K. W., Sham, M. H., Koopman, P., Tam, P. P. and Cheah, K. S. (1997). SOX9 directly regulates the type-II collagen gene. *Nat. Genet.* **16**, 174-178.
- Bergsland, M., Werme, M., Malewicz, M., Perlmann, T. and Muhr, J. (2006). The establishment of neuronal properties is controlled by Sox4 and Sox11. *Genes Dev.* **20**, 3475-3486.
- Bergsland, M., Ramsköld, D., Zaouter, C., Klum, S., Sandberg, R. and Muhr, J. (2011). Sequentially acting Sox transcription factors in neural lineage development. *Genes Dev.* **25**, 2453-2464.
- Bernard, P. and Harley, V. R. (2010). Acquisition of SOX transcription factor specificity through protein-protein interaction, modulation of Wnt signalling and post-translational modification. *Int. J. Biochem. Cell Biol.* **42**, 400-410.
- Bernard, P., Tang, P., Liu, S., Dewing, P., Harley, V. R. and Vilain, E. (2003). Dimerization of SOX9 is required for chondrogenesis, but not for sex determination. *Hum. Mol. Genet.* **12**, 1755-1765.
- Betancur, P., Bronner-Fraser, M. and Sauka-Spengler, T. (2010). Genomic code for Sox10 activation reveals a key regulatory enhancer for cranial neural crest. *Proc. Natl. Acad. Sci. USA* **107**, 3570-3575.
- Bhattaram, P., Penzo-Méndez, A., Sock, E., Colmenares, C., Kaneko, K. J., Vassilev, A., Depamphilis, M. L., Wegner, M. and Lefebvre, V. (2010). Organogenesis relies on SoxC transcription factors for the survival of neural and mesenchymal progenitors. *Nat Commun* **1**, 9.
- Bi, W., Huang, W., Whitworth, D. J., Deng, J. M., Zhang, Z., Behringer, R. R. and de Crombrughe, B. (2001). Haploinsufficiency of Sox9 results in defective cartilage primordia and premature skeletal mineralization. *Proc. Natl. Acad. Sci. USA* **98**, 6698-6703.
- Bondurand, N., Pingault, V., Goerich, D. E., Lemort, N., Sock, E., Le Caignec, C., Wegner, M. and Goossens, M. (2000). Interaction among SOX10, PAX3 and MITF, three genes altered in Waardenburg syndrome. *Hum. Mol. Genet.* **9**, 1907-1917.
- Bondurand, N., Girard, M., Pingault, V., Lemort, N., Dubourg, O. and Goossens, M. (2001). Human Connexin 32, a gap junction protein altered in the X-linked form of Charcot-Marie-Tooth disease, is directly regulated by the transcription factor SOX10. *Hum. Mol. Genet.* **10**, 2783-2795.
- Bowles, J., Schepers, G. and Koopman, P. (2000). Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators. *Dev. Biol.* **227**, 239-255.
- Boyer, L. A., Lee, T. I., Cole, M. F., Johnstone, S. E., Levine, S. S., Zucker, J. P., Guenther, M. G., Kumar, R. M., Murray, H. L., Jenner, R. G. et al. (2005). Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* **122**, 947-956.
- Bridgewater, L. C., Walker, M. D., Miller, G. C., Ellison, T. A., Holsinger, L. D., Potter, J. L., Jackson, T. L., Chen, R. K., Winkel, V. L., Zhang, Z. et al. (2003). Adjacent DNA sequences modulate Sox9 transcriptional activation at paired Sox sites in three chondrocyte-specific enhancer elements. *Nucleic Acids Res.* **31**, 1541-1553.
- Bylund, M., Andersson, E., Novitch, B. G. and Muhr, J. (2003). Vertebrate neurogenesis is counteracted by Sox1-3 activity. *Nat. Neurosci.* **6**, 1162-1168.
- Cajal, M., Lawson, K. A., Hill, B., Moreau, A., Rao, J., Ross, A., Collignon, J. and Camus, A. (2012). Clonal and molecular analysis of the prospective anterior neural boundary in the mouse embryo. *Development* **139**, 423-436.
- Chen, X., Xu, H., Yuan, P., Fang, F., Huss, M., Vega, V. B., Wong, E., Orlov, Y. L., Zhang, W., Jiang, J. et al. (2008). Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. *Cell* **133**, 1106-1117.
- Cheng, L. C., Pastrana, E., Tavazoie, M. and Doetsch, F. (2009). miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nat. Neurosci.* **12**, 399-408.
- Cheung, M. and Briscoe, J. (2003). Neural crest development is regulated by the transcription factor Sox9. *Development* **130**, 5681-5693.
- Clavel, C., Grisanti, L., Zemla, R., Rezza, A., Barros, R., Sennett, R., Mazloom, A. R., Chung, C. Y., Cai, X., Cai, C. L. et al. (2012). Sox2 in the dermal papilla niche controls hair growth by fine-tuning BMP signaling in differentiating hair shaft progenitors. *Dev. Cell* **23**, 981-994.
- Collignon, J., Sockanathan, S., Hacker, A., Cohen-Tannoudji, M., Norris, D., Rastan, S., Stevanovic, M., Goodfellow, P. N. and Lovell-Badge, R. (1996). A



- comparison of the properties of Sox-3 with Sry and two related genes, Sox-1 and Sox-2. *Development* **122**, 509-520.
- Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A. et al. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819-823.
- Dabdoub, A., Puligilla, C., Jones, J. M., Fritzsich, B., Cheah, K. S., Pevny, L. H. and Kelley, M. W. (2008). Sox2 signaling in prosensory domain specification and subsequent hair cell differentiation in the developing cochlea. *Proc. Natl. Acad. Sci. USA* **105**, 18396-18401.
- De Santa Barbara, P., Bonneaud, N., Boizet, B., Desclozeaux, M., Moniot, B., Sudbeck, P., Scherer, G., Poulat, F. and Berta, P. (1998). Direct interaction of SRY-related protein SOX9 and steroidogenic factor 1 regulates transcription of the human anti-Müllerian hormone gene. *Mol. Cell. Biol.* **18**, 6653-6665.
- Dee, C. T., Hirst, C. S., Shih, Y. H., Tripathi, V. B., Patient, R. K. and Scotting, P. J. (2008). Sox3 regulates both neural fate and differentiation in the zebrafish ectoderm. *Dev. Biol.* **320**, 289-301.
