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Sox6, Jack of all trades: A versatile regulatory protein in vertebrate development

Nobuko Hagiwara, Ph.D.

Division of Cardiovascular Medicine, Department of Internal Medicine, University of California, Davis, One Shields Ave., Davis, CA 95616, (530) 792-0389

Nobuko Hagiwara: nhagiwara@ucdavis.edu

Abstract

Approximately twenty thousand genes are encoded in our genome, one tenth of which are thought to be transcription factors. Considering the complexity and variety of cell types generated during development, many transcription factors likely play multiple roles. Uncovering the versatile roles of Sox6 in vertebrate development sheds some light on how an organism efficiently utilizes the limited resources of transcription factors. The structure of the Sox6 gene itself may dictate its functional versatility. First, Sox6 contains no known regulatory domains; instead, it utilizes various cofactors. Second, Sox6 has a long 3'-UTR that contains multiple microRNA targets, thus its protein level is duly adjusted by cell type-specific microRNAs. Just combining these two characteristics alone makes Sox6 extremely versatile. To date, Sox6 has been reported to regulate differentiation of tissues of mesoderm, ectoderm, and endoderm origins, making Sox6 a truly multifaceted transcription factor.

Keywords

Sox6; Sox family of transcription factors; terminal differentiation; vertebrate development

Introduction

When we embark on assigning specific roles to individual genes, gene families present a challenging task because of their often hard-to-define functional redundancies. Teasing out specific roles for individual members of a gene family is made difficult because gene families evolve through the process of repeated gene duplications and subsequent gene loss. Therefore, the more recent the duplication event (i.e. younger family members), the greater the likelihood that functional redundancy will be seen. In the Sox gene family, Sox6 is one of these young bloods. The Sox family of transcription factors currently consists of about a dozen genes in invertebrates and 20 genes in vertebrates, relative to the complexity of the body plan (Bowels et al. 2000; Schepers et al. 2002; Phochanukul and Russell 2010). Vertebrate Sox genes have been grouped into eight groups (A-H) based on similarities in their amino acid sequences (Schepers et al. 2002). Sox6 (along with Sox5 and Sox13) belongs to the SoxD family. This family appears to have evolved from a single ancestral SoxD gene, appearing first in Bilateria (Bowels et al. 2000; Larroux et al. 2008; Phochanukul and Russell 2010). Invertebrates such as *Drosophila*, *C. elegans*, and sea urchin possess only one SoxD gene (Bowels et al. 2000; Phochanukul and Russell 2010), as does the lamprey of the taxon cyclostomata (jawless fishes) (Ohtani et al. 2008). A single copy of three members of the SoxD family (Sox5, Sox6 and Sox13) has been identified in model organisms of

gnathostoma (vertebrates with jaws) (Bowels et al. 2000), except for fugu (puffer fish) which contains two Sox6 genes (Sox6A and Sox6B) (Koopman et al. 2004). Reflecting the evolutionary proximity of the vertebrate SoxD genes, many overlapping developmental functions have been reported for Sox5 and Sox6 in multiple tissues (Lefebvre et al. 1998; Stolt et al. 2006; Lefebvre 2010). Expression of the three SoxD genes has been detected in a wide variety of vertebrate tissues (Roose et al. 1998; Hagiwara et al. 2000; Kasimiotis et al. 2000; Cohen-Barak et al. 2001; Ikeda et al. 2002), and it is likely that there are a great deal of unknown functions shared by these SoxD genes. One of the main challenges then lies in discerning between the overlapping and distinct roles this family plays during vertebrate development. In spite of this challenge, our understanding of the roles of the SoxD genes in vertebrate development is slowly but steadily growing.

Though expressed in multiple tissues throughout the life of the organisms, Sox6's role in the adult remains a mystery. Therefore, in this review I will restrict my focus to discussing the roles of the Sox6 gene during development. A picture of Sox6 as an important player for cell type specification of many cell lineages during vertebrate development is emerging. In this review, I define cell type specification (within the flow of the developmental processes) as the changes that take place during the transition from lineage specific progenitor cells to the post-mitotic, terminally differentiated, functionally mature cells. In this process of cell type specification, an important feature of Sox6's structure is utilized, namely, that there are apparently no transcriptional regulatory domains (activator or repressor) in the Sox6 protein. Accordingly, Sox6 must interact with different partner proteins in order to activate or repress gene transcription (Kamachi et al. 2000). As will be seen, this is a blessing in disguise. The presumed inability to independently regulate transcription actually brings in an incredible flexibility to the functions of Sox6, allowing it to be used in multiple tissue types at different developmental stages to effect many different results. As the developmental process becomes exponentially complex throughout evolution, the repertoires of transcription factors also have expanded to specify the increasing number of cell types and respond to more intricate cellular signaling. Rather than innovating totally new mechanisms for each new level of complexity, the pairing of transcription factors with different partner proteins at different places at different times is a more economical and swift way to achieve this task.

In the following sections, I will first briefly describe the expression patterns, structure, and identified cofactors of Sox6. I will then discuss the fascinating functions Sox6 plays in various tissues during vertebrate development based on the reports accumulated by a broad community of developmental biologists.

Expression, Protein Structure and Cofactors of Sox6

Sox6 expression is found in a wide range of tissues throughout the life cycle of the mouse. In the developing mouse embryo, by using in situ hybridization, Sox6 mRNA has been detected in the central nervous system (both brain and spinal cord), otic vesicle, somites, branchial arches, thymus, notochord, craniofacial mesenchyme, limb buds, and liver (Connor et al. 1995; Lefebvre et al. 1998; Murakami et al. 2001; Smits and Lefebvre 2003; Yi et al. 2006). In the adult mouse and human tissues, using Northern hybridization, Sox6 mRNA has been detected in tissues such as the brain, heart, lung, liver, spleen, pancreas, skeletal muscle, kidney, and testis (Hagiwara et al. 2000; Cohen-Barak et al. 2001). This widespread expression of Sox6 in adult tissues is also observed in the rainbow trout (Takamatsu et al. 1995). These observations suggest that Sox6 may have functions in the maintenance of a wide array of adult tissues, a subject which is relatively unexplored but ripe for discovery.

Structurally, subgrouping of the numerous Sox genes is determined by the similarities in their amino acid sequences (Wegner 1999; Bowels et al. 2000). The SoxD proteins (Sox5, Sox6 and Sox13) are set apart from the rest of the Sox proteins by the presence of a leucine zipper (LZ) and glutamine-rich (Q-box) domains located in the N-terminal half of the protein (Wegner 1999; Kamachi et al. 2000). In Sox6 specifically, the currently identified functional domains include one DNA binding domain (the HMG box), and two coiled-coil domains where the LZ and Q-boxes are located (Connor et al. 1995; Lefebvre et al. 1998). The more N-terminus coiled-coil domain has been shown to be involved in protein-protein interactions with multiple proteins (Lefebvre et al. 1998; Yamashita et al. 2000; Cohen-Barak et al. 2003; Iguchi et al. 2007; Ohe et al. 2009), as has, to a lesser extent, the Sox6 HMG box (Iguchi et al. 2005; 2007). The amino acid sequence of Sox6 is evolutionarily highly conserved, as exemplified by the sequence alignment of the human and zebrafish Sox6 proteins shown in Fig. 1. The characteristic structural features of Sox6 (the HMG box and the two coiled-coil domains) are all extremely well conserved between the two organisms. It is conventionally assumed that structural conservation is an indication of functional conservation. As it will be discussed later in this review in more detail, an evolutionarily conserved Sox6 function in skeletal muscle has been observed between mice and fish, with only a slight species specific difference (Hagiwara et al. 2007; von Hofsten et al. 2008). As more information about the functions of Sox6 in non-mammalian vertebrates becomes available in the future, we will be able to gain greater insight in the degrees of similarity and dissimilarity in Sox6 function in vertebrate development shaped by evolution.

