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Review

Having it both ways: Sox protein function between conservation and innovation

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Abstract. Transcription factors of the Sox family arose around the advent of multicellularity in animals, arguing that their ability to regulate the expression of extracellular matrix, cell adhesion and signaling molecules may have been instrumental in the generation of metazoans. In particular, during vertebrate evolution, the Sox family experienced a phase of expansion that led to the appearance of groups of highly homologous Sox proteins and the division of existing Sox protein functions among group members. It furthermore allowed Sox transcription factors to acquire numerous novel functions. These past events of subfunctionalization and neofunctionalization can still be recognized today in all groups of the Sox family. They have led to partial functional redundancies, but also to interesting species-specific variations in the developmental roles of Sox proteins as shown here for the SoxB and the SoxE groups.

Keywords. Transcription factor, Sox, Sry, high-mobility-group, evolution, redundancy, multicellularity, species-specificity.

Introduction

In 1990, *Sry* was identified as the first member of the Sox gene family [1, 2]. *Sry* is quite an unusual Sox gene that occurs only in mammals where it is responsible for determination of the male sex. Its corresponding location in the sex-determining region on the Y chromosome led to the gene's acronym. Hallmark of the *Sry* gene is a high-mobility-group (HMG) box that codes for the DNA-binding domain of the protein. For this reason, Sry also belongs to the superfamily of HMG domain-containing proteins. Among HMG domain proteins, some possess multiple HMG domains that bind DNA in a sequence-unspecific manner (Hmgb/Ubf branch), whereas others contain a single HMG domain and bind in a sequence-specific manner [3, 4]. Sry belongs to this latter Tcf/Sox/Mata branch.

Proteins whose HMG domains have an approximately 50 % or higher amino acid similarity to that of Sry are referred to as Sox proteins (Sry-related HMG-box), and the corresponding genes as Sox genes (for review, see [4, 5]). Sox proteins all bind to the minor groove of DNA and recognize the common consensus motif 5'-(A/T)(A/T)CAA(A/T)G-3'. As this binding is associated with a widening of the minor groove, Sox proteins invariably cause DNA bending, which has led to the assumption that they may function as architectural proteins similar to their Tcf/Lef relatives [6]. Evidence for such an architectural function is still sparse, however [7].

Sox genes have been identified throughout the animal kingdom and are grouped according to the similarities

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in their encoded protein sequences [4, 8]. Within the Sox gene family, ten groups named SoxA to SoxH have been defined (Fig. 1A). Sox proteins of the same group usually exhibit an amino acid identity of at least 70%. In contrast, Sox proteins from different groups share very little sequence identity outside their HMG domain [4].

During the last few years, Sox proteins have been primarily characterized as important transcriptional regulators of many developmental processes and are known for their roles in stem cells, early embryogenesis, gastrulation, neural induction, formation of various organs and tissues as well as for their impact on specification and differentiation of many cell types. Whereas these have been intensely reviewed [4, 5, 9–12], little effort has so far been undertaken to put the knowledge on Sox proteins in an evolutionary perspective.

In this review we focus on these evolutionary aspects by comparing Sox proteins and their function in different species and by addressing questions of functional conservation, divergence and partitioning. A picture emerges in which some Sox protein functions are highly conserved. Additionally, a whole plethora of novel functions has been acquired by Sox proteins at different times during evolution to serve the specific developmental requirements of a particular group of animals. As a consequence, Sox proteins perform a mix of well-conserved and specifically tailored functions. The acquisition of novel functions may also have been a major driving force for the expansion of the Sox gene family in several animal groups. The generation of new Sox genes in turn caused old functions to be partitioned or to be jointly performed by several Sox proteins, the latter leading to partial functional redundancy. Although these processes could be described for all major Sox groups, we focus in this review on the SoxB and SoxE groups for which most data are available.

