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**Abstract**

Sex determining region Y-box 2 (Sox2), a member of the SoxB1 transcription factor family, is an important transcriptional regulator in pluripotent stem cells (PSCs). Together with octamer-binding transcription factor 4 and Nanog, they co-operatively control gene expression in PSCs and maintain their pluripotency. Furthermore, Sox2 plays an essential role in somatic cell reprogramming, reversing the epigenetic configuration of differentiated cells back to a pluripotent embryonic state. In addition to its role in regulation of pluripotency, Sox2 is also a critical factor for directing the differentiation of PSCs to neural progenitors and for maintaining the properties of neural progenitor stem cells. Here, we review recent findings concerning the involvement of Sox2 in pluripotency, somatic cell reprogramming and neural differentiation as well as the molecular mechanisms underlying these roles.

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**Key words:** Sex determining region Y-box 2; Pluripotent stem cells; Pluripotency; Neural differentiation; Reprogramming

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**Core tip:** Sex determining region Y-box 2 (Sox2) plays important roles in pluripotent stem cells, not only for maintaining their pluripotency but also for directing their neural differentiation. There have been many intensive studies in the last decade, which serve to ascertain the function of Sox2 in these processes. In this review, we have summarized the recent progress made regarding the involvement of Sox2 in pluripotency, somatic cell reprogramming and neural differentiation as well as the molecular mechanisms underlying these roles.

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**INTRODUCTION**

Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), possess two important properties: indefinite self-renewal in culture and the ability to generate most, if not all, cell types in the human body *via* differentiation into one of the three embryonic germ layers<sup>[1,2]</sup>. These unique properties of hPSCs make them an invaluable cell resource not only for regenerative medicine, disease modelling and drug development, but also for the study of early human development, serving as a cell model to elucidate the molecular mechanisms regulating embryonic cell proliferation and differentiation. Understanding the mechanisms underlying the self-renewal and differentiation of hPSCs is fundamentally important for the subsequent utilization of these cells. In the past

decade, increasing evidence has shown that cell fate determination of a pluripotent stem cell, either maintaining pluripotency or differentiating into one of the three germ layers, is controlled by both extrinsic and intrinsic factors<sup>[5]</sup>. Intrinsic factors refer mainly to transcription factors that play an essential role in the direct control of gene expression in cells, while extrinsic factors, including growth factors, extracellular matrices and cytokines, have considerable effects on expression levels of intrinsic transcription factors through various signalling pathways. The core intrinsic factors for regulating pluripotency have been identified as octamer-binding transcription factor 4 (Oct4), sex determining region Y-box 2 (Sox2) and Nanog<sup>[4,5]</sup>, while Oct4 and Sox2 are also proposed as lineage specifiers to regulate mesendoderm and ectoderm differentiation, respectively<sup>[6,7]</sup>. Thus, Sox2 is one of the critical factors that control both pluripotency and neural differentiation of hPSCs.

In this review, we place particular emphasis on the biological functions of Sox2 in regulating pluripotency and early neural differentiation of ESCs and summarize the recent findings on the role that Sox2 plays in the regulation of PSC fate.

## SOX2 IS INDISPENSIBLE DURING EARLY EMBRYONIC DEVELOPMENT

Sox2 is a member of the Sox family of transcription factors. The Sox gene family was first defined by the discovery of the mammalian testis-determining factor, Sry<sup>[8,9]</sup>. Proteins of the Sox family all share a highly conserved high-mobility-group (HMG) DNA binding domain. To date, 20 different Sox genes have been identified in mouse and human<sup>[10]</sup>, which are divided into subgroups, according to the degree of homology within the HMG domain and other structural motifs. Sox2 is classified as a member of SoxB1 group, which also includes Sox1 and Sox3. Although Sox1, Sox2 and Sox3 share more than 80% sequence similarity and are functionally redundant, Sox2 can exert distinct functions in a biologically context-dependent manner and is indispensable for embryonic development. Many factors have been shown to influence binding of Sox proteins to their target genes, leading to diverse functional effects. One such factor is the interaction between Sox proteins and various cofactors. Interaction with various cofactors confers upon Sox2 greater functional versatility during developmental processes<sup>[11]</sup>.