As mentioned previously, it appears that for Sox6 to function as a transcription factor, it must partner with other proteins. In the search for these partners, multiple cofactors have been identified for the mammalian Sox6 proteins using yeast two hybrid screenings. Table 1 summarizes the known Sox6 cofactors to date and the regions in the Sox6 protein that are involved in their interactions. The majority of cofactors bind Sox6 through the N-terminus coiled-coil (leucine zipper and the first Q-box). Fewer proteins have been found to interact with the HMG box or the outside of the defined domains (Table 1). In our laboratory we have also conducted Sox6 yeast two hybrid assays using mammalian muscle cDNA libraries and identified far more cofactor candidates for the N-terminus half of Sox6 (with the Q-box sequences) than the C-terminus half containing the HMG box (Ganio, Hiroki, and Hagiwara, unpublished results). Therefore, as previously suggested (Lefebvre et al. 1998; Kamachi et al. 2000), the coiled-coil domains appear to play the central role in the bulk of Sox6 protein-protein interactions. The effects of these Sox6-partner protein interactions on gene expression are known for some cases, as summarized in Table 1. For example, partnering with Sox5, Sox6 participates in the activation of chondrocyte specific genes in concert with the activator Sox9 (Lefebvre et al. 1998; Han and Lefebvre 2008). Conversely, when partnered with the transcriptional repressor CtBP2, Sox6 negatively regulates the expression of the Fgf3 gene (Murakami et al. 2001). The fact that Sox6 is involved in both activation and suppression of gene transcription reflects the functional versatility of the Sox6 protein, and may be one of the reasons that Sox6 is expressed in so many cell types throughout development.

In the following sections, I will discuss the current knowledge regarding the multifaceted roles of Sox6 in vertebrate development.

Multifaceted Roles of Sox6 in Vertebrate Development

Complexity of the vertebrate body plan is achieved by the emergence and coordination of functionally diverse cell types. To generate literally millions of functionally specialized cell types with a limited number of transcription factors (e.g. ~2,000 in humans, Vaquerizas et al. 2009), it is becoming a common scenario to see the same transcription factor frequently

playing multiple roles in the differentiation of varying cell types during development. However, our understanding of the combinatorial control of transcription is still limited. In light of this, Sox6 can serve as a model transcription factor to investigate this aspect of transcriptional regulation. Another well known example is the Sox2 transcription factor. It plays a key role in maintaining pluripotency of embryonic stem cells (Avilion et al. 2003) and is one of the four factors that have been shown to be capable of reprogramming differentiated somatic cells into pluripotent stem cells (the others being Oct3/4, c-Myc, and Klf4) (Takahashi et al. 2006; 2007). In embryonic stem cells, it has been reported that Sox2 cooperates with Oct3/4 to transcriptionally activate genes important for maintaining the pluripotent cell state (Yuan et al. 1995; Nishimoto et al. 1999; Ambrosetti et al. 1997). Later in development, Sox2, with its cofactor Pax6, participates in the induction of the delta-crystallin gene in lens (Kamachi et al. 2001). This is just one example demonstrating that the function of a transcription factor is highly context dependent during development, both influencing and being influenced by numerous factors such as co-regulators and chromatin environment. It is becoming increasingly clear that Sox6 is one of these multi-tasking transcription factors. Below, I will discuss the functions of Sox6 focusing mainly on the development of chondrocytes, skeletal muscle, oligodendrocytes, cortical neurons, and red blood cells. It appears that Sox6 is a major factor in two critical decision making processes: 1) whether to stay or exit from the cell cycle and proceed from lineage specific progenitors to post-mitotic cells, and 2) the terminal differentiation of post-mitotic cells (Fig. 2). The following section also briefly touches on the other cell types in which Sox6 has been shown to be involved in cell type specific gene expression and differentiation.

In discussing the role of Sox6 in mammalian development, there are three recurring themes. The first is the functional redundancy between Sox6 and Sox5. The aspect will be discussed in the development of chondrocyte and oligodendrocyte, where the roles of Sox6 and Sox5 heavily overlap (Smits et al. 2001; Stolt et al. 2006). The second is the highly specialized, non-overlapping role of Sox6. An example is cell type specification in the neocortex. The expression patterns as well as roles of Sox6 and Sox5 are highly distinct in the development of telencephalon (Azim et al. 2009). The third theme is the dance between members of the SoxD (Sox5 and Sox6) and SoxE family (Sox9 and Sox10). Regarding the first two themes, why is it that Sox6 and Sox5 share functions in some tissues but not in others? If speculation is allowed, the answer may lie in the interplay between the evolution of the SoxD gene family and the evolutionary age of the tissue. In vertebrate Sox gene evolution, the Sox5 and Sox6 are closest to each other based on sequence similarity (Koopman et al. 2004). Functional redundancies between Sox5 and Sox6 (theme 1) are often observed in the vertebrate tissues of greater evolutionary age, such as the bones (Vickaryous et al. 2009) and glial cells (Rowitch and Kriegstein 2010). On the other hand, non-redundant functions (theme 2) are seen in tissues that are evolutionarily more recent, such as the neocortex (Azim et al. 2009). Therefore, it appears that in general the more ancient the structure is, the more redundancy exists for the functions of Sox5 and Sox6. It is thus possible that, in vertebrate development, commonly shared functions among the all three SoxD genes will soon be found, based on the observation that Sox13 expression during mouse development overlaps with both Sox5 and Sox6 (Wang et al. 2006). The third theme revolves around the interactions between the two groups of Sox proteins, SoxD and SoxE in transcriptional regulation. As mentioned above, the SoxD proteins contain no regulatory domains, whereas the SoxE proteins contain an activator domain (Wegner 1999; Kamachi et al. 2000). In chondrocyte development, Sox5, Sox6 (SoxD) and Sox9 (SoxE) (commonly referred to as the Sox trio) act in concert to activate expression of cartilage specific genes (Lefebvre et al. 1998; Akiyama et al. 2002). In contrast, during oligodendrocyte development, Sox5 and Sox6 antagonize Sox9 and Sox10's function as transcriptional activators (Stolt et al. 2006). It seems that SoxD has developed a pact to work with SoxE proteins. Whether this pact is a coincidence or has functional relevance will be answered by further uncovering the

interactions between these two groups of proteins in different tissues and different species, as viewed through the lens of evolution.

1. The Sox6 protein plays an important role in differentiation of mesenchymal tissues

Chondrocyte differentiation

For the bones to grow, the role of cartilage is essential. The cartilage primarily consists of chondrocytes which secrete extracellular matrix proteins to form a scaffolding structure for the osteoblasts to lay out the bone matrix. Chondrogenesis is initiated by the condensation of mesenchymal cells and subsequently goes through three basic developmental stages: proliferative chondroblast, pre-hypertrophic (post-mitotic), and hypertrophic chondrocyte stages, all of which significantly affect bone growth (Kornenberg 2003). It is now well established that Sox6, in conjunction with Sox5, plays an important role during the proliferative chondroblast stage of cartilage differentiation (Lefebvre et al. 1998; Smits et al. 2001; Akiyama et al. 2002; Lefebvre and Smits 2005). These two SoxD proteins are abundantly expressed in the proliferating chondroblasts and are known to have at least two important functions: regulating cell proliferation and activating extracellular matrix protein gene expression (Lefebvre et al. 1998; Smits et al. 2004). Sox6 and Sox5 function together to suppress precocious exit from the cell cycle, keeping chondroblasts from entering the pre-hypertrophic (post-mitotic) stage (Smits et al. 2004). Two possible mechanisms for this have been posited: first, in conjunction with Sox5, Sox6 may negatively regulate (possible cofactors are yet to be identified) the transcription factor Runx2 which promotes the pre-hypertrophic differentiation (Smits et al. 2004), and second, as part of the Sox trio (Sox5, Sox6, and Sox9), Sox6 activates expression of the calcium binding proteins S100A1 and S100B, which in turn suppress hypertrophic differentiation and mineralization of chondrocytes (Saito et al. 2007). Sox6's other important known function in chondrogenesis is transcriptional activation of the genes encoding extracellular matrix proteins. In proliferating chondroblasts, as part of the Sox trio, Sox6 directly activates transcription of type II collagen, aggrecan, and matrilin-1 (Lefebvre et al. 1998; Han and Lefebvre 2008; Nagy et al. 2011).