- Dickmeis, T., Mourrain, P., Saint-Etienne, L., Fischer, N., Aanstad, P., Clark, M., Strähle, U. and Rosa, F. (2001). A crucial component of the endoderm formation pathway, CASANOVA, is encoded by a novel sox-related gene. *Genes Dev.* **15**, 1487-1492.
- Donner, A. L., Episkopou, V. and Maas, R. L. (2007). Sox2 and Pou2f1 interact to control lens and olfactory placode development. *Dev. Biol.* **303**, 784-799.
- Downes, M., Francois, M., Ferguson, C., Parton, R. G. and Koopman, P. (2009). Vascular defects in a mouse model of hypotrichosis-lymphedema-telangiectasia syndrome indicate a role for SOX18 in blood vessel maturation. *Hum. Mol. Genet.* **18**, 2839-2850.
- Driskell, R. R., Giangreco, A., Jensen, K. B., Mulder, K. W. and Watt, F. M. (2009). Sox2-positive dermal papilla cells specify hair follicle type in mammalian epidermis. *Development* **136**, 2815-2823.
- Dugas, J. C., Cuellar, T. L., Scholze, A., Ason, B., Ibrahim, A., Emery, B., Zamanian, J. L., Foo, L. C., McManus, M. T. and Barres, B. A. (2010). Dicer1 and miR-219 Are required for normal oligodendrocyte differentiation and myelination. *Neuron* **65**, 597-611.
- Dy, P., Penzo-Méndez, A., Wang, H., Pedraza, C. E., Macklin, W. B. and Lefebvre, V. (2008). The three SoxC proteins—Sox4, Sox11 and Sox12—exhibit overlapping expression patterns and molecular properties. *Nucleic Acids Res.* **36**, 3101-3117.
- Dy, P., Wang, W., Bhattaram, P., Wang, Q., Wang, L., Ballock, R. T. and Lefebvre, V. (2012). Sox9 directs hypertrophic maturation and blocks osteoblast differentiation of growth plate chondrocytes. *Dev. Cell* **22**, 597-609.
- Ekonomou, A., Kazanis, I., Malas, S., Wood, H., Alifragis, P., Denaxa, M., Karagozeos, D., Constanti, A., Lovell-Badge, R. and Episkopou, V. (2005). Neuronal migration and ventral subtype identity in the telencephalon depend on SOX1. *PLoS Biol.* **3**, e186.
- Engelen, E., Akinci, U., Bryne, J. C., Hou, J., Gontan, C., Moen, M., Szumska, D., Kockx, C., van Ijcken, W., Dekkers, D. H. et al. (2011). Sox2 cooperates with Chd7 to regulate genes that are mutated in human syndromes. *Nat. Genet.* **43**, 607-611.
- Evens, L., Sugahara, S., Uchikawa, M., Kondoh, H. and Wu, D. K. (2013). Progression of neurogenesis in the inner ear requires inhibition of Sox2 transcription by neurogenin1 and neurod1. *J. Neurosci.* **33**, 3879-3890.
- Fantauzzo, K. A., Kurban, M., Levy, B. and Christiano, A. M. (2012). Trps1 and its target gene Sox9 regulate epithelial proliferation in the developing hair follicle and are associated with hypertrichosis. *PLoS Genet.* **8**, e1003002.
- Favaro, R., Valotta, M., Ferri, A. L., Latorre, E., Mariani, J., Giachino, C., Lancini, C., Tosetti, V., Ottolenghi, S., Taylor, V. et al. (2009). Hippocampal development and neural stem cell maintenance require Sox2-dependent regulation of Shh. *Nat. Neurosci.* **12**, 1248-1256.
- Ferri, A. L., Cavallaro, M., Braidà, D., Di Cristofano, A., Canta, A., Vezzani, A., Ottolenghi, S., Pandolfi, P. P., Sala, M., DeBiasi, S. et al. (2004). Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. *Development* **131**, 3805-3819.
- Festuccia, N., Osorno, R., Halbritter, F., Karwacki-Neisius, V., Navarro, P., Colby, D., Wong, F., Yates, A., Tomlinson, S. R. and Chambers, I. (2012). Esrrb is a direct Nanog target gene that can substitute for Nanog function in pluripotent cells. *Cell Stem Cell* **11**, 477-490.
- Foster, J. W. and Graves, J. A. (1994). An SRY-related sequence on the marsupial X chromosome: implications for the evolution of the mammalian testis-determining gene. *Proc. Natl. Acad. Sci. USA* **91**, 1927-1931.
- Foster, J. W., Dominguez-Steglich, M. A., Guioli, S., Kwok, C., Weller, P. A., Stevanović, M., Weissenbach, J., Mansour, S., Young, I. D., Goodfellow, P. N. et al. (1994). Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. *Nature* **372**, 525-530.
- François, M., Caprini, A., Hosking, B., Orsenigo, F., Wilhelm, D., Browne, C., Paavonen, K., Karnezis, T., Shayan, R., Downes, M. et al. (2008). Sox18 induces development of the lymphatic vasculature in mice. *Nature* **456**, 643-647.
- Francois, M., Koopman, P. and Beltrame, M. (2010). SoxF genes: Key players in the development of the cardio-vascular system. *Int. J. Biochem. Cell Biol.* **42**, 445-448.
- Ghislain, J. and Charnay, P. (2006). Control of myelination in Schwann cells: a Krox20 cis-regulatory element integrates Oct6, Brn2 and Sox10 activities. *EMBO Rep.* **7**, 52-58.
- Graham, V., Khudyakov, J., Ellis, P. and Pevny, L. (2003). SOX2 functions to maintain neural progenitor identity. *Neuron* **39**, 749-765.
- Gubbay, J., Collignon, J., Koopman, P., Capel, B., Economou, A., Münsterberg, A., Vivian, N., Goodfellow, P. and Lovell-Badge, R. (1990). A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature* **346**, 245-250.
- Han, Y. and Lefebvre, V. (2008). L-Sox5 and Sox6 drive expression of the aggrecan gene in cartilage by securing binding of Sox9 to a far-upstream enhancer. *Mol. Cell. Biol.* **28**, 4999-5013.
- Hoeth, M., Niederleithner, H., Hofer-Warbinek, R., Bilban, M., Mayer, H., Resch, U., Lemberger, C., Wagner, O., Hofer, E., Petzelbauer, P. et al. (2012). The transcription factor SOX18 regulates the expression of matrix metalloproteinase 7 and guidance molecules in human endothelial cells. *PLoS ONE* **7**, e30982.
- Hoser, M., Potzner, M. R., Koch, J. M., Bösl, M. R., Wegner, M. and Sock, E. (2008). Sox12 deletion in the mouse reveals nonreciprocal redundancy with the related Sox4 and Sox11 transcription factors. *Mol. Cell. Biol.* **28**, 4675-4687.