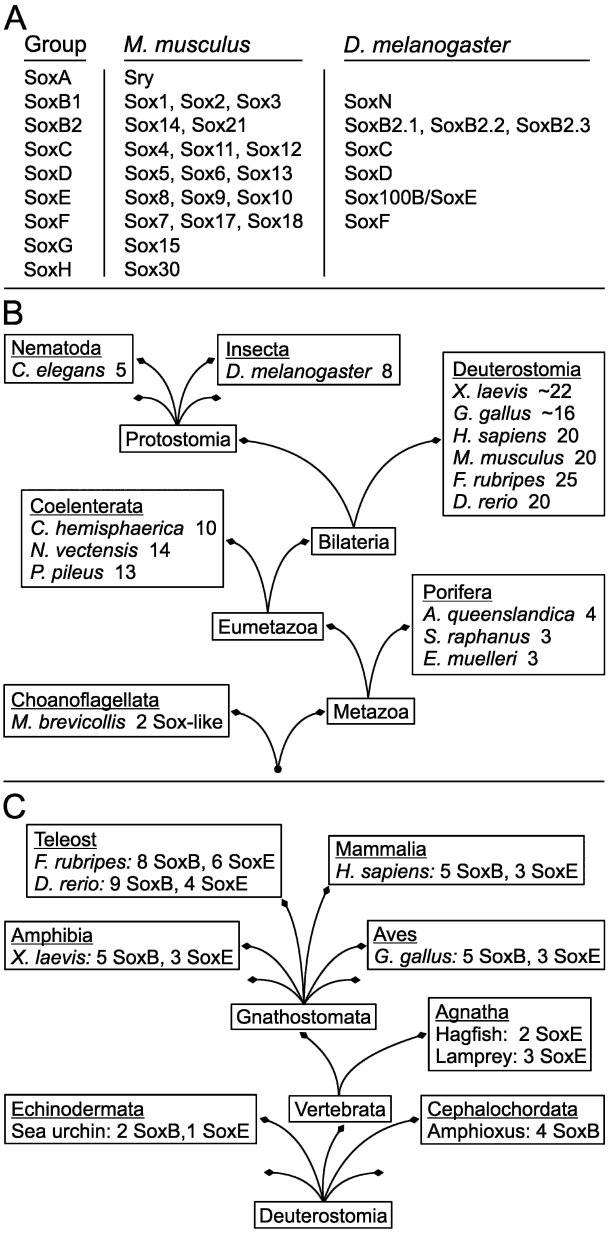

Figure 1. Sox gene phylogeny. (*A*) Overview of the Sox groups with their members in mouse and *Drosophila*. (*B*) Several species (italics) from the main metazoan branches are depicted in their phylogenetic relationships and with the total number of determined Sox genes. (*C*) SoxB and SoxE gene numbers are also separately listed for several representative species from different deuterostome lineages.

Phylogeny of the Sox gene family

Until recently, Sox genes were believed to exist only in multicellular organisms of the animal kingdom (metazoans). The *Monosiga brevicollis* genome sequence, however, revealed two Sox-like genes in this unicellular choanoflagellate [13], with the predicted HMG domains exhibiting 49–50% amino acid identity to the HMG domain of mouse Sry and human SRY. As choanoflagellates are the closest known relatives of metazoans (Fig. 1B), the origin of Sox proteins predates multicellularity. The fact that choanoflagellates express Sox-like proteins in addition to some of the signaling and adhesion molecules that are characteristic of metazoans, may even hint at a causal role of Sox proteins in the generation of multicellularity in the common unicellular ancestor of choanoflagellates and metazoans.

The mostly random distribution of Sox genes throughout the genome and the absence of Sox gene clusters argue against a model in which divergence has arisen from simple tandem duplication events [4, 5]. Instead, it is thought that following the duplication of a

common ancestor, there must have been dispersal, mutation, and acquisition events [5]. At different time points during metazoan evolution, duplicated HMG box-containing genome fragments probably recruited coding regions for novel functional domains in their neighborhood and thereby generated new potential Sox genes [14, 15]. The functional ones survived, whereas the non-functional ones were again lost from the genome [16].