An understanding of how Sox6 expression itself is regulated in chondrogenesis is still developing. It has been shown that Sox9 is required for the expression of Sox6 and Sox5 (Akiyama et al. 2002). Among the numerous signaling pathways critical to mammalian development, so far only the bone morphogenetic protein (BMP) signaling pathway has been implicated in stimulating Sox6 expression during chondrogenesis. In vitro, BMP-4 and BMP-2 have been shown to upregulate Sox6 transcription in sclerotome primary cultures and the 10T1/2 cell line, respectively (Sohn et al. 2010; Fernandez-Lloris et al. 2003). In vivo, adenovirus-mediated BMP-2 gene transfer to fractured bone led to the induction of the Sox trio, and subsequent upregulation of pre-hypertrophic chondrocyte specific genes at the fracture site (Uusitalo et al. 2001). Further evidence for the regulation of Sox6 via the BMP pathway came from embryonic mouse limbs. It has been shown that direct administration of BMP-7 and noggin (a BMP antagonist) had the effect of upregulating and downregulating Sox6 expression, respectively (Chimal-Monroy et al. 2003). Added evidence for the connection between the BMP signaling pathway and Sox6 (or Sox trio induced chondrogenesis), came from a report showing that cartilage specific BMP receptor double KO mice lost expression of the Sox trio and displayed severe chondrodysplasia (Yoon et al. 2005). These observations suggest that as part of the Sox trio complex, Sox6 is induced by BMPs and plays a key role both in early chondrogenesis and during fracture healing to produce and maintain healthy bone structure.

Skeletal muscle differentiation

Skeletal muscle effects motor neuron signaling into locomotion. To respond to the constantly changing functional demands, the physiological characteristics of adult skeletal muscle is highly adjustable (Pete and Staron, 2000; Schiaffino et al. 2007). This adjustability of adult muscle is achieved through the process of skeletal muscle fiber type switching (between slow-twitch and fast-twitch) in response to motor neuron input, a process termed fiber type plasticity (Schiaffino et al. 2007). Several transcription factors that regulate fiber type plasticity in response to motor neuron activity in adult have been identified (Schiaffino et al. 2007), but until recently, the factors specifying fetal fiber types in the absence of the mature motor neurons remained elusive (Francis-West et al. 2003). It has now been shown, in two evolutionarily distant organisms, mice and zebrafish, that the factor specifying the muscle fiber type in the absence of motor neuron input is Sox6 (Hagiwara et al. 2005; 2007; von Hofsten et al. 2008). In both animals, the loss of Sox6 activity led to the upregulation of numerous slow fiber specific genes, suggesting that Sox6 functions as a transcriptional suppressor of slow fiber specific genes (Hagiwara et al. 2007; von Hofsten et al. 2008). In addition, overexpression of Sox6 resulted in a decrease in expression of slow fiber specific genes in mice as well as zebrafish (van Rooij et al. 2009; von Hofsten et al. 2008). Whether Sox6 also influences fiber type plasticity in adult skeletal muscle has yet to be explored.

How is the expression of Sox6 in skeletal muscle regulated? Transcriptional regulation of Sox6 is better understood in zebrafish than mice. In zebrafish skeletal muscle, slow fibers differentiate first through the process regulated by Hedgehog (Hh) signaling (Baxendale et al. 2004). It has been shown that the transcription factor Blimp-1/PRDM-1 which is activated by Hh signaling suppresses Sox6 expression and allows slow fiber specific gene expression (von Hofsten et al. 2008; Liew et al. 2008). In mammalian myogenesis, it is not clear whether Hh signaling plays as definitive a role in regulation of Sox6 expression as it does in fish (Li et al. 2004). Though the transcriptional regulation of Sox6 is not known, post-transcriptional regulation of Sox6 expression by microRNA has been recently reported in muscle (van Rooij et al. 2009; McCarthy et al. 2009). MicroRNAs regulate protein expression by targeting the non-coding sequences of mRNA and interfering with translation (Bartel 2009). In case of Sox6, the muscle-specific microRNA miR-499 encoded in the Myh7b intron (Bell et al. 2009; van Rooij et al. 2009; Rossi et al. 2010) and miR-208b encoded in the MyHC- β intron (van Rooij et al. 2009) have been shown to suppress Sox6 protein expression. MicroRNAs which are expressed in a cell type-specific manner are known to further adjust the protein levels of cell type-specific genes (Bartel 2009). Therefore, employing microRNA as a regulatory mechanism seems to be a clever way to achieve cell type-specific expression of broadly expressed proteins like Sox6. However, the story does not end there and regulation of Sox6 expression in skeletal muscle is far more complicated. First, muscle specific overexpression of Sox6 significantly reduces expression of miR-499 and its parental mRNA encoded by the MyHC-7b gene, suggesting Sox6 functions as a transcriptional suppressor of its own suppressor (van Rooij et al. 2009). Secondly, the question as to whether miR-499 is an active participant in fiber type differentiation of fetal muscle or is simply reinforcing a pre-existing fiber type layout in adult skeletal muscle, remains unanswered. So far, no mechanism has been shown to be involved in regulation of fiber type specific genes both in fetal and adult muscle (Oh et al. 2005; Issa et al. 2006). Therefore, if Sox6 turns out to be involved in the regulation of fiber type gene expression in both adult and fetal muscle, the Sox6-miR499 feedback loop may become the first mechanism known to be regulating fiber type plasticity in adult muscle in response to motor neuron input as well as fiber type specification in developing muscle independent of neuronal influence. It seems that the story of Sox6 in muscle will keep developing.

2. The roles of Sox6 in the development of the central nervous system

Recent reports show that Sox6 plays a role in development of the central nervous system by regulating differentiation of both neurons and glia. In oligodendrocyte development, the role of Sox6 is similar to that in chondrogenesis. Sox6, together with Sox5, regulates proliferation of oligodendrocyte progenitors and prevents precocious exit from the cell cycle. Their functions are also entwined with those of SoxE proteins. In contrast, in neuronal development, expression of Sox5 and Sox6 does not overlap and they appear to regulate specification of distinct sets of neurons in the mammalian cortex.

Oligodendrocyte differentiation

Myelin is a crucial structural unit for maintaining the health of neuronal signaling circuits. In the central nervous system, oligodendrocytes are the myelin forming glial cells. To build the fully operative brain, proliferation of oligodendrocyte progenitor cells (OPCs), their migration, and the timing of terminal differentiation are all highly coordinated (Richardson et al. 2006; Nishiyama et al. 2009; Emery 2010). The effort to identify the factors regulating oligodendrocyte development has recently added Sox6 to the growing list of transcription factors for OPCs differentiation. It has been shown that Sox6, in concert with Sox5, inhibits OPCs from exiting the cell cycle (Stolt et al. 2006). Similar to chondrogenesis, Sox6 is highly expressed in proliferating OPCs, but not in post-mitotic differentiating oligodendrocytes (Stolt et al. 2006), thus suggesting that Sox6 is likely involved in OPCs' exit from the cell cycle during terminal differentiation (Fig. 2). In the spinal cord of Sox5 and Sox6 double knockout mice (also in a lesser degree in Sox6 knockout mice), the number of prematurely differentiating post-mitotic oligodendrocytes significantly increased (Stolt et al. 2006), supporting the notion that Sox5 and Sox6 are pro-mitotic factors in oligodendrocyte differentiation. As for the mechanism, it appears that Sox6 and Sox5 antagonize the activities of Sox9 and Sox10, which are required for specification of OPCs (Sox9) and terminal differentiation of oligodendrocytes (Sox10) (Stolt et al. 2002; 2003). These SoxE proteins are functionally redundant during the development of OPCs, but not in the specification and terminal differentiation of oligodendrocytes (Finzsch et al. 2008). Stolt et al. (2006) have shown that overexpression of Sox5 and Sox6 in glial and neuroblastoma cell lines in vitro results in significantly reduced transcription from the myelin specific gene promoters, which otherwise are activated by Sox9 and Sox10. Though detailed mechanisms remain to be uncovered, these results strongly suggest that Sox5 and Sox6 are functioning as repressors of oligodendrocyte terminal differentiation by hindering the activator functions of Sox9 and Sox10.

How is Sox6 expression regulated during oligodendrocyte development? Similar to skeletal muscle, it appears that microRNAs play an important role. It has been shown that miR-219, which is required for oligodendrocyte differentiation, targets Sox6 and reduces Sox6 protein level, thus allowing terminal differentiation of oligodendrocytes (Dugas et al. 2010; Zhao et al. 2010).