- Hosking, B. M., Wang, S. C., Chen, S. L., Penning, S., Koopman, P. and Muscat, G. E. (2001). SOX18 directly interacts with MEF2C in endothelial cells. *Biochem. Biophys. Res. Commun.* **287**, 493-500.
- Hosking, B. M., Wang, S. C., Downes, M., Koopman, P. and Muscat, G. E. (2004). The VCAM-1 gene that encodes the vascular cell adhesion molecule is a target of the Sry-related high mobility group box gene, Sox18. *J. Biol. Chem.* **279**, 5314-5322.
- Hudson, C., Clements, D., Friday, R. V., Stott, D. and Woodland, H. R. (1997). Xsox1 alpha and -beta mediate endoderm formation in *Xenopus*. *Cell* **91**, 397-405.
- Hutchins, A. P., Choo, S. H., Mistri, T. K., Rahmani, M., Woon, C. T., Ng, C. K., Jauch, R. and Robson, P. (2013). Co-motif discovery identifies an Esrrb-Sox2-DNA ternary complex as a mediator of transcriptional differences between mouse embryonic and epiblast stem cells. *Stem Cells* **31**, 269-281.
- Inoue, M., Kamachi, Y., Matsunami, H., Imada, K., Uchikawa, M. and Kondoh, H. (2007). PAX6 and SOX2-dependent regulation of the Sox2 enhancer N-3 involved in embryonic visual system development. *Genes Cells* **12**, 1049-1061.
- Iwafuchi-Doi, M., Yoshida, Y., Onichtchouk, D., Leichenring, M., Driever, W., Takemoto, T., Uchikawa, M., Kamachi, Y. and Kondoh, H. (2011). The Pou5f1/Pou3f-dependent but Sox8-independent regulation of conserved enhancer N2 initiates Sox2 expression during epiblast to neural plate stages in vertebrates. *Dev. Biol.* **352**, 354-366.
- Iwafuchi-Doi, M., Matsuda, K., Murakami, K., Niwa, H., Tesar, P. J., Aruga, J., Matsuo, I. and Kondoh, H. (2012). Transcriptional regulatory networks in epiblast stem cells and during anterior neural plate development as modeled in epiblast stem cells. *Development* **139**, 3926-3937.
- Jang, H., Kim, T. W., Yoon, S., Choi, S. Y., Kang, T. W., Kim, S. Y., Kwon, Y. W., Cho, E. J. and Youn, H. D. (2012). O-GlcNAc regulates pluripotency and reprogramming by directly acting on core components of the pluripotency network. *Cell Stem Cell* **11**, 62-74.
- Jeong, C. H., Cho, Y. Y., Kim, M. O., Kim, S. H., Cho, E. J., Lee, S. Y., Jeon, Y. J., Lee, K. Y., Yao, K., Keum, Y. S. et al. (2010). Phosphorylation of Sox2 cooperates in reprogramming to pluripotent stem cells. *Stem Cells* **28**, 2141-2150.
- Kamachi, Y., Sockanathan, S., Liu, Q., Breitman, M., Lovell-Badge, R. and Kondoh, H. (1995). Involvement of SOX proteins in lens-specific activation of crystallin genes. *EMBO J.* **14**, 3510-3519.
- Kamachi, Y., Uchikawa, M., Collignon, J., Lovell-Badge, R. and Kondoh, H. (1998). Involvement of Sox1, 2 and 3 in the early and subsequent molecular events of lens induction. *Development* **125**, 2521-2532.
- Kamachi, Y., Cheah, K. S. and Kondoh, H. (1999). Mechanism of regulatory target selection by the SOX high-mobility-group domain proteins as revealed by comparison of SOX1/2/3 and SOX9. *Mol. Cell. Biol.* **19**, 107-120.
- Kamachi, Y., Uchikawa, M. and Kondoh, H. (2000). Pairing SOX off: with partners in the regulation of embryonic development. *Trends Genet.* **16**, 182-187.
- Kamachi, Y., Uchikawa, M., Tanouchi, A., Sekido, R. and Kondoh, H. (2001). Pax6 and SOX2 form a co-DNA-binding partner complex that regulates initiation of lens development. *Genes Dev.* **15**, 1272-1286.
- Kamachi, Y., Iwafuchi, M., Okuda, Y., Takemoto, T., Uchikawa, M. and Kondoh, H. (2009). Evolution of non-coding regulatory sequences involved in the developmental process: reflection of differential employment of paralogous genes as highlighted by Sox2 and group B1 Sox genes. *Proc. Jpn. Acad., Ser. B, Phys. Biol. Sci.* **85**, 55-68.
- Kanai-Azuma, M., Kanai, Y., Gad, J. M., Tajima, Y., Taya, C., Kurohmaru, M., Sanai, Y., Yonekawa, H., Yazaki, K., Tam, P. P. et al. (2002). Depletion of definitive gut endoderm in Sox17-null mutant mice. *Development* **129**, 2367-2379.

- Kang, P., Lee, H. K., Glasgow, S. M., Finley, M., Donti, T., Gaber, Z. B., Graham, B. H., Foster, A. E., Novitsch, B. G., Gronostajski, R. M. et al. (2012). Sox9 and NFIA coordinate a transcriptional regulatory cascade during the initiation of gliogenesis. *Neuron* **74**, 79–94.
- Kelberman, D., Rizzotti, K., Avilion, A., Bitner-Glindzicz, M., Cianfarani, S., Collins, J., Chong, W. K., Kirk, J. M., Achermann, J. C., Ross, R. et al. (2006). Mutations within Sox2/SOX2 are associated with abnormalities in the hypothalamo-pituitary-gonadal axis in mice and humans. *J. Clin. Invest.* **116**, 2442–2455.
- Kellerer, S., Schreiner, S., Stolt, C. C., Scholz, S., Bösl, M. R. and Wegner, M. (2006). Replacement of the Sox10 transcription factor by Sox8 reveals incomplete functional equivalence. *Development* **133**, 2875–2886.
- Kiernan, A. E., Pelling, A. S., Leung, K. K., Tang, A. S., Bell, D. M., Tease, C., Lovell-Badge, R., Steel, K. P. and Cheah, K. S. (2005). Sox2 is required for sensory organ development in the mammalian inner ear. *Nature* **434**, 1031–1035.