Comparing the numbers and groups of Sox genes in different species gives important hints on ancestral genes, duplication events, and gene losses (Fig. 1). Metazoans can be divided in porifera (sponges) on the one side, and eumetazoans, including coelenterates and bilaterians, on the other. Three to four Sox genes were detected in three poriferan species, the freshwater sponge *Ephydatia muelleri*, the calcareous sponge *Sycon raphanus* and the demosponge *Amphimedon queenslandica* [17, 18]. These Sox genes either fall into the SoxB or SoxF groups or cannot be easily grouped (Fig. 1B). Coelenterates, as basic eumetazoans, have a much higher number of Sox genes, ranging from 10 in the cnidarian *Clytia hemisphaerica* to 13 in the ctenophore *Pleurobrachia pileus* and 14 in the cnidarian *Nematostella vectensis* (Fig. 1B) [17, 19]. Again, some of the coelenterate Sox genes cannot be easily placed into the commonly known major Sox groups. Those that can, fall into groups SoxB, SoxE and SoxF. These results have been taken as evidence that a first event of modest Sox gene expansion already occurred at the base of the metazoan tree before the divergence of the poriferan and coelenterate lineages and that the SoxB, SoxE and SoxF groups are phylogenetically old. Taking the simple body plan of these non-bilaterian metazoans and the absence of complex tissues into consideration, Sox genes must serve basic functions for multicellularity in these organisms quite distinct from the functions in organ development or sex determination for which they are best known in vertebrates.

Clear evidence for the SoxC and SoxD groups (Fig. 1A) was obtained in all bilaterian metazoans, arguing that the full complement of the major subgroups of the Sox gene family was already established early in the bilaterian branch. There is, however, a dramatic difference in the number of Sox genes between the two main bilaterian groups, the protostomes and the deuterostomes. Model protostomes such as the dipteran *Drosophila melanogaster* or the nematode *Caenorhabditis elegans* have few Sox genes, with numbers ranging between 5 and 8, corresponding roughly to one gene per Sox group (Fig. 1A, B) [20].

During vertebrate evolution, in contrast, an original set of Sox genes, which covered all major groups, probably duplicated twice and diversified again early in the lineage [21]. In agreement with such a model, there are often three closely related Sox genes for the SoxB, SoxC, SoxD, SoxE and SoxF groups (Fig. 1A). Additionally, other mechanisms were probably at work to increase Sox gene diversity. The SoxB group, in particular, has further expanded in many animal groups. In mammals, for instance, there are five SoxB genes that can be further separated into a SoxB1 and a SoxB2 subgroup. Additionally, mammals possess one SoxA (*Sry*), one SoxG (*Sox15*) and one SoxH (*Sox30*) gene, bringing the total number of Sox genes per mammalian species to 20 (Fig. 1A, B) [8]. The analysis of Sox genes in teleost fish generally supports a model in which a basic Sox gene set was duplicated twice during early vertebrate evolution. Further expansion of the Sox gene family, however, occurred in teleosts because of an additional whole genome duplication before their immense radiation [22]. As a consequence, gene pairs can in theory exist in teleosts for every gene in the major Sox groups of other vertebrates. Which of the pairs survived, differs among teleost species. In the pufferfish *Fugu rubripes*, for instance, 25 Sox genes were identified with the mammalian *Sox1, Sox4, Sox6, Sox8, Sox9, Sox10* and *Sox14* occurring as duplicated paralogs [20]. In contrast, *Sox8* and *Sox10* are not duplicated in the zebrafish *Danio rerio*.

There are two reasons for keeping duplicated genes under the selective pressure of evolution. During subfunctionalization, the functions of the original gene are split between the duplicated gene copies such that each takes over a defined subset [23]. During neofunctionalization the original gene function is passed onto one of the duplicated derivatives so that the other copy is first freed from functional constraints and then adopts new functions that bring it again back under selective pressure [24]. Often, subfunctionalization and neofunctionalization are not strictly separated so that aspects of both can be realized simultaneously. During the process of sub- and neofunctionalization, there must be a period in which both gene copies are co-expressed and share functions. For genes with functions in multiple tissues, it is conceivable that some functions are not (yet) partitioned and remain under the joint control of both gene copies.

In agreement with these considerations, genes of the same Sox group often have overlapping, but not identical, expression patterns. Sites where only one group member is expressed, usually rely heavily in their development on this particular Sox gene as evident from loss-of-function studies. Sites of co-expression, in contrast, are often less reliant on a

single Sox gene because the encoded proteins are functionally similar or even equivalent and therefore able to partially or fully compensate the loss of each other. There is no compelling reason to exclude the possibility that developmental roles could be differently partitioned among functionally equivalent Sox genes in different species. As shown below, ample evidence exists for all of this.