Before switching gears to Sox6 and neuronal differentiation, mentioning astrocytes, the most abundant glial cells in the brain is warranted. Sox6 may be also involved in astrocyte differentiation. It has been reported that overexpression of Sox6 in adult rat neuronal stem cells can shift differentiation from neurons to astrocytes even under the culture condition that favors neuronal differentiation (Scheel et al. 2005). Although it is still preliminary, Sox6 may play a role in the decision making process of neural stem cell differentiation in the adult brain. Understanding how Sox6 induces astrocyte differentiation from adult neural stem cells would make a significant contribution to regenerative medicine. It has also been reported that Sox6 expression is significantly upregulated in human gliomas (Ueda et al. 2004; Schlierf et al. 2007). These reports indicate that Sox6 plays an important role in

proliferation of macroglial progenitor cells as well as their differentiation from neural stem cells.

Neuronal differentiation

Sox6 also plays a role in creating neuronal diversity in the central nervous system. Unlike chondrocyte and oligodendrocyte development, in which Sox5 and Sox6 have redundant functions, in cortical neuron development these two SoxD proteins appear to regulate differentiation of discrete sets of neurons. The cerebral cortex contains, broadly categorized, two classes of neurons, projection neurons (send out axons to distant targets, glutamate producing) and interneurons (local signal modulation, γ -aminobutyric acid, GABA producing) (Wonders and Anderson 2006; Molyneaux et al. 2007). Both projection and interneurons consist of multiple subtypes of neurons (Molyneaux 2007; Wonders and Anderson 2006). Interneuron subtypes in particular are quite diverse, varying in characteristics such as morphology, ion channel and neuropeptide expression (Wonders and Anderson 2006).

Recent reports indicate that Sox6 is expressed in the dorsal telencephalic progenitors to specify interneurons (Azim et al. 2009) whereas Sox5 is expressed in the ventral progenitors to specify the projection neurons (Lai et al. 2008), suggesting possible cross-repressive interactions between Sox5 and Sox6 in neuronal development (Azim et al. 2009). Reflecting this observation, the loss of Sox6 function leads to ectopic Sox5 expression and mixed dorsal-ventral identity (Azim et al. 2009). Sox6 also plays a role in specification of post-mitotic interneurons. It has been shown that Sox6 is highly expressed in the post-mitotic interneurons expressing parvalbumin (PV) and somatostatin (SST) which originate from the medial ganglion eminence (MGE), a substructure in the telencephalon (Azim et al. 2009; Batista-Brito 2009). In the absence of Sox6, the majority of these MGE derived interneurons fell short of reaching their final destinations in the cortex and ended up expressing different types of neuropeptides (Azim et al. 2009; Batista-Brito 2009). These observations hint at exciting new roles for the SoxD proteins in the specification of diverse types of neurons in the mammalian neocortex. Currently, how Sox6 expression is regulated in neuronal development is unknown.

3. The role of Sox6 in erythropoiesis

Differentiation of red blood cells in higher vertebrates (i.e. birds and mammals) is highly regulated both spatially and temporally (Baron and Fraser 2005; Palis 2008; McGrath and Palis 2008). Developmentally, production of red blood cells first starts in the yolk sac (primitive erythropoiesis, starting ~E7 in mice), and moves to the fetal liver (fetal definitive erythropoiesis, starting ~E12 in mice) (Baron and Fraser 2005; Palis 2008). After birth, the adult definitive erythroblasts are generated in the bone marrow (Palis 2008).

Recently, it has been shown that Sox6 plays a crucial role in both the proliferation and maturation of definitive red blood cells (Dumitriu et al. 2006; Yi et al. 2006; Cohen-Barak et al. 2007; Dumitriu et al. 2010). Sox6 is expressed in the fetal liver and bone marrow, and also in purified adult definitive erythroblasts (Dumitriu et al. 2006; Yi et al. 2006; 2010, Cantu et al. 2010; Dumitriu et al. 2010; Xu et al. 2010). When the Sox6 gene is experimentally inactivated in definitive erythroblasts, their proliferation and survival is severely compromised, leading to a delay in red blood cell maturation (Yi et al. 2006; Dumitriu et al. 2006; 2010). How does Sox6 regulate survival of definitive red blood cells? The recently identified Sox6 target gene, Bcl-X, likely holds a key to uncover Sox6's role in this matter. Bcl-X is an anti-apoptosis factor shown to be required for the survival of red blood cells in mice (Wagner et al. 2000). Mice deficient in Bcl-X are anemic, which is recapitulated by erythroid-specific Sox6 deficient mice (Dumitriu et al. 2006; 2010). It has

been shown that Sox6 directly binds to the regulatory regions of the Bcl-X gene and upregulates its transcription, thus aiding in the survival of red blood cells (Dumitirui et al. 2010). Since the Bcl-X gene sequence regions that Sox6 binds to are the regulatory elements controlling the response to the erythropoietin signaling (Socolovsky et al. 1999; Tian et al. 2003), identification of Sox6 cofactors will help to uncover the molecular mechanisms of erythrocyte proliferation regulated by erythropoietin.

Sox6 also plays an important role in developmentally regulated globin gene switching. Globin gene isoform switching is a well known phenomenon observed in the transition from primitive to definitive erythropoiesis (Sankaran et al. 2010). Expression of the embryonic globin genes is limited to primitive red blood cells produced in the yolk sac and becomes silenced in the fetal liver where fetal definitive erythroblasts are generated (Trimborn et al. 1999). It has been shown that Sox6 plays a significant role in silencing the transcription of the mouse embryonic β -globin $\epsilon\gamma$ gene (Yi et al. 2006). This Sox6 function is cell-autonomous as shown by the substantial increase in $\epsilon\gamma$ expression reproduced in the Sox6 null hematopoietic stem cells that were engrafted to adult mouse bone marrow (Cohen-Barak et al. 2007). In addition, Sox6 takes part in suppression of human fetal β -globin isoform genes in adult definitive red blood cells (Xu et al. 2010). It has been shown that Sox6 silences transcription of both mouse embryonic and human fetal β -globin genes in concert with the BCL11A protein (Xu et al. 2010), a protein known to function as a repressor (Liu et al. 2006). The authors (Xu et al. 2010) propose that Sox6 and BCL11A physically interact, thus the binding of Sox6 to the promoter region of the embryonic and fetal β -globin genes provides an anchoring point for the BCL11A-repressor complex to associate with the regulatory regions of these globin genes. These new findings have provided the biological basis to the genome wide association studies of human fetal globin (HbF) and significantly advanced our understanding of the globin gene switching in erythropoiesis (Sankaran et al. 2008; 2010).

The regulation of Sox6 expression in erythroid cells appears to be mediated by both extracellular signals and subsequent intracellular feedback mechanisms. First, different cytokine conditions can alter Sox6 expression levels in human erythroid cell cultures (Sripichai et al. 2009). Second, it has been shown that in differentiating erythroblast cultures, the Sox6 protein functions as its own transcriptional suppressor by binding to its own promoter (Cantu et al. 2010). Interestingly, the Sox6 binding sequences reported for the erythroblast DNA (Cantu et al. 2010) are distinct from those we found for the skeletal muscle DNA (An and Hagiwara, unpublished results). Therefore, the chromatin environment of the Sox6 gene region could be specific to each cell type, resulting in cell type specific auto-regulation of Sox6 expression.

4. Other cell and tissue types

In this section, I will briefly discuss differentiation of additional cell types where Sox6 has been reported to play a role.

Pancreatic β -cells

In the pancreas, Sox6 has been identified as a regulator of glucose-stimulated insulin secretion from β -cells (Iguchi et al. 2005). It has been shown that Sox6 physically interferes with the activator domain of the PDX1 protein and leads to reduced transcription from the insulin II gene promoter (Iguchi et al. 2005). This suppression is likely caused by deacetylation of histone in the insulin II promoter region (Iguchi et al. 2005). Subsequently, Iguchi et al (2007) have further demonstrated that Sox6 inhibits proliferation cells such as insulinoma (INS-1E and MIN6) and fibroblasts (NIH-3T3). Inhibition of cell proliferation is likely achieved through transcriptional suppression of the cyclin-D1 gene by HDAC1. The

authors suggest that Sox6-HDAC1 complex is recruited to the cyclin-D1 promoter via physical interaction between Sox6 and β -catenin (Iguchi et al. 2007).