- Kikuchi, Y., Agathon, A., Alexander, J., Thisse, C., Waldron, S., Yelon, D., Thisse, B. and Stainier, D. Y. (2001). *casanova* encodes a novel Sox-related protein necessary and sufficient for early endoderm formation in zebrafish. *Genes Dev.* **15**, 1493–1505.
- King, N., Westbrook, M. J., Young, S. L., Kuo, A., Abedin, M., Chapman, J., Fairclough, S., Hellsten, U., Isogai, Y., Letunic, I. et al. (2008). The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature* **451**, 783–788.
- Kishi, M., Mizuseki, K., Sasai, N., Yamazaki, H., Shiota, K., Nakanishi, S. and Sasai, Y. (2000). Requirement of Sox2-mediated signaling for differentiation of early Xenopus neuroectoderm. *Development* **127**, 791–800.
- Kiso, M., Tanaka, S., Saba, R., Matsuda, S., Shimizu, A., Ohyama, M., Okano, H. J., Shiroishi, T., Okano, H. and Saga, Y. (2009). The disruption of Sox21-mediated hair shaft cuticle differentiation causes cyclic alopecia in mice. *Proc. Natl. Acad. Sci. USA* **106**, 9292–9297.
- Kobayashi, D., Jindo, T., Naruse, K. and Takeda, H. (2006). Development of the endoderm and gut in medaka, *Oryzias latipes*. *Dev. Growth Differ.* **48**, 283–295.
- Kondoh, H. and Kamachi, Y. (2010). SOX-partner code for cell specification: Regulatory target selection and underlying molecular mechanisms. *Int. J. Biochem. Cell Biol.* **42**, 391–399.
- Kondoh, H., Uchikawa, M. and Kamachi, Y. (2004). Interplay of Pax6 and SOX2 in lens development as a paradigm of genetic switch mechanisms for cell differentiation. *Int. J. Dev. Biol.* **48**, 819–827.
- Kuhlbrodt, K., Herbarth, B., Sock, E., Hermans-Borgmeyer, I. and Wegner, M. (1998). Sox10, a novel transcriptional modulator in glial cells. *J. Neurosci.* **18**, 237–250.
- Kwan, K. Y., Lam, M. M., Krsnik, Z., Kawasawa, Y. I., Lefebvre, V. and Sestan, N. (2008). SOX5 postmitotically regulates migration, postmigratory differentiation, and projections of subplate and deep-layer neocortical neurons. *Proc. Natl. Acad. Sci. USA* **105**, 16021–16026.
- Kwon, G. S., Viotti, M. and Hadjantonakis, A. K. (2008). The endoderm of the mouse embryo arises by dynamic widespread intercalation of embryonic and extraembryonic lineages. *Dev. Cell* **15**, 509–520.
- Lai, T., Jabaudon, D., Molyneaux, B. J., Azim, E., Arlotta, P., Menezes, J. R. and Macklis, J. D. (2008). SOX5 controls the sequential generation of distinct corticofugal neuron subtypes. *Neuron* **57**, 232–247.
- LeBlanc, S. E., Ward, R. M. and Svaren, J. (2007). Neuropathy-associated Egr2 mutants disrupt cooperative activation of myelin protein zero by Egr2 and Sox10. *Mol. Cell Biol.* **27**, 3521–3529.
- Lee, H. J., Göring, W., Ochs, M., Mühlfeld, C., Steding, G., Paprotta, I., Engel, W. and Adham, I. M. (2004). Sox15 is required for skeletal muscle regeneration. *Mol. Cell Biol.* **24**, 8428–8436.
- Lee, P. C., Taylor-Jaffe, K. M., Nordin, K. M., Prasad, M. S., Lander, R. M. and LaBonne, C. (2012). SUMOylated SoxE factors recruit Grg4 and function as transcriptional repressors in the neural crest. *J. Cell Biol.* **198**, 799–813.
- Lefebvre, V. (2010). The SoxD transcription factors—Sox5, Sox6, and Sox13—are key cell fate modulators. *Int. J. Biochem. Cell Biol.* **42**, 429–432.
- Lefebvre, V., Huang, W., Harley, V. R., Goodfellow, P. N. and de Crombrughe, B. (1997). SOX9 is a potent activator of the chondrocyte-specific enhancer of the pro alpha1(II) collagen gene. *Mol. Cell Biol.* **17**, 2336–2346.
- Lefebvre, V., Li, P. and de Crombrughe, B. (1998). A new long form of Sox5 (L-Sox5), Sox6 and Sox9 are coexpressed in chondrogenesis and cooperatively activate the type II collagen gene. *EMBO J.* **17**, 5718–5733.
- Leung, V. Y., Gao, B., Leung, K. K., Melhado, I. G., Wynn, S. L., Au, T. Y., Dung, N. W., Lau, J. Y., Mak, A. C., Chan, D. et al. (2011). SOX9 governs differentiation stage-specific gene expression in growth plate chondrocytes via direct concomitant transactivation and repression. *PLoS Genet.* **7**, e1002356.
- Liber, D., Domaschenz, R., Holmqvist, P. H., Mazzarella, L., Georgiou, A., Leleu, M., Fisher, A. G., Labosky, P. A. and Dillon, N. (2010). Epigenetic priming of a pre-B cell-specific enhancer through binding of Sox2 and Foxd3 at the ESC stage. *Cell Stem Cell* **7**, 114–126.
- Liu, J., Wu, R., Yan, C., Chau, B., So, H., Chan, A., Cheah, K. S., Briscoe, J. and Cheung, M. (2013). Phosphorylation of Sox9 is required for neural crest delamination and is regulated downstream of BMP and canonical Wnt signaling. *Proc. Natl. Acad. Sci. USA* **110**, 2882–2887.
- Ludwig, A., Rehberg, S. and Wegner, M. (2004). Melanocyte-specific expression of dopachrome tautomerase is dependent on synergistic gene activation by the Sox10 and Mitf transcription factors. *FEBS Lett.* **556**, 236–244.
- Mak, A. C., Szeto, I. Y., Fritsch, B. and Cheah, K. S. (2009). Differential and overlapping expression pattern of SOX2 and SOX9 in inner ear development. *Gene Expr. Patterns* **9**, 444–453.
- Maka, M., Stolt, C. C. and Wegner, M. (2005). Identification of Sox8 as a modifier gene in a mouse model of Hirschsprung disease reveals underlying molecular defect. *Dev. Biol.* **277**, 155–169.
- Malki, S., Boizet-Bonhoure, B. and Poulat, F. (2010). Shuttling of SOX proteins. *Int. J. Biochem. Cell Biol.* **42**, 411–416.