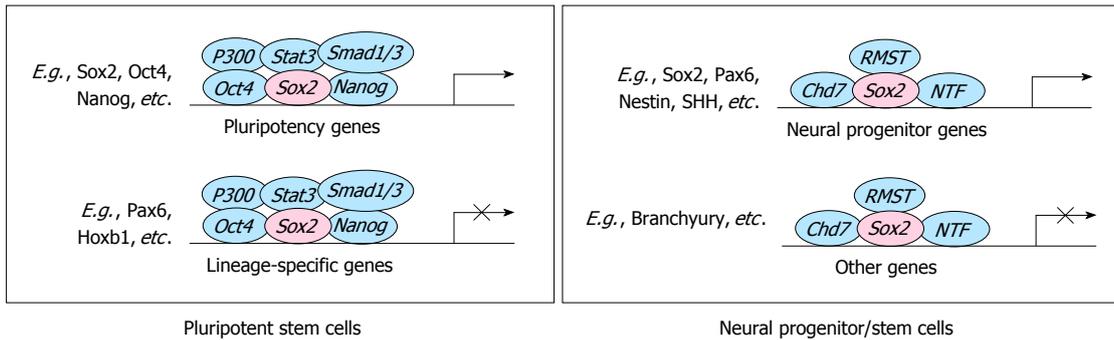
During mouse embryogenesis, a totipotent zygote undergoes cleavage to increase the cell number and the resulting multi-cellular morula further develops to form the blastocyst, in which the cells, for the first time, appear to acquire spatially derived identities, segregating into the inner cell mass (ICM) and trophectoderm. Cells in the ICM give rise to the embryo proper, differentiating into all cell types found within the body and are thus classified as pluripotent. Conversely, trophoblast cells develop into placental tissues, assisting with implantation and nourishment of the embryo during development. Sox2

expression is initially detected in cells at the morula stage, becoming more specifically located in the ICM of blastocyst and epiblast<sup>[12]</sup> during the latter stages. This implies that Sox2 may have important roles in the formation of early pluripotent embryonic cells. Indeed, zygotic deletion of Sox2 is embryonically lethal due to the failure to form pluripotent epiblast whilst the absence of Sox2 has little effect on the formation of trophectoderm<sup>[12]</sup>. Therefore, Sox2 is an essential factor in the formation of pluripotent cells in early embryos and ultimately an critical factor for embryonic development.

## CRITICAL ROLE OF SOX2 IN MAINTAINING PLURIPOTENCY OF ESCS AND GENERATION OF IPSCS

ESCs, derived from the ICM of preimplantation embryos, share many characteristics with the ICM cells. One major similarity is their pluripotent capability, being able to give rise to all cell types of the adult body. However, ESCs are not identical to the cells in the ICM as ESCs are able to amplify themselves during extended culture without compromising their pluripotency. Consistent with the data in pre-implantation embryos, Sox2 is highly expressed in ESCs. Depletion of Sox2 by either gene-knockout or RNA interference considerably compromises the pluripotent state of both mouse and human ESCs as shown by the changes in cell morphology, loss of pluripotent marker expression and their differentiation primarily into trophectoderm<sup>[2,13]</sup>. However, forced expression of Oct4 in Sox2-null mouse ESCs (mESCs) can rescue the pluripotency of these cells, indicating that the role of Sox2 in maintaining the pluripotent state of ESCs is primarily to sustain a sufficient level of Oct4 expression<sup>[2,13]</sup>. Collectively, these results demonstrate that Sox2 is crucial in the maintenance of pluripotent ESCs, possibly through promoting and maintaining Oct4 expression.