Cardiomyocytes

Sox6 appears to play a similar role in cardiomyocyte progenitor cells as reported for chondroblasts and oligodendrocyte progenitors, namely maintaining mitotic activity of the lineage specific progenitor cells. Sox6 is highly expressed in proliferating cardiomyocyte progenitor cells, and reduced Sox6 expression results in the exit from the cell cycle and differentiation (Sluijter et al. 2010). A decrease in Sox6 expression coincides with a significant increase in miR-499 expression in differentiating cardiomyocytes, indicating that this microRNA functions as a suppressor of Sox6 protein expression in cardiomyocytes (Sluijter et al. 2010). Though information is still limited, these observations suggest that the Sox6-miR499 network may play an important role in cardiomyocyte development as has been shown in skeletal muscle.

Testis

Although the Sox6 cDNA was initially isolated from a testis cDNA library (Connor et al. 1995), not much is known about the role of Sox6 in spermatogenesis. The major form of the Sox6 mRNA expressed in testis is the short form (~3kb) and lacks the long 3'-UTR sequence found in the long form (~9kb) expressed in other tissues (Lefebvre et al. 1998; Hagiwara et al. 2000; Cohen-Barak et al. 2001). The short and long Sox6 messages share the same coding sequence. The 3kb Sox6 mRNA expression is upregulated around four weeks after birth in the rodent testis and also cycles during the spermatogenesis, indicating that Sox6 may play a role in maturation of sperm (Takamatsu et al. 2000; Ohe et al. 2009). A new piece of information that may be relevant to the possible role of Sox6 in regulating spermatogenesis is that the previously reported Sox6 cofactor, Solt (Yamashita et al. 2000), has been identified as a chromatin centromere associated protein CEMP-K (Okada et al. 2006). Since CEMP-K is required for the progression of cell cycle and its inactivation stalls cell division, Sox6 might regulate cell cycle during spermatogenesis.

5. Perspectives

In the flow of development, no one gene can control the process; rather they are all participating in a complex network of interactions influencing, and in turn, being influenced (Nijhout 1990). Transcription factors, more often than others, undeniably play a significant role in the decision making processes during development. As described above, changes in Sox6 expression initiate a cascade of events, leading cells to an alternate route in developmental pathways. Thus, although it could be said that Sox6 controls the development of certain tissues, it is not much different from the rocks in the river bed changing the flow of the water. The phrase "Jack of all trades, master of none" is conventionally used as an unwelcomed characteristic of an individual. However, when it is considered as a description for a transcription factor, it fits the reality of transcriptional regulation positively. First, no one transcription factor can function alone, independent of external stimuli, cellular conditions, cofactors, etc. Second, increasingly individual transcription factors are found to be part of the regulatory networks shaping the development of multiple cell types. Among them, Sox6 is truly a multifaceted transcription factor with important regulatory functions in the development of the mesoderm, ectoderm, and endodermal tissues. Although its function covers a broad range of cell types, there are reoccurring themes in the way Sox6 plays its role in development. As discussed above, Sox6 exerts its function during the time period where progenitors are about to exit the cell cycle to terminally differentiate, or when gene switching is occurring during cell type specification. Sox6 might have become a specialist in

this arena during vertebrate evolution as exemplified by its role in skeletal muscle fiber specification conserved in fish and mammals.

To function as a regulator of terminal differentiation, coordinated regulation of numerous genes becomes necessary. To achieve this task, Sox6 also brings in epigenetic regulation to its turf. Interaction of Sox6 with histone deacetylase 1 (HDAC1) has been reported (Iguchi et al. 2007), and it is expected that more chromatin modifying enzymes will be found to be interacting with Sox6. It is known that Sox proteins bind to the minor groove of DNA and induce a bend in the bound DNA. Because of this trait, Sox proteins are thought to function as architectural proteins and facilitate interactions of transcriptional complexes on the chromatin (Wegner 1999; Weiss 2001). Therefore, Sox6 can extend the effect of its interaction with the chromatin modifying enzymes to distant locations. In regulating terminal differentiation, Sox6 likely influences expression of multiple genes by changing the chromatin environment. The regulation of chromatin environments by Sox6 will be an exciting subject in vertebrate development.

On top of this, Sox6 may not be just a transcription factor. There have been reports indicating that Sox6 plays a role in pre-mRNA splicing (Ohe et al. 2002; 2009). Since transcription and RNA processing are highly interconnected (Moore and Proudfoot, 2009), Sox6 may streamline these two critical regulatory processes of gene expression.

We have just started to uncover the trades that Sox6 is involved in. Sox6 has a great potential to serve as a model transcription factor for uncovering the mechanisms of coordinated gene regulation in multiple cell types that are likely conserved through vertebrate evolution, at the chromatin, transcriptional, and post-translational levels.

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References

- Akiyama H, Chaboissier MC, Martin JF, Schedl A, de Crombrughe B. The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. *Genes Dev.* 2002; 16:2813–2828. [PubMed: 12414734]
- Ambrosetti DC, Basilico C, Dailey L. 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.* 1997; 17:6321–6329. [PubMed: 9343393]
- Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R. Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* 2003; 17:126–140. [PubMed: 12514105]
- Azim E, Jabaudon D, Fame RM, Macklis JD. SOX6 controls dorsal progenitor identity and interneuron diversity during neocortical development. *Nat Neurosci.* 2009; 12:1238–1247. [PubMed: 19657336]
- Baron MH, Fraser ST. The specification of early hematopoiesis in the mammal. *Curr Opin Hematol.* 2005; 12:217–221. [PubMed: 15867578]
- Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell.* 2009; 136:215–233. [PubMed: 19167326]

- Batista-Brito R, Rossignol E, Hjerling-Leffler J, Denaxa M, Wegner M, Lefebvre V, Pachnis V, Fishell G. The cell-intrinsic requirement of Sox6 for cortical interneuron development. *Neuron*. 2009; 63:466–481. [PubMed: 19709629]
- Baxendale S, Davison C, Muxworthy C, Wolff C, Ingham PW, Roy S. The B-cell maturation factor Blimp-1 specifies vertebrate slow-twitch muscle fiber identity in response to Hedgehog signaling. *Nat Genet*. 2004; 36:88–93. [PubMed: 14702044]
- Bell ML, Buvoli M, Leinwand LA. Uncoupling of expression of an intronic microRNA and its myosin host gene by exon skipping. *Mol Cell Biol*. 2010; 30:1937–1945. [PubMed: 20154144]
- Bowles J, Schepers G, Koopman P. Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators. *Dev Biol*. 2000; 227:239–255. [PubMed: 11071752]
- Cantu C, Grande V, Alborelli I, Cassinelli L, Cantu I, Colzani MT, Ierardi R, Ronzoni L, Cappellini MD, Ferrari G, Ottolenghi S, Ronchi A. A highly conserved SOX6 double binding site mediates SOX6 gene downregulation in erythroid cells. *Nucleic Acids Res*. 2010
- Chimal-Monroy J, Rodriguez-Leon J, Montero JA, Ganan Y, Macias D, Merino R, Hurle JM. Analysis of the molecular cascade responsible for mesodermal limb chondrogenesis: Sox genes and BMP signaling. *Dev Biol*. 2003; 257:292–301. [PubMed: 12729559]
- Cohen-Barak O, Erickson DT, Badowski MS, Fuchs DA, Klassen CL, Harris DT, Brilliant MH. Stem cell transplantation demonstrates that Sox6 represses epsilon y globin expression in definitive erythropoiesis of adult mice. *Exp Hematol*. 2007; 35:358–367. [PubMed: 17309816]
- Cohen-Barak O, Hagiwara N, Arlt MF, Horton JP, Brilliant MH. Cloning, characterization and chromosome mapping of the human SOX6 gene. *Gene*. 2001; 265:157–164. [PubMed: 11255018]
- Cohen-Barak O, Yi Z, Hagiwara N, Monzen K, Komuro I, Brilliant MH. Sox6 regulation of cardiac myocyte development. *Nucleic Acids Res*. 2003; 31:5941–5948. [PubMed: 14530442]
- Connor F, Wright E, Denny P, Koopman P, Ashworth A. The Sry-related HMG box-containing gene Sox6 is expressed in the adult testis and developing nervous system of the mouse. *Nucleic Acids Res*. 1995; 23:3365–3372. [PubMed: 7567444]
- Dugas JC, Cuellar TL, Scholze A, Ason B, Ibrahim A, Emery B, Zamanian JL, Foo LC, McManus MT, Barres BA. Dicer1 and miR-219 Are required for normal oligodendrocyte differentiation and myelination. *Neuron*. 2010; 65:597–611. [PubMed: 20223197]
- Dumitriu B, Bhattaram P, Dy P, Huang Y, Quayum N, Jensen J, Lefebvre V. Sox6 is necessary for efficient erythropoiesis in adult mice under physiological and anemia-induced stress conditions. *PLoS One*. 2010; 5:e12088. [PubMed: 20711497]
- Dumitriu B, Patrick MR, Petschek JP, Cherukuri S, Klingmuller U, Fox PL, Lefebvre V. Sox6 cell-autonomously stimulates erythroid cell survival, proliferation, and terminal maturation and is thereby an important enhancer of definitive erythropoiesis during mouse development. *Blood*. 2006; 108:1198–1207. [PubMed: 16627753]
- Emery B. Regulation of oligodendrocyte differentiation and myelination. *Science*. 2010; 330:779–782. [PubMed: 21051629]
- Fernandez-Lloris R, Vinals F, Lopez-Rovira T, Harley V, Bartrons R, Rosa JL, Ventura F. Induction of the Sry-related factor SOX6 contributes to bone morphogenetic protein-2-induced chondroblastic differentiation of C3H10T1/2 cells. *Mol Endocrinol*. 2003; 17:1332–1343. [PubMed: 12677004]
- Finzsch M, Stolt CC, Lommes P, Wegner M. Sox9 and Sox10 influence survival and migration of oligodendrocyte precursors in the spinal cord by regulating PDGF receptor alpha expression. *Development*. 2008; 135:637–646. [PubMed: 18184726]
- Francis-West PH, Antoni L, Anakwe K. Regulation of myogenic differentiation in the developing limb bud. *J Anat*. 2003; 202:69–81. [PubMed: 12587922]
- Hagiwara N, Klewer SE, Samson RA, Erickson DT, Lyon MF, Brilliant MH. Sox6 is a candidate gene for p100H myopathy, heart block, and sudden neonatal death. *Proc Natl Acad Sci U S A*. 2000; 97:4180–4185. [PubMed: 10760285]
- Hagiwara N, Ma B, Ly A. Slow and fast fiber isoform gene expression is systematically altered in skeletal muscle of the Sox6 mutant, p100H. *Dev Dyn*. 2005; 234:301–311. [PubMed: 16124007]