- Mallanna, S. K., Ormsbee, B. D., Iacovino, M., Gilmore, J. M., Cox, J. L., Kyba, M., Washburn, M. P. and Rizzino, A. (2010). Proteomic analysis of Sox2-associated proteins during early stages of mouse embryonic stem cell differentiation identifies Sox21 as a novel regulator of stem cell fate. *Stem Cells* **28**, 1715–1727.
- Martello, G., Sugimoto, T., Diamanti, E., Joshi, A., Hannah, R., Ohtsuka, S., Göttgens, B., Niwa, H. and Smith, A. (2012). Esrrb is a pivotal target of the Gsk3/Tcf3 axis regulating embryonic stem cell self-renewal. *Cell Stem Cell* **11**, 491–504.
- Maruyama, M., Ichisaka, T., Nakagawa, M. and Yamanaka, S. (2005). Differential roles for Sox15 and Sox2 in transcriptional control in mouse embryonic stem cells. *J. Biol. Chem.* **280**, 24371–24379.
- Matsuda, S., Kuwako, K., Okano, H. J., Tsutsumi, S., Aburatani, H., Saga, Y., Matsuzaki, Y., Akaike, A., Sugimoto, H. and Okano, H. (2012). Sox21 promotes hippocampal adult neurogenesis via the transcriptional repression of the Hes5 gene. *J. Neurosci.* **32**, 12543–12557.
- Matsumata, M., Uchikawa, M., Kamachi, Y. and Kondoh, H. (2005). Multiple N-cadherin enhancers identified by systematic functional screening indicate its Group B1 SOX-dependent regulation in neural and placodal development. *Dev. Biol.* **286**, 601–617.
- Meeson, A. P., Shi, X., Alexander, M. S., Williams, R. S., Allen, R. E., Jiang, N., Adham, I. M., Goetsch, S. C., Hammer, R. E. and Garry, D. J. (2007). Sox15 and Fhl3 transcriptionally coactivate Foxk1 and regulate myogenic progenitor cells. *EMBO J.* **26**, 1902–1912.
- Mitsunaga, K., Araki, K., Mizusaki, H., Morohashi, K., Haruna, K., Nakagata, N., Giguère, V., Yamamura, K. and Abe, K. (2004). Loss of PGC-specific expression of the orphan nuclear receptor ER-R-beta results in reduction of germ cell number in mouse embryos. *Mech. Dev.* **121**, 237–246.
- Miyagi, S., Nishimoto, M., Saito, T., Ninomiya, M., Sawamoto, K., Okano, H., Muramatsu, M., Oguro, H., Iwama, A. and Okuda, A. (2006). The Sox2 regulatory region 2 functions as a neural stem cell-specific enhancer in the telencephalon. *J. Biol. Chem.* **281**, 13374–13381.
- Murakami, A., Ishida, S., Thurlow, J., Revest, J. M. and Dickson, C. (2001). SOX6 binds CtBP2 to repress transcription from the Fgf-3 promoter. *Nucleic Acids Res.* **29**, 3347–3355.
- Murisier, F., Guichard, S. and Beermann, F. (2007). The tyrosinase enhancer is activated by Sox10 and Mitf in mouse melanocytes. *Pigment Cell Res.* **20**, 173–184.
- Murugan, S., Shan, J., Kühl, S. J., Tata, A., Pietilä, I., Kühl, M. and Vainio, S. J. (2012). WT1 and Sox11 regulate synergistically the promoter of the Wnt4 gene that encodes a critical signal for nephrogenesis. *Exp. Cell Res.* **318**, 1134–1145.
- Neves, J., Parada, C., Chamizo, M. and Giraldez, F. (2011). Jagged 1 regulates the restriction of Sox2 expression in the developing chicken inner ear: a mechanism for sensory organ specification. *Development* **138**, 735–744.
- Neves, J., Uchikawa, M., Bigas, A. and Giraldez, F. (2012). The prosensory function of Sox2 in the chicken inner ear relies on the direct regulation of Atoh1. *PLoS ONE* **7**, e30871.
- Ng, L. J., Wheatley, S., Muscat, G. E., Conway-Campbell, J., Bowles, J., Wright, E., Bell, D. M., Tam, P. P., Cheah, K. S. and Koopman, P. (1997). SOX9 binds DNA, activates transcription, and coexpresses with type II collagen during chondrogenesis in the mouse. *Dev. Biol.* **183**, 108–121.
- Nishiguchi, S., Wood, H., Kondoh, H., Lovell-Badge, R. and Episkopou, V. (1998). Sox1 directly regulates the gamma-crystallin genes and is essential for lens development in mice. *Genes Dev.* **12**, 776–781.
- Nishimoto, M., Fukushima, A., Okuda, A. and Muramatsu, M. (1999). The gene for the embryonic stem cell coactivator UTF1 carries a regulatory element which selectively interacts with a complex composed of Oct-3/4 and Sox-2. *Mol. Cell Biol.* **19**, 5453–5465.
- Nishimura, N., Kamimura, Y., Ishida, Y., Takemoto, T., Kondoh, H. and Uchikawa, M. (2012). A systematic survey and characterization of enhancers that regulate Sox3 in neurosensory development in comparison with Sox2 enhancers. *Biology* **1**, 714–735.
- Okubo, T., Pevny, L. H. and Hogan, B. L. (2006). Sox2 is required for development of taste bud sensory cells. *Genes Dev.* **20**, 2654–2659.

- Okuda, Y., Yoda, H., Uchikawa, M., Furutani-Seiki, M., Takeda, H., Kondoh, H. and Kamachi, Y. (2006). Comparative genomic and expression analysis of group B1 sox genes in zebrafish indicates their diversification during vertebrate evolution. *Dev. Dyn.* **235**, 811-825.
- Okuda, Y., Ogura, E., Kondoh, H. and Kamachi, Y. (2010). B1 SOX coordinate cell specification with patterning and morphogenesis in the early zebrafish embryo. *PLoS Genet.* **6**, e1000936.
- Onichtchouk, D., Geier, F., Polok, B., Messerschmidt, D. M., Mössner, R., Wendik, B., Song, S., Taylor, V., Timmer, J. and Driever, W. (2010). Zebrafish Pou5f1-dependent transcriptional networks in temporal control of early development. *Mol. Syst. Biol.* **6**, 354.
- Oosterveen, T., Kurdija, S., Alekseenko, Z., Uhde, C. W., Bergsland, M., Sandberg, M., Andersson, E., Dias, J. M., Muhr, J. and Ericson, J. (2012). Mechanistic differences in the transcriptional interpretation of local and long-range Shh morphogen signaling. *Dev. Cell* **23**, 1006-1019.