Interestingly, to maintain pluripotency of stem cells, levels of Sox2 expression need to be stringently regulated, with either higher or lower Sox2 expression leading to the loss of pluripotency in ESCs. This could be attributed to the fact that both low and high levels of Sox2 reduce the promoter/enhancer activity of Sox2-Oct4 target genes<sup>[14,15]</sup>. The expression level of Sox2 needs to be retained in a dynamic equilibrium with other synergistic factors in order to maintain pluripotency. This concept is also supported by the finding that Sox2 cooperates with other highly dose-dependent transcription factors, such as Oct4 and Nanog, in the regulation of pluripotency<sup>[4]</sup>. In human and mouse ESCs, Oct4, Sox2 and Nanog form a core transcriptional regulatory circuitry in pluripotent stem cells to maintain their self-renewal. Oct4 and Sox2 co-occupy a large number of enhancers/promoters and regulate the expression levels of their target genes (Figure 1). They activate the expression of pluripotent genes, including Nanog and themselves, whilst repressing the ex-



**Figure 1 Sex determining region Y-box 2 regulates gene expression in pluripotent stem cells and neural progenitor/stem cells.** Sex determining region Y-box 2 (Sox2) interacts with Oct4 and other nuclear factors to activate pluripotent gene expression and repress differentiation gene expression in pluripotent stem cells. In neural progenitor/stem cells, Sox2 interacts with neural transcription factors, such as Brn2 and Pax6, to activate neural progenitor gene expression. Oct4: Octamer-binding transcription factor 4; Pax6: Paired-box protein 6.

pression of key genes that are responsible for the *in vitro* differentiation and *in vivo* developmental processes, such as paired-box protein 6 (Pax6) and gastrulation brain homeobox 2<sup>[4,5]</sup>. These transcriptional regulatory activities of Sox2 and Oct4 have been shown to require their direct interaction<sup>[6]</sup>. The cooperative interaction between HMG of Sox2 and pit-oct-unc (POU) homeodomain of Oct4 is thought to be critical in regulation of ESC pluripotency<sup>[17]</sup>.

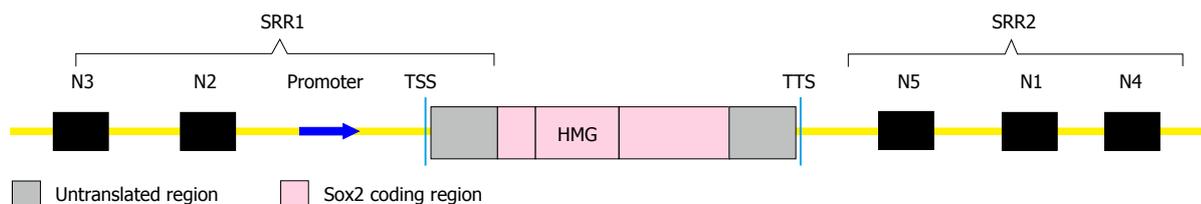
iPSCs are generated from various somatic cell types by ectopically expressing transcription factors that are important for ESC pluripotency. The most commonly used factors are Oct4, Sox2, Kruppel-like factor 4 (Klf4) and c-Myc<sup>[18]</sup>. These factors are able to reprogram the somatic cells back to their embryonic state, making them share the dual properties of pluripotency and long-term self-renewal much like their ESC counterparts. Given that Sox2 is essential in the maintenance of pluripotency in ESCs, it is conceivable that Sox2 is one of the key factors for the generation of iPSCs<sup>[18]</sup>. In fact, by analysing gene expression profile on a single cell level during reprogramming, it has been found that the activation of endogenous Sox2 is a relative early event, which initiates a cascade of transcriptional changes, leading to the formation of iPSCs<sup>[19]</sup>. Interestingly, based on the shared biological properties between *SoxB1* genes, Sox2 can be replaced by closely related Sox family members, Sox1 and Sox3, in the generation of iPSCs, but not by more distant members, like Sox7 and Sox15<sup>[20]</sup>. However, it has been reported that Sox17 is able to replace Sox2 in the successful generation of iPSCs after it is genetically modified in which two amino acids in the Sox17 HMG domain are converted to those of Sox2. This conversion does not alter Sox17 HMG DNA binding motif but confers its ability to interact with Oct4. The modified Sox17 is able to interact with Oct4 and the resulting Sox17-Oct4 complex can cooperatively bind to the canonical subset of Sox-Oct motifs and successfully reprogram somatic cells<sup>[21]</sup>. Taken together, Sox2 is therefore important for the successful reprogramming of somatic cells to iPSCs. As such, the physical interaction between Sox2 and Oct4 is likely to be critical in induction and maintenance of