- Hagiwara N, Yeh M, Liu A. Sox6 is required for normal fiber type differentiation of fetal skeletal muscle in mice. *Dev Dyn.* 2007; 236:2062–2076. [PubMed: 17584907]
- Han Y, Lefebvre V. 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.* 2008; 28:4999–5013. [PubMed: 18559420]
- Iguchi H, Ikeda Y, Okamura M, Tanaka T, Urashima Y, Ohguchi H, Takayasu S, Kojima N, Iwasaki S, Ohashi R, Jiang S, Hasegawa G, Ioka RX, Magoori K, Sumi K, Maejima T, Uchida A, Naito M, Osborne TF, Yanagisawa M, Yamamoto TT, Kodama T, Sakai J. SOX6 attenuates glucose-stimulated insulin secretion by repressing PDX1 transcriptional activity and is down-regulated in hyperinsulinemic obese mice. *J Biol Chem.* 2005; 280:37669–37680. [PubMed: 16148004]
- Iguchi H, Urashima Y, Inagaki Y, Ikeda Y, Okamura M, Tanaka T, Uchida A, Yamamoto TT, Kodama T, Sakai J. SOX6 suppresses cyclin D1 promoter activity by interacting with beta-catenin and histone deacetylase 1, and its down-regulation induces pancreatic beta-cell proliferation. *J Biol Chem.* 2007; 282:19052–19061. [PubMed: 17412698]
- Ikeda T, Zhang J, Chano T, Mabuchi A, Fukuda A, Kawaguchi H, Nakamura K, Ikegawa S. Identification and characterization of the human long form of Sox5 (L-SOX5) gene. *Gene.* 2002; 298:59–68. [PubMed: 12406576]
- Issa LL, Palmer SJ, Guven KL, Santucci N, Hodgson VR, Popovic K, Joya JE, Hardeman EC. MusTRD can regulate postnatal fiber-specific expression. *Dev Biol.* 2006; 293:104–115. [PubMed: 16494860]
- Kamachi Y, Uchikawa M, Kondoh H. Pairing SOX off: with partners in the regulation of embryonic development. *Trends Genet.* 2000; 16:182–187. [PubMed: 10729834]
- Kamachi Y, Uchikawa M, Tanouchi A, Sekido R, Kondoh H. Pax6 and SOX2 form a co-DNA-binding partner complex that regulates initiation of lens development. *Genes Dev.* 2001; 15:1272–1286. [PubMed: 11358870]
- Kasimiotis H, Myers MA, Argentaro A, Mertin S, Fida S, Ferraro T, Olsson J, Rowley MJ, Harley VR. Sex-determining region Y-related protein SOX13 is a diabetes autoantigen expressed in pancreatic islets. *Diabetes.* 2000; 49:555–561. [PubMed: 10871192]
- Koopman P, Schepers G, Brenner S, Venkatesh B. Origin and diversity of the SOX transcription factor gene family: genome-wide analysis in *Fugu rubripes*. *Gene.* 2004; 328:177–186. [PubMed: 15019997]
- Kronenberg HM. Developmental regulation of the growth plate. *Nature.* 2003; 423:332–336. [PubMed: 12748651]
- Lai T, Jabaudon D, Molyneaux BJ, Azim E, Arlotta P, Menezes JR, Macklis JD. SOX5 controls the sequential generation of distinct corticofugal neuron subtypes. *Neuron.* 2008; 57:232–247. [PubMed: 18215621]
- Larroux C, Luke GN, Koopman P, Rokhsar DS, Shimeld SM, Degnan BM. Genesis and expansion of metazoan transcription factor gene classes. *Mol Biol Evol.* 2008; 25:980–996. [PubMed: 18296413]
- Lefebvre V. The SoxD transcription factors--Sox5, Sox6, and Sox13--are key cell fate modulators. *Int J Biochem Cell Biol.* 2010; 42:429–432. [PubMed: 19647094]
- Lefebvre V, Li P, de Crombrughe B. 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.* 1998; 17:5718–5733. [PubMed: 9755172]
- Lefebvre V, Smits P. Transcriptional control of chondrocyte fate and differentiation. *Birth Defects Res C Embryo Today.* 2005; 75:200–212. [PubMed: 16187326]
- Li X, Blagden CS, Bildsoe H, Bonnin MA, Duprez D, Hughes SM. Hedgehog can drive terminal differentiation of amniote slow skeletal muscle. *BMC Dev Biol.* 2004; 4:9. [PubMed: 15238161]
- Liew HP, Choksi SP, Wong KN, Roy S. Specification of vertebrate slow-twitch muscle fiber fate by the transcriptional regulator Blimp1. *Dev Biol.* 2008; 324:226–235. [PubMed: 18948093]
- Liu H, Ippolito GC, Wall JK, Niu T, Probst L, Lee BS, Pulford K, Banham AH, Stockwin L, Shaffer AL, Staudt LM, Das C, Dyer MJ, Tucker PW. Functional studies of BCL11A: characterization of the conserved BCL11A-XL splice variant and its interaction with BCL6 in nuclear paraspeckles of germinal center B cells. *Mol Cancer.* 2006; 5:18. [PubMed: 16704730]