- Osaki, E., Nishina, Y., Inazawa, J., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Ohsugi, M., Tezuka, T., Yoshida, M. and Semba, K. (1999). Identification of a novel Sry-related gene and its germ cell-specific expression. *Nucleic Acids Res.* **27**, 2503-2510.
- Peng, C., Li, N., Ng, Y. K., Zhang, J., Meier, F., Theis, F. J., Merckenschlager, M., Chen, W., Wurst, W. and Prakash, N. (2012). A unilateral negative feedback loop between miR-200 microRNAs and Sox2/E2F3 controls neural progenitor cell-cycle exit and differentiation. *J. Neurosci.* **32**, 13292-13308.
- Pennisi, D., Bowles, J., Nagy, A., Muscat, G. and Koopman, P. (2000a). Mice null for sox18 are viable and display a mild coat defect. *Mol. Cell. Biol.* **20**, 9331-9336.
- Pennisi, D., Gardner, J., Chambers, D., Hosking, B., Peters, J., Muscat, G., Abbott, C. and Koopman, P. (2000b). Mutations in Sox18 underlie cardiovascular and hair follicle defects in ragged mice. *Nat. Genet.* **24**, 434-437.
- Peterson, K. A., Nishi, Y., Ma, W., Vedenko, A., Shokri, L., Zhang, X., McFarlane, M., Baizabal, J. M., Junker, J. P., van Oudenaarden, A. et al. (2012). Neural-specific Sox2 input and differential Gli-binding affinity provide context and positional information in Shh-directed neural patterning. *Genes Dev.* **26**, 2802-2816.
- Pettersson, K., Svensson, K., Mattsson, R., Carlsson, B., Ohlsson, R. and Berkenstam, A. (1996). Expression of a novel member of estrogen response element-binding nuclear receptors is restricted to the early stages of chorion formation during mouse embryogenesis. *Mech. Dev.* **54**, 211-223.
- Pevny, L. H. and Lovell-Badge, R. (1997). Sox genes find their feet. *Curr. Opin. Genet. Dev.* **7**, 338-344.
- Pevny, L. H. and Nicolis, S. K. (2010). Sox2 roles in neural stem cells. *Int. J. Biochem. Cell Biol.* **42**, 421-424.
- Pevny, L. and Placzek, M. (2005). SOX genes and neural progenitor identity. *Curr. Opin. Neurobiol.* **15**, 7-13.
- Phochanukul, N. and Russell, S. (2010). No backbone but lots of Sox: Invertebrate Sox genes. *Int. J. Biochem. Cell Biol.* **42**, 453-464.
- Pozniak, C. D., Langseth, A. J., Dijkgraaf, G. J., Choe, Y., Werb, Z. and Pleasure, S. J. (2010). Sox10 directs neural stem cells toward the oligodendrocyte lineage by decreasing Suppressor of Fused expression. *Proc. Natl. Acad. Sci. USA* **107**, 21795-21800.
- Pulgilla, C., Dabdoub, A., Brenowitz, S. D. and Kelley, M. W. (2010). Sox2 induces neuronal formation in the developing mammalian cochlea. *J. Neurosci.* **30**, 714-722.
- Que, J., Okubo, T., Goldenring, J. R., Nam, K. T., Kurotani, R., Morrisey, E. E., Taranova, O., Pevny, L. H. and Hogan, B. L. (2007). Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. *Development* **134**, 2521-2531.
- Que, J., Luo, X., Schwartz, R. J. and Hogan, B. L. (2009). Multiple roles for Sox2 in the developing and adult mouse trachea. *Development* **136**, 1899-1907.
- Rajaram, N. and Kerppola, T. K. (2004). Synergistic transcription activation by Maf and Sox and their subnuclear localization are disrupted by a mutation in Maf that causes cataract. *Mol. Cell. Biol.* **24**, 5694-5709.
- Reiprich, S., Kriesch, J., Schreiner, S. and Wegner, M. (2010). Activation of Krox20 gene expression by Sox10 in myelinating Schwann cells. *J. Neurochem.* **112**, 744-754.
- Reményi, A., Lins, K., Nissen, L. J., Reinbold, R., Schöler, H. R. and Wilmanns, M. (2003). Crystal structure of a POU/HMG/DNA ternary complex suggests differential assembly of Oct4 and Sox2 on two enhancers. *Genes Dev.* **17**, 2048-2059.
- Reményi, A., Schöler, H. R. and Wilmanns, M. (2004). Combinatorial control of gene expression. *Nat. Struct. Mol. Biol.* **11**, 812-815.
- Rizzoti, K. and Lovell-Badge, R. (2007). SOX3 activity during pharyngeal segmentation is required for craniofacial morphogenesis. *Development* **134**, 3437-3448.
- Rizzoti, K., Brunelli, S., Carmignac, D., Thomas, P. Q., Robinson, I. C. and Lovell-Badge, R. (2004). SOX3 is required during the formation of the hypothalamo-pituitary axis. *Nat. Genet.* **36**, 247-255.
- Saint-Germain, N., Lee, Y. H., Zhang, Y., Sargent, T. D. and Saint-Jeannet, J. P. (2004). Specification of the otic placode depends on Sox9 function in *Xenopus*. *Development* **131**, 1755-1763.
- Sakai, D., Suzuki, T., Osumi, N. and Wakamatsu, Y. (2006). Cooperative action of Sox9, Snail2 and PKA signaling in early neural crest development. *Development* **133**, 1323-1333.
- Sakamoto, Y., Hara, K., Kanai-Azuma, M., Matsui, T., Miura, Y., Tsunekawa, N., Kurohmaru, M., Saijoh, Y., Koopman, P. and Kanai, Y. (2007). Redundant roles of Sox17 and Sox18 in early cardiovascular development of mouse embryos. *Biochem. Biophys. Res. Commun.* **360**, 539-544.
- Sandberg, M., Källström, M. and Muhr, J. (2005). Sox21 promotes the progression of vertebrate neurogenesis. *Nat. Neurosci.* **8**, 995-1001.
- Sato, N., Kamachi, Y., Kondoh, H., Shima, Y., Morohashi, K., Horikawa, R. and Ogata, T. (2007). Hypogonadotropic hypogonadism in an adult female with a heterozygous hypomorphic mutation of SOX2. *Eur. J. Endocrinol.* **156**, 167-171.
- Schepers, G. E., Teasdale, R. D. and Koopman, P. (2002). Twenty pairs of sox: extent, homology, and nomenclature of the mouse and human sox transcription factor gene families. *Dev. Cell* **3**, 167-170.