Phylogeny of SoxB genes

In many organisms, the SoxB group can be further divided into a SoxB1 and a SoxB2 subgroup. Where this division is not readily possible, as in non-bilaterian metazoans [17–19], it is usually the SoxB2 subgroup that is poorly defined.

In *Drosophila* as an example for protostomes, there is only a single SoxB1 member, i.e., *SoxNeuro*, whereas three representatives exist for the SoxB2 subgroup (Fig. 1A). These *SoxB2.1/Dichaete/Fish-hook*, *SoxB2.2*, and *SoxB2.3* genes are physically linked on the same chromosome arguing that they arose recently by lineage-specific tandem duplication and diversification with *SoxB2.1* probably representing the ancestral SoxB2 gene (Fig. 2A) [25].

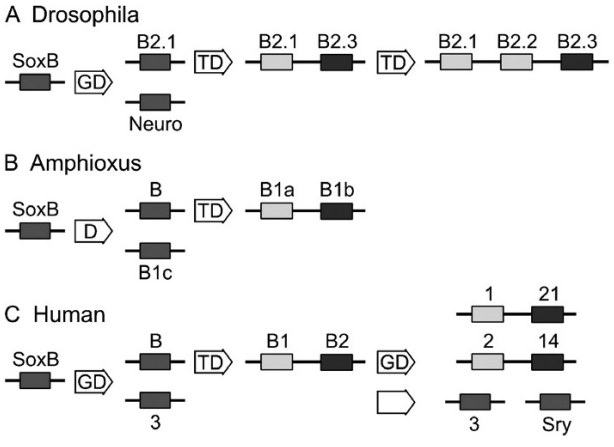

Figure 2. Hypothetical evolutionary pedigrees of SoxB genes in *Drosophila melanogaster* (*A*) amphioxus (*B*) and human (*C*) from the ancestral SoxB gene. Both whole genome duplications (GD) and tandem duplications (TD) are postulated as types of duplication (*D*). *Sry* likely developed from a *Sox3* allele during divergence of X and Y chromosomes.

Orthologs to protostome SoxB genes cannot be readily assigned in deuterostomes. Even among deuterostome lineages, orthologs are not easily identified as independent duplication events most likely occurred in the different lineages [26]. In the sea urchin *Strongylocentrotus purpuratus* and the urochordate *Ciona intestinalis*, only a single *SoxB1* gene is present. Amphioxus as a primitive cephalochordate has three SoxB1 genes and a single SoxB2 gene [26]. The first duplication event in amphioxus generated *SoxB1c* and a second gene whose consecutive duplication led to *SoxB1a* and *SoxB1b* (Fig. 2B). None of the SoxB1 genes of amphioxus is, however, a direct ortholog of a vertebrate SoxB1 gene.

The ancestral SoxB gene in vertebrates had to undergo at least three duplication events to give rise to the SoxB group in mammals (Fig. 2C). From the pair of SoxB genes generated during the first duplication, one was probably located on the chromosome that gave rise to the gonosomes. This SoxB gene evolved into *Sox3* on the X chromosome. During the differentiation event of the Y chromosome, it also gave rise to the *Sry* gene [27]. The other SoxB gene produced the hypothetical and closely spaced SoxB1 and SoxB2 genes by tandem duplication and divergence (Fig. 2C). A further joint duplication event led to the evolution of *Sox1* and *Sox21* on one chromosome and *Sox2* and *Sox14* on the other [27–29]. As a consequence, mammals possess three SoxB1 and two SoxB2 genes.

The SoxB group underwent even further expansion in teleosts. Zebrafish has three SoxB2 genes (*Sox14*, *Sox21a* and *Sox21b*) and six SoxB1 genes (*Sox1a*, *Sox1b*, *Sox2*, *Sox3*, *Sox19a* and *Sox19b*). Starting from the ancestral SoxB gene at least four duplication events must have happened. Interestingly, there is no direct SoxB ortholog for teleost *Sox19a/b* in other vertebrates. Instead, the highly divergent mammalian SoxG gene *Sox15* and *Xenopus SoxD* appear to be the closest relatives [8, 30].