pluripotency.

## SOX2 IN NEURAL DIFFERENTIATION AND MAINTENANCE OF NEURAL PROGENITOR/STEM CELLS

During embryonic development, Sox2 is persistently expressed, initially in the epiblast of preimplantation embryos, then more predominantly in the central nervous system after gastrulation, hinting at a possible function for Sox2 in neural commitment<sup>[22]</sup>. Recently, it has been suggested that the three core pluripotent transcription factors Sox2, Oct4 and Nanog not only play an important role in the induction and maintenance of pluripotency, but also in functioning as lineage specifiers, regulating the differentiation of ESCs to specific lineages<sup>[6,23]</sup>. Sox2 governs ESC specification to neuroectoderm while Oct4 and Nanog promote their differentiation to mesoderm, a common precursor of mesoderm and definitive endoderm<sup>[7]</sup>. Sox2 induces neural induction and enhances neural differentiation by repressing key regulators of other lineage fates, for example brachyury<sup>[7,23,24]</sup>. Therefore, Sox2 appears to be an important regulator in controlling PSC neural initiation and differentiation.

In addition to its role in regulating neural induction, Sox2 also functions to maintain the self-renewal of neural progenitor stem cells *in vitro* as well as *in vivo*. Sox2 is highly expressed in proliferating neural progenitor cells (NPCs) and is downregulated upon differentiation to post-mitotic neuronal and glial cells. Reduction of Sox2 in neural progenitor stem cells hinders their self-renewal and proliferation, promoting their earlier exit from cell cycle and terminal differentiation; whereas ectopic expression of Sox2 inhibits the differentiation of NPCs into neurons and glia<sup>[25,26]</sup>. The fact that ectopic expression of Sox2 alone or in combination with other neural transcription factors can directly reprogram fibroblasts to multipotent neural progenitor stem cells further highlights the essential role Sox2 plays in these cells<sup>[27,28]</sup>. Taken together, Sox2 is therefore a key factor in both the establishment and maintenance of neural progenitor



**Figure 2** Transcriptional expression of sex determining region Y-box 2 is regulated by multiple enhancers located in the sex determining region Y-box 2 locus. The sex determining region Y-box 2 (*Sox2*) gene locus is illustrated in yellow box, in which *Sox2* exon and location of the N1 to N5 enhancers are indicated. TSS and TTS represent transcription starting and termination sites, respectively. SRR1 and 2 are *Sox2* regulatory regions 1 and 2.

properties.

## MOLECULAR MECHANISMS UNDERLYING THE ROLES OF SOX2 IN PLURIPOTENCY AND NEURAL DIFFERENTIATION

In the last decade, intensive studies have been carried out in order to elucidate the molecular mechanisms that control pluripotency and lineage specification. Although considerable progress has been made, the mechanisms are still not fully understood. Given that *Sox2* functions in both PSCs and NPCs, it is thought that stringently regulated *Sox2* expression is necessary to govern both pluripotency and initiation of neural differentiation in PSCs. Furthermore, differentially orchestrated mechanisms are required to control distinct functions of *Sox2* in self-renewal of PSCs and during their neural differentiation.