- McCarthy JJ, Esser KA, Peterson CA, Dupont-Versteegden EE. Evidence of MyomiR network regulation of beta-myosin heavy chain gene expression during skeletal muscle atrophy. *Physiol Genomics*. 2009; 39:219–226. [PubMed: 19690046]
- McGrath K, Palis J. Ontogeny of erythropoiesis in the mammalian embryo. *Curr Top Dev Biol*. 2008; 82:1–22. [PubMed: 18282515]
- Molyneaux BJ, Arlotta P, Menezes JR, Macklis JD. Neuronal subtype specification in the cerebral cortex. *Nat Rev Neurosci*. 2007; 8:427–437. [PubMed: 17514196]
- Moore MJ, Proudfoot NJ. Pre-mRNA processing reaches back to transcription and ahead to translation. *Cell*. 2009; 136:688–700. [PubMed: 19239889]
- Murakami A, Ishida S, Thurlow J, Revest JM, Dickson C. SOX6 binds CtBP2 to repress transcription from the Fgf-3 promoter. *Nucleic Acids Res*. 2001; 29:3347–3355. [PubMed: 11504872]
- Nagy A, Kenesi E, Rentsendorj O, Molnar A, Szenasi T, Sinko I, Zvara A, Oommen ST, Barta E, Puskas LG, Lefebvre V, Kiss I. Evolutionarily conserved, growth plate zone-specific regulation of the matrilin-1 promoter: L-Sox5/Sox6 and Nfi factors bound near TATA finely tune activation by Sox9. *Mol Cell Biol*. 2011; 31:686–699. [PubMed: 21173167]
- Nijhout HF. Metaphors and the role of genes in development. *Bioessays*. 1990; 12:441–446. [PubMed: 1979486]
- Nishimoto M, Fukushima A, Okuda A, Muramatsu M. 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*. 1999; 19:5453–5465. [PubMed: 10409735]
- Nishiyama A, Komitova M, Suzuki R, Zhu X. Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. *Nat Rev Neurosci*. 2009; 10:9–22. [PubMed: 19096367]
- Oh M, Rybkin, Copeland V, Czubryt MP, Shelton JM, van Rooij E, Richardson JA, Hill JA, De Windt LJ, Bassel-Duby R, Olson EN, Rothmel BA. Calcineurin is necessary for the maintenance but not embryonic development of slow muscle fibers. *Mol Cell Biol*. 2005; 25:6629–6638. [PubMed: 16024798]
- Ohe K, Lalli E, Sassone-Corsi P. A direct role of SRY and SOX proteins in pre-mRNA splicing. *Proc Natl Acad Sci U S A*. 2002; 99:1146–1151. [PubMed: 11818535]
- Ohe K, Tamai KT, Parvinen M, Sassone-Corsi P. DAX-1 and SOX6 molecular interplay results in an antagonistic effect in pre-mRNA splicing. *Dev Dyn*. 2009; 238:1595–1604. [PubMed: 19384854]
- Ohtani K, Yao T, Kobayashi M, Kusakabe R, Kuratani S, Wada H. Expression of Sox and fibrillar collagen genes in lamprey larval chondrogenesis with implications for the evolution of vertebrate cartilage. *J Exp Zool B Mol Dev Evol*. 2008; 310:596–607. [PubMed: 18702077]
- Okada M, Cheeseman IM, Hori T, Okawa K, McLeod IX, Yates JR 3rd, Desai A, Fukagawa T. The CENP-H-I complex is required for the efficient incorporation of newly synthesized CENP-A into centromeres. *Nat Cell Biol*. 2006; 8:446–457. [PubMed: 16622420]
- Palis J. Ontogeny of erythropoiesis. *Curr Opin Hematol*. 2008; 15:155–161. [PubMed: 18391778]
- Pette D, Staron RS. Myosin isoforms, muscle fiber types, and transitions. *Microsc Res Tech*. 2000; 50:500–509. [PubMed: 10998639]
- Phochanukul N, Russell S. No backbone but lots of Sox: Invertebrate Sox genes. *Int J Biochem Cell Biol*. 2010; 42:453–464. [PubMed: 19589395]
- Richardson WD, Kessaris N, Pringle N. Oligodendrocyte wars. *Nat Rev Neurosci*. 2006; 7:11–18. [PubMed: 16371946]
- Roose J, Korver W, Oving E, Wilson A, Wagenaar G, Markman M, Lamers W, Clevers H. High expression of the HMG box factor sox-13 in arterial walls during embryonic development. *Nucleic Acids Res*. 1998; 26:469–476. [PubMed: 9421502]
- Rossi AC, Mammucari C, Argentini C, Reggiani C, Schiaffino S. Two novel/ancient myosins in mammalian skeletal muscles: MYH14/7b and MYH15 are expressed in extraocular muscles and muscle spindles. *J Physiol*. 2010; 588:353–364. [PubMed: 19948655]
- Rowitch DH, Kriegstein AR. Developmental genetics of vertebrate glial-cell specification. *Nature*. 2010; 468:214–222. [PubMed: 21068830]
- Saito T, Ikeda T, Nakamura K, Chung UI, Kawaguchi H. S100A1 and S100B, transcriptional targets of SOX trio, inhibit terminal differentiation of chondrocytes. *EMBO Rep*. 2007; 8:504–509. [PubMed: 17396138]

- Sankaran VG, Menne TF, Xu J, Akie TE, Lettre G, Van Handel B, Mikkola HK, Hirschhorn JN, Cantor AB, Orkin SH. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. *Science*. 2008; 322:1839–1842. [PubMed: 19056937]
- Sankaran VG, Xu J, Orkin SH. Advances in the understanding of haemoglobin switching. *Br J Haematol*. 2010; 149:181–194. [PubMed: 20201948]
- Scheel JR, Ray J, Gage FH, Barlow C. Quantitative analysis of gene expression in living adult neural stem cells by gene trapping. *Nat Methods*. 2005; 2:363–370. [PubMed: 15846364]
- Schepers GE, Teasdale RD, Koopman P. Twenty pairs of sox: extent, homology, and nomenclature of the mouse and human sox transcription factor gene families. *Dev Cell*. 2002; 3:167–170. [PubMed: 12194848]
- Schiaffino S, Sandri M, Murgia M. Activity-dependent signaling pathways controlling muscle diversity and plasticity. *Physiology (Bethesda)*. 2007; 22:269–278. [PubMed: 17699880]
- Schlierf B, Friedrich RP, Roerig P, Felsberg J, Reifenberger G, Wegner M. Expression of SoxE and SoxD genes in human gliomas. *Neuropathol Appl Neurobiol*. 2007; 33:621–630. [PubMed: 17961134]
- Sluijter JP, van Mil A, van Vliet P, Metz CH, Liu J, Doevendans PA, Goumans MJ. MicroRNA-1 and -499 regulate differentiation and proliferation in human-derived cardiomyocyte progenitor cells. *Arterioscler Thromb Vasc Biol*. 2010; 30:859–868. [PubMed: 20081117]
- Smits P, Dy P, Mitra S, Lefebvre V. Sox5 and Sox6 are needed to develop and maintain source, columnar, and hypertrophic chondrocytes in the cartilage growth plate. *J Cell Biol*. 2004; 164:747–758. [PubMed: 14993235]
- Smits P, Lefebvre V. Sox5 and Sox6 are required for notochord extracellular matrix sheath formation, notochord cell survival and development of the nucleus pulposus of intervertebral discs. *Development*. 2003; 130:1135–1148. [PubMed: 12571105]
- Smits P, Li P, Mandel J, Zhang Z, Deng JM, Behringer RR, de Crombrughe B, Lefebvre V. The transcription factors L-Sox5 and Sox6 are essential for cartilage formation. *Dev Cell*. 2001; 1:277–290. [PubMed: 11702786]
- Socolovsky M, Fallon AE, Wang S, Brugnara C, Lodish HF. Fetal anemia and apoptosis of red cell progenitors in Stat5a^{-/-}5b^{-/-} mice: a direct role for Stat5 in Bcl-X(L) induction. *Cell*. 1999; 98:181–191. [PubMed: 10428030]
- Sohn P, Cox M, Chen D, Serra R. Molecular profiling of the developing mouse axial skeleton: a role for Tgfb2 in the development of the intervertebral disc. *BMC Dev Biol*. 2010; 10:29. [PubMed: 20214815]
- Sripichai O, Kiefer CM, Bhanu NV, Tanno T, Noh SJ, Goh SH, Russell JE, Rognerud CL, Ou CN, Oneal PA, Meier ER, Gantt NM, Byrnes C, Lee YT, Dean A, Miller JL. Cytokine-mediated increases in fetal hemoglobin are associated with globin gene histone modification and transcription factor reprogramming. *Blood*. 2009; 114:2299–2306. [PubMed: 19597182]
- Stolt CC, Lommes P, Sock E, Chaboissier MC, Schedl A, Wegner M. The Sox9 transcription factor determines glial fate choice in the developing spinal cord. *Genes Dev*. 2003; 17:1677–1689. [PubMed: 12842915]
- Stolt CC, Rehberg S, Ader M, Lommes P, Riethmacher D, Schachner M, Bartsch U, Wegner M. Terminal differentiation of myelin-forming oligodendrocytes depends on the transcription factor Sox10. *Genes Dev*. 2002; 16:165–170. [PubMed: 11799060]
- Stolt CC, Schlierf A, Lommes P, Hillgartner S, Werner T, Kosian T, Sock E, Kessaris N, Richardson WD, Lefebvre V, Wegner M. SoxD proteins influence multiple stages of oligodendrocyte development and modulate SoxE protein function. *Dev Cell*. 2006; 11:697–709. [PubMed: 17084361]
- Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131:861–872. [PubMed: 18035408]
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]