- Schilham, M. W., Oosterwegel, M. A., Moerer, P., Ya, J., de Boer, P. A., van de Wetering, M., Verbeek, S., Lamers, W. H., Kruijsbeek, A. M., Cumano, A. et al. (1996). Defects in cardiac outflow tract formation and pro-B-lymphocyte expansion in mice lacking Sox-4. *Nature* **380**, 711-714.
- Schlosser, G., Awtry, T., Brugmann, S. A., Jensen, E. D., Neilson, K., Ruan, G., Stammler, A., Voelker, D., Yan, B., Zhang, C. et al. (2008). Eya1 and Six1 promote neurogenesis in the cranial placodes in a SoxB1-dependent fashion. *Dev. Biol.* **320**, 199-214.
- Schnetz, M. P., Handoko, L., Akhtar-Zaidi, B., Bartels, C. F., Pereira, C. F., Fisher, A. G., Adams, D. J., Flicek, P., Crawford, G. E., Laframboise, T. et al. (2010). CHD7 targets active gene enhancer elements to modulate ES cell-specific gene expression. *PLoS Genet.* **6**, e1001023.
- Scott, C. E., Wynn, S. L., Sesay, A., Cruz, C., Cheung, M., Gomez Gaviro, M. V., Booth, S., Gao, B., Cheah, K. S., Lovell-Badge, R. et al. (2010). SOX9 induces and maintains neural stem cells. *Nat. Neurosci.* **13**, 1181-1189.
- Sekido, R. (2010). SRY: A transcriptional activator of mammalian testis determination. *Int. J. Biochem. Cell Biol.* **42**, 417-420.
- Sekido, R. and Lovell-Badge, R. (2008). Sex determination involves synergistic action of SRY and SF1 on a specific Sox9 enhancer. *Nature* **453**, 930-934.
- Shih, Y. H., Kuo, C. L., Hirst, C. S., Dee, C. T., Liu, Y. R., Laghari, Z. A. and Scotting, P. J. (2010). SoxB1 transcription factors restrict organizer gene expression by repressing multiple events downstream of Wnt signalling. *Development* **137**, 2671-2681.
- Sinclair, A. H., Berta, P., Palmer, M. S., Hawkins, J. R., Griffiths, B. L., Smith, M. J., Foster, J. W., Frischauf, A. M., Lovell-Badge, R. and Goodfellow, P. N. (1990). A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* **346**, 240-244.
- Smith, A. N., Miller, L. A., Radice, G., Ashery-Padan, R. and Lang, R. A. (2009). Stage-dependent modes of Pax6-Sox2 epistasis regulate lens development and eye morphogenesis. *Development* **136**, 2977-2985.
- Smits, P., Li, P., Mandel, J., Zhang, Z., Deng, J. M., Behringer, R. R., de Crombrughe, B. and Lefebvre, V. (2001). The transcription factors L-Sox5 and Sox6 are essential for cartilage formation. *Dev. Cell* **1**, 277-290.
- Sock, E., Pagon, R. A., Keymolen, K., Lissens, W., Wegner, M. and Scherer, G. (2003). Loss of DNA-dependent dimerization of the transcription factor SOX9 as a cause for campomelic dysplasia. *Hum. Mol. Genet.* **12**, 1439-1447.
- Sock, E., Rettig, S. D., Enderich, J., Bösl, M. R., Tamm, E. R. and Wegner, M. (2004). Gene targeting reveals a widespread role for the high-mobility-group transcription factor Sox11 in tissue remodeling. *Mol. Cell. Biol.* **24**, 6635-6644.
- Srivastava, M., Begovic, E., Chapman, J., Putnam, N. H., Hellsten, U., Kawashima, T., Kuo, A., Mitros, T., Salamov, A., Carpenter, M. L. et al. (2008). The Trichoplax genome and the nature of placozoans. *Nature* **454**, 955-960.
- Stolt, C. C. and Wegner, M. (2010). SoxE function in vertebrate nervous system development. *Int. J. Biochem. Cell Biol.* **42**, 437-440.
- Stolt, C. C., Rehberg, S., Ader, M., Lommes, P., Riethmacher, D., Schachner, M., Bartsch, U. and Wegner, M. (2002). Terminal differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10. *Genes Dev.* **16**, 165-170.
- Stolt, C. C., Lommes, P., Sock, E., Chaboissier, M. C., Schedl, A. and Wegner, M. (2003). The Sox9 transcription factor determines glial fate choice in the developing spinal cord. *Genes Dev.* **17**, 1677-1689.
- Stolt, C. C., Schmitt, S., Lommes, P., Sock, E. and Wegner, M. (2005). Impact of transcription factor Sox8 on oligodendrocyte specification in the mouse embryonic spinal cord. *Dev. Biol.* **281**, 309-317.
- Stolt, C. C., Schlierf, A., Lommes, P., Hillgärtner, S., Werner, T., Kosian, T., Sock, E., Kessar, N., Richardson, W. D., Lefebvre, V. et al. (2006). SoxD proteins influence multiple stages of oligodendrocyte development and modulate SoxE protein function. *Dev. Cell* **11**, 697-709.
- Suh, H., Consiglio, A., Ray, J., Sawai, T., D'Amour, K. A. and Gage, F. H. (2007). In vivo fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippocampus. *Cell Stem Cell* **1**, 515-528.



- Takahashi, K. and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676.
- Takemoto, T., Uchikawa, M., Yoshida, M., Bell, D. M., Lovell-Badge, R., Papaioannou, V. E. and Kondoh, H. (2011). Tbx6-dependent Sox2 regulation determines neural or mesodermal fate in axial stem cells. *Nature* **470**, 394-398.
- Tanaka, S., Kamachi, Y., Tanouchi, A., Hamada, H., Jing, N. and Kondoh, H. (2004). Interplay of SOX and POU factors in regulation of the Nestin gene in neural primordial cells. *Mol. Cell. Biol.* **24**, 8834-8846.
- Taylor, K. M. and Labonne, C. (2005). SoxE factors function equivalently during neural crest and inner ear development and their activity is regulated by SUMOylation. *Dev. Cell* **9**, 593-603.
- Thevenet, L., Méjean, C., Moniot, B., Bonneaud, N., Galéotti, N., Aldrian-Herrada, G., Poulat, F., Berta, P., Benkirane, M. and Boizet-Bonhoure, B. (2004). Regulation of human SRY subcellular distribution by its acetylation/deacetylation. *EMBO J.* **23**, 3336-3345.