Like other transcription factors, the expression of *Sox2* is regulated by both intrinsic factors and extrinsic signalling pathways. It has been identified that several regulatory regions in the *Sox2* locus are responsible for controlling *Sox2* expression, which include *Sox2* core promoter<sup>[29]</sup> and a number of enhancers located both upstream and downstream of the *Sox2* gene (Figure 2)<sup>[30-33]</sup>. All of these regulatory regions are highly conserved across species, responding to different factors and signalling pathways<sup>[30]</sup>. In ESCs, several laboratories have clearly demonstrated that *Sox2* interacts with Oct4 to form a regulatory complex, which binds to *Sox2* regulatory region 2 to activate *Sox2* transcription<sup>[4,5,34,35]</sup>, indicating that *Sox2* is positively auto-regulated by the *Sox2*-Oct4 complex in ESCs. In addition to Oct4, several other transcriptional factors, including Nanog, mothers against decapentaplegic homolog 1 (*Smad1*) and signal transducer and activator of transcription 3 (*Stat3*), are also identified to be involved in the formation of the autoregulatory complex in mESCs, which activate *Sox2* as well as other pluripotent genes<sup>[5]</sup>. In this complex, *Stat3* and *Smad1*, which are the key components of the bone morphogenetic protein and leukemia inhibitory factor signalling pathways of mESCs, allow the core transcriptional network integrated into external signalling pathways of mESCs. In hESCs, *Sox2*-Oct4 complex co-occupies their target genes with mothers against decapentaplegic homolog 3 (*Smad3*) protein, a downstream effector of the

transforming growth factor beta signalling pathway which is required for hESC maintenance<sup>[36]</sup>. In NPCs, *Sox2* expression is promoted by transcriptional factors that are highly expressed during neural development and differentiation, such as activating protein 2, prospero homeobox protein 1 and *Pax6*<sup>[29]</sup>. Signalling pathways, such as phosphatidylinositol-4,5-bisphosphate 3-kinase/Akt and *Stat3*, also function to regulate *Sox2* expression<sup>[37,38]</sup>. Recently, cell cycle regulators *E2f3a* and *E2f3b* have been reported to regulate *Sox2* expression and control neural progenitor cell proliferation in adult brain<sup>[39,40]</sup>. *E2f3a* and *E2f3b* are shown to differentially regulate *Sox2* expression in neural progenitor cells, thus affecting adult neurogenesis<sup>[39]</sup>. *E2f3a* cooperates with the pRb family member p107 to repress *Sox2* expression, reducing neural progenitor self-renewal and promoting terminal differentiation, whereas *E2f3b* activates *Sox2* expression by recruiting RNA polymerase II to its promoter, leading to increased self-renewal and neural progenitor/stem cell expansion. Cyclin-dependent kinase inhibitor P21 has also been found to directly bind to a *Sox2* enhancer and repress *Sox2* expression in NPCs<sup>[40]</sup>. The various enhancers/regulatory regions of *Sox2* work together to stringently regulate the expression of *Sox2* from early preimplantation embryos to various neural progenitor cells<sup>[30,35,41]</sup>.

Other than transcriptional regulation, *Sox2* expression and activity are also regulated by post-transcriptional and translational mechanisms. MicroRNA-145 has been demonstrated to negatively affect the expression of pluripotent transcription factors, including Oct4, *Sox2* and *Klf4*<sup>[42]</sup>. *Sox2* protein can be modulated by methylation, acetylation, sumoylation and phosphorylation, which subsequently affect its activities as a transcriptional regulator. Three phosphorylation sites, S249, S250 and S251, have been identified in *Sox2*, the phosphorylation of which promotes sumoylation of *Sox2*, subsequently inhibiting the binding of *Sox2* to DNA motifs<sup>[43,44]</sup>. Acetylation of *Sox2* by a histone acetyltransferase, p300, induces its nuclear export in ESCs, leading to increased ubiquitination and proteasomal degradation of *Sox2* protein<sup>[45]</sup>.