- Takamatsu N, Kanda H, Tsuchiya I, Yamada S, Ito M, Kabeno S, Shiba T, Yamashita S. A gene that is related to SRY and is expressed in the testes encodes a leucine zipper-containing protein. *Mol Cell Biol.* 1995; 15:3759–3766. [PubMed: 7791783]
- Tian C, Gregoli P, Bondurant M. The function of the bcl-x promoter in erythroid progenitor cells. *Blood.* 2003; 101:2235–2242. [PubMed: 12411308]
- Trimborn T, Gribnau J, Grosveld F, Fraser P. Mechanisms of developmental control of transcription in the murine alpha- and beta-globin loci. *Genes Dev.* 1999; 13:112–124. [PubMed: 9887104]
- Ueda R, Yoshida K, Kawakami Y, Kawase T, Toda M. Expression of a transcriptional factor, SOX6, in human gliomas. *Brain Tumor Pathol.* 2004; 21:35–38. [PubMed: 15696967]
- Uusitalo H, Hiltunen A, Ahonen M, Gao TJ, Lefebvre V, Harley V, Kahari VM, Vuorio E. Accelerated up-regulation of L-Sox5, Sox6, and Sox9 by BMP-2 gene transfer during murine fracture healing. *J Bone Miner Res.* 2001; 16:1837–1845. [PubMed: 11585348]
- van Rooij E, Quiat D, Johnson BA, Sutherland LB, Qi X, Richardson JA, Kelm RJ Jr, Olson EN. A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. *Dev Cell.* 2009; 17:662–673. [PubMed: 19922871]
- Vaquerizas JM, Kummerfeld SK, Teichmann SA, Luscombe NM. A census of human transcription factors: function, expression and evolution. *Nat Rev Genet.* 2009; 10:252–263. [PubMed: 19274049]
- Vickaryous MK, Sire JY. The integumentary skeleton of tetrapods: origin, evolution, and development. *J Anat.* 2009; 214:441–464. [PubMed: 19422424]
- von Hofsten J, Elworthy S, Gilchrist MJ, Smith JC, Wardle FC, Ingham PW. Prdm1- and Sox6-mediated transcriptional repression specifies muscle fibre type in the zebrafish embryo. *EMBO Rep.* 2008; 9:683–689. [PubMed: 18535625]
- Wagner KU, Claudio E, Rucker EB 3rd, Riedlinger G, Broussard C, Schwartzberg PL, Siebenlist U, Hennighausen L. Conditional deletion of the Bcl-x gene from erythroid cells results in hemolytic anemia and profound splenomegaly. *Development.* 2000; 127:4949–4958. [PubMed: 11044408]
- Wang Y, Ristevski S, Harley VR. SOX13 exhibits a distinct spatial and temporal expression pattern during chondrogenesis, neurogenesis, and limb development. *J Histochem Cytochem.* 2006; 54:1327–1333. [PubMed: 16835393]
- Wegner M. From head to toes: the multiple facets of Sox proteins. *Nucleic Acids Res.* 1999; 27:1409–1420. [PubMed: 10037800]
- Weiss MA. Floppy SOX: mutual induced fit in hmg (high-mobility group) box-DNA recognition. *Mol Endocrinol.* 2001; 15:353–362. [PubMed: 11222737]
- Wonders CP, Anderson SA. The origin and specification of cortical interneurons. *Nat Rev Neurosci.* 2006; 7:687–696. [PubMed: 16883309]
- Xu J, Sankaran VG, Ni M, Menne TF, Puram RV, Kim W, Orkin SH. Transcriptional silencing of {gamma}-globin by BCL11A involves long-range interactions and cooperation with SOX6. *Genes Dev.* 2010; 24:783–798. [PubMed: 20395365]
- Yamashita A, Ito M, Takamatsu N, Shiba T. Characterization of Solt, a novel SoxLZ/Sox6 binding protein expressed in adult mouse testis. *FEBS Lett.* 2000; 481:147–151. [PubMed: 10996314]
- Yi Z, Cohen-Barak O, Hagiwara N, Kingsley PD, Fuchs DA, Erickson DT, Epner EM, Palis J, Brilliant MH. Sox6 directly silences epsilon globin expression in definitive erythropoiesis. *PLoS Genet.* 2006; 2:e14. [PubMed: 16462943]
- Yoon BS, Ovchinnikov DA, Yoshii I, Mishina Y, Behringer RR, Lyons KM. Bmpr1a and Bmpr1b have overlapping functions and are essential for chondrogenesis in vivo. *Proc Natl Acad Sci U S A.* 2005; 102:5062–5067. [PubMed: 15781876]
- Yuan H, Corbi N, Basilico C, Dailey L. Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. *Genes Dev.* 1995; 9:2635–2645. [PubMed: 7590241]
- Zhao X, He X, Han X, Yu Y, Ye F, Chen Y, Hoang T, Xu X, Mi QS, Xin M, Wang F, Appel B, Lu QR. MicroRNA-mediated control of oligodendrocyte differentiation. *Neuron.* 2010; 65:612–626. [PubMed: 20223198]

[illegible]

Fig. 1. Alignment of the human and zebrafish Sox6 amino acid sequences (CLUSTAL 2.0 multiple sequence alignment)

The human (AAK26115.1, 808 aa) and zebrafish (NP_001116481.1, 768 aa) Sox6 proteins were aligned to examine the amino acid sequence conservation between the two organisms. Overall amino acid identity is 79%. Gaps are allowed to obtain the maximum score for the alignment. The software is available at <http://www.ebi.ac.uk/Tools/clustalw2/index.html>. The stars indicate identical amino acid and caps indicate substitutions within the same subgroups of amino acids. The leucine zipper (LZ) motif and the “PLNLSS” motif are indicated by a solid-line box and a dotted-line box, respectively. The two Q-boxes and the HMG-box are highlighted with gray and black background, respectively. The two coiled-coil domains are underlined.

Fig. 2. Summary of the Sox6 protein expression patterns in different cell lineages

Sox6 expression levels are schematically expressed for the five cell lineages discussed in detail in the text. In the development of these cell types, Sox6 plays a key role during the transition from mitotic progenitors to post-mitotic cells. References used to deduce the Sox6 expression levels are: chondrocyte (Lefebvre et al. 1998), erythrocyte (Cantu et al. 2010), oligodendrocyte (Stolt et al. 2006), cortical interneuron (Azim et al. 2009; Batista-Brito 2009), and skeletal muscle (Hagiwara et al. 2007).

Table 1
The list of known Sox6 cofactors

This table shows the collection of Sox6 cofactors that are verified to physically interact with the Sox6 protein. The region of the Sox6 protein which is involved in the interaction with the cofactor and the target gene whose transcription is directly regulated by Sox6 (if it is known) are also listed. The amino acid sequence motif, “PLNLSS”, engaged in the interaction with the CtBP2 protein, is shown in the Fig. 1. The Solt protein which was originally identified as a cofactor of Sox6 expressed in the testis has been recently identified as the chromatin centromere protein CEMP-K (Okada et al. 2006).

Partner proteins	Known characteristics	Interacting Sox6 domains	Target gene and effect of Sox6	References
Sox5	transcription factor	1st coiled-coil domain	Col 2a 1, activation	Lefebvre et al., 1998
			Aggrecan, activation	Han and Lefebvre, 2008
			Matn1, activation	Nagy et al., 2011
Solt/CEMP-K	centromeric protein	leucine zipper domain		Yamashita et al., 2000
CtBP2	transcriptional corepressor	“PLNLSS” motif (Fig. 1)	Fgf3, suppression	Murakami et al., 2001
Prtb/DAZAP2	prolin-rich protein	1st coiled-coil domain		Cohen-Barak et al., 2003
PDX1	transcriptional activator	HMG box	insullin II, suppression	Iguchi et al., 2005
HDAC1	histon deacetylase	HMG box	cyclin-D1, suppression	Iguchi et al., 2007
β-catenin	armadillo family protein	1st coiled-coil domain	cyclin-D1, suppression	Iguchi et al., 2007
DAX-1	nuclear hormone receptor	1st coiled-coil domain		Ohe et al. 2009