- Tomioka, M., Nishimoto, M., Miyagi, S., Katayanagi, T., Fukui, N., Niwa, H., Muramatsu, M. and Okuda, A. (2002). Identification of Sox-2 regulatory region which is under the control of Oct-3/4-Sox-2 complex. *Nucleic Acids Res.* **30**, 3202-3213.
- Tsuruzoe, S., Ishihara, K., Uchimura, Y., Watanabe, S., Sekita, Y., Aoto, T., Saitoh, H., Yuasa, Y., Niwa, H., Kawasuji, M. et al. (2006). Inhibition of DNA binding of Sox2 by the SUMO conjugation. *Biochem. Biophys. Res. Commun.* **351**, 920-926.
- Uchikawa, M., Kamachi, Y. and Kondoh, H. (1999). Two distinct subgroups of Group B Sox genes for transcriptional activators and repressors: their expression during embryonic organogenesis of the chicken. *Mech. Dev.* **84**, 103-120.
- Uchikawa, M., Yoshida, M., Iwafuchi-Doi, M., Matsuda, K., Ishida, Y., Takemoto, T. and Kondoh, H. (2011). B1 and B2 Sox gene expression during neural plate development in chicken and mouse embryos: universal versus species-dependent features. *Dev. Growth Differ.* **53**, 761-771.
- Uwanogho, D., Rex, M., Cartwright, E. J., Pearl, G., Healy, C., Scotting, P. J. and Sharpe, P. T. (1995). Embryonic expression of the chicken Sox2, Sox3 and Sox11 genes suggests an interactive role in neuronal development. *Mech. Dev.* **49**, 23-36.
- Van Hoof, D., Muñoz, J., Braam, S. R., Pinkse, M. W., Linding, R., Heck, A. J., Mummery, C. L. and Krijgsveld, J. (2009). Phosphorylation dynamics during early differentiation of human embryonic stem cells. *Cell Stem Cell* **5**, 214-226.
- Vergier, A. and Duterque-Coquillaud, M. (2002). When Ets transcription factors meet their partners. *BioEssays* **24**, 362-370.
- Vidal, V. P., Chaboissier, M. C., Lutzkendorf, S., Cotsarelis, G., Mill, P., Hui, C. C., Ortonne, N., Ortonne, J. P. and Schedl, A. (2005). Sox9 is essential for outer root sheath differentiation and the formation of the hair stem cell compartment. *Curr. Biol.* **15**, 1340-1351.
- Wagner, T., Wirth, J., Meyer, J., Zabel, B., Held, M., Zimmer, J., Pasantes, J., Bricarelli, F. D., Keutel, J., Hustert, E. et al. (1994). Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. *Cell* **79**, 1111-1120.
- Wat, M. J., Beck, T. F., Hernández-García, A., Yu, Z., Veenma, D., García, M., Holder, A. M., Wat, J. J., Chen, Y., Mohila, C. A. et al. (2012). Mouse model reveals the role of SOX7 in the development of congenital diaphragmatic hernia associated with recurrent deletions of 8p23.1. *Hum. Mol. Genet.* **21**, 4115-4125.
- Weiss, J., Meeks, J. J., Hurley, L., Raverot, G., Frassetto, A. and Jameson, J. L. (2003). Sox3 is required for gonadal function, but not sex determination, in males and females. *Mol. Cell. Biol.* **23**, 8084-8091.
- Wiebe, M. S., Nowling, T. K. and Rizzino, A. (2003). Identification of novel domains within Sox-2 and Sox-11 involved in autoinhibition of DNA binding and partnership specificity. *J. Biol. Chem.* **278**, 17901-17911.
- Wilhelm, D., Hiramatsu, R., Mizusaki, H., Widjaja, L., Combes, A. N., Kanai, Y. and Koopman, P. (2007). SOX9 regulates prostaglandin D synthase gene transcription in vivo to ensure testis development. *J. Biol. Chem.* **282**, 10553-10560.
- Williamson, K. A., Hever, A. M., Rainger, J., Rogers, R. C., Magee, A., Fiedler, Z., Keng, W. T., Sharkey, F. H., McGill, N., Hill, C. J. et al. (2006). Mutations in SOX2 cause anophthalmia-esophageal-genital (AEG) syndrome. *Hum. Mol. Genet.* **15**, 1413-1422.
- Wood, H. B. and Episkopou, V. (1999). Comparative expression of the mouse Sox1, Sox2 and Sox3 genes from pre-gastrulation to early somite stages. *Mech. Dev.* **86**, 197-201.
- Xu, J., Watts, J. A., Pope, S. D., Gadue, P., Kamps, M., Plath, K., Zaret, K. S. and Smale, S. T. (2009a). Transcriptional competence and the active marking of tissue-specific enhancers by defined transcription factors in embryonic and induced pluripotent stem cells. *Genes Dev.* **23**, 2824-2838.
- Xu, N., Papagiannakopoulos, T., Pan, G., Thomson, J. A. and Kosik, K. S. (2009b). MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. *Cell* **137**, 647-658.
- Yuan, H., Corbi, N., Basilico, C. and Dailey, L. (1995). Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. *Genes Dev.* **9**, 2635-2645.
- Zhao, Q., Eberspaecher, H., Lefebvre, V. and De Crombrughe, B. (1997). Parallel expression of Sox9 and Col2a1 in cells undergoing chondrogenesis. *Dev. Dyn.* **209**, 377-386.
- Zhao, X., He, X., Han, X., Yu, Y., Ye, F., Chen, Y., Hoang, T., Xu, X., Mi, Q. S., Xin, M. et al. (2010). MicroRNA-mediated control of oligodendrocyte differentiation. *Neuron* **65**, 612-626.
- Zhao, H. Y., Zhang, Y. J., Dai, H., Zhang, Y. and Shen, Y. F. (2011). CARM1 mediates modulation of Sox2. *PLoS ONE* **6**, e27026.
- Zhao, L., Zavallos, S. E., Rizzotti, K., Jeong, Y., Lovell-Badge, R. and Epstein, D. J. (2012). Disruption of SoxB1-dependent Sonic hedgehog expression in the hypothalamus causes septo-optic dysplasia. *Dev. Cell* **22**, 585-596.
- Zhou, G., Zheng, Q., Engin, F., Munivez, E., Chen, Y., Sebald, E., Krakow, D. and Lee, B. (2006). Dominance of SOX9 function over RUNX2 during skeletogenesis. *Proc. Natl. Acad. Sci. USA* **103**, 19004-19009.