*Sox2* regulates distinct target genes in pluripotent ESCs and during neural differentiation. Although the exact mechanisms that govern its selection on target genes are not fully elucidated, transcription factors that function as *Sox2* interacting partners may play an important role in this selection. *Sox2* is similar to all *Sox* family members, in that achieving their regulatory functions requires

**Table 1** Sex determining region Y-box 2-pairing partners and their functions

Sox2 binding partner	Species	Function	Target genes	Ref.
Oct3/4	Human, mouse	Maintain pluripotency in ES and repress genes involved in developmental process	<i>Sox2, Oct3/4, Nanog, Egf4, Utl1, FbX15</i>	[4,34,35]
Pax6	Chicken, mouse	Initiate lens development	<i>Sox2, Pax6, delta crystallin</i>	[42,47]
Brn2	Mouse	Regulate <i>Nestin</i> gene in neural primordial cells	<i>Sox2, Nestin</i>	[48]
Oct1	Mouse	Regulate Pax6 expression which is required for the lens and olfactory placode development	<i>Sox2, Pax6</i>	[49]
Chd7	Mouse	Involved in the regulation of neural stem cells	<i>Jag1, Gli3, Mycn</i>	[50]

Sox2: Sex determining region Y-box 2; Oct3/4: Octamer-binding transcription factor 3/4; Pax6: Paired-box protein 6.

pairing and coordination with other transcription factors to form complexes<sup>[46]</sup>. The Sox transcription factor and its interacting partner bind to adjacent DNA sequences in promoter/enhancer of target genes to regulate their expression. Several Sox2 partners have been identified in various cell types (Table 1)<sup>[4,34,35,41,47-50]</sup>. The most studied Sox2 partner is Oct4 in PSCs. As discussed earlier, Sox2 has been shown to interact directly with Oct4 in PSCs and the Sox2-Oct4 complex binds to adjacent DNA motifs located in the enhancer/promoter regions of thousands of genes genome-wide to either activate or repress the expression of these genes. They cooperatively activate pluripotent genes whilst repressing lineage-specific ones, hence maintaining pluripotency in these cells (Figure 1)<sup>[4,34,35]</sup>. However, it is less clear which transcription factors serve as the Sox2 binding partners during the neural differentiation of PSCs. In early murine neural progenitors, Sox2 is shown to interact with the brain-specific POU domain-containing transcription factor Brn2 to activate the NPC-associated *Nestin* gene expression<sup>[48]</sup>. During lens development, Sox2 and Pax6 form a complex which binds to lens-specific enhancer elements to initiate lens development<sup>[41]</sup>. Recently, it is also reported that Sox2 is able to interact with long non-coding RNA rhabdomyosarcoma 2 associated transcript to activate the expression of their neural target genes and to promote neural differentiation<sup>[51]</sup>. It is possible that Sox2 requires different partners in different neural progenitor cells, which regulate expression of different gene sets, leading to the formation of different neural cell types.

## CONCLUSION

Sox2 is one of the key transcription factors that play an essential role in maintaining pluripotency of stem cells. Sox2 interacts with Oct4 to form a binary complex, which then recruits other nuclear factors to activate pluripotent gene expression and repress genes involved in differentiation. Furthermore, Sox2 is also a critical factor for initiating the neural induction and maintaining neural progenitor stem cell properties throughout neural differentiation. Recently, it has been reported that Sox2 is expressed in adult stem cells of several epithelial tissues and regulates trophoblast stem cell differentiation<sup>[47,52]</sup>. However, how Sox2 achieves these pleiotropic functions remains to be elucidated. Sox2, like other Sox family members, performs

its regulatory functions more efficiently when paired with an interacting partner. Although Oct4 has been well demonstrated as being such a partner in pluripotent stem cells, the identities of Sox2 partners in other tissues are largely unknown. Understanding the molecular mechanisms governing Sox2 functions will facilitate the use of pluripotent stem cells for clinical and biomedical applications, with particular relevance to the modelling and treatment of various neurological disorders.

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