SoxB genes and early embryogenesis

SoxB genes perform a wide range of functions. They are best known for their role in pluripotency and self-renewal, but have also been implicated in specification, maturation and terminal differentiation events (Fig. 3A).

Although not systematically studied, at least one SoxB gene appears to be expressed in germ cells and to be cell-autonomously involved in gametogenesis, as shown for *Sox3* in the mouse [31]. Additionally, SoxB transcripts and proteins are laid down as maternal components in the mature oocyte. In the case of mouse *Sox2* (Fig. 3B), sea urchin *SoxB1* and *Drosophila SoxB2.1*, maternal protein is present; for zebrafish *Sox19b*, *Sox21a* and *Xenopus Sox3*, maternal transcripts were predominantly found [30, 32–36]. The deposition of maternal SoxB components is probably indicative of an essential role for SoxB genes in the very early stages of development.

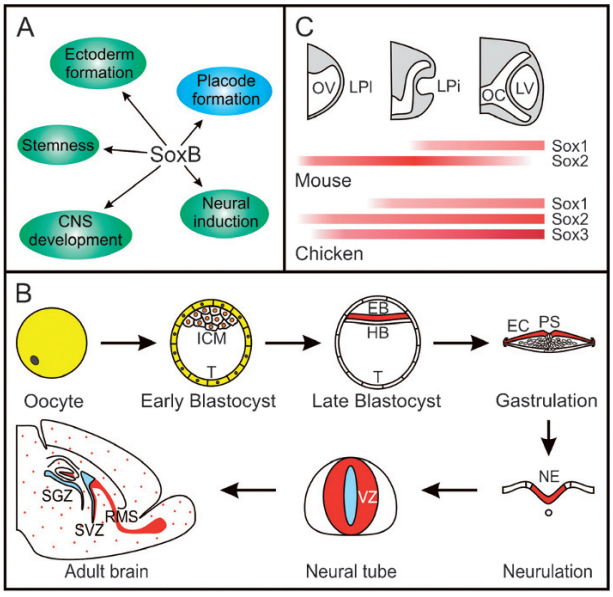

Figure 3. Developmental expression and function of SoxB genes. (*A*) Overview of ancient (green) and newly acquired, vertebrate-specific (blue) SoxB gene functions. (*B*) Sox2 expression during mammalian development. Maternal Sox2 protein (yellow) from the oocyte is still dominant in the early blastocyst, although embryonic expression (red) in cells of the inner cell mass (ICM) has set in. Note that Sox2 is nuclear in cells of the inner cell mass (ICM) and cytoplasmic in trophoblast cells (T). In the late blastocyst, Sox2 occurs in epiblast (EB), but is absent from the hypoblast (HB). Upon gastrulation, Sox2 becomes restricted to the ectoderm (EC) and is no longer present in those cells of the epiblast that enter through the primitive streak (PS) to become endoderm or mesoderm. Further confinement to the neuroectoderm (NE) occurs upon neurulation. With the onset of differentiation in the neural tube, Sox2 is found in the ventricular zone (VZ), which harbors the proliferating stem cell population. Stem cells in the adult brain retain Sox2 expression and are predominantly found in the subgranular zone of the hippocampus (SGZ), the subventricular zone (SVZ) of the lateral ventricles (blue) and the rostral migratory stream (RMS). Select populations of mature neurons also express Sox2 proteins, as indicated by dots. (*C*) Comparison of SoxB1 gene expression during lens development in mouse and chicken. Differences in the expression pattern from early placode to optic cup stage translate into different roles of SoxB1 genes in the two species and present an example of species-specific subfunctionalization. LPl, lens placode; LPi, lens pit; LV, lens vesicle; OC, optic cup; OV, optic vesicle.

In the mouse embryo, maternal Sox2 protein enters the nuclei in the two-cell stage and persists throughout preimplantation development [32]. It is sufficient to support embryonic development until implantation so that loss of *Sox2* in the embryo goes unnoticed during this phase. Embryonic *Sox2* expression sets in at the morula stage and is jointly driven by two enhancers, one upstream (SRR1) and one downstream of the gene (SRR2) [37, 38]. In the blastocyst, Sox2 occurs in the cells of the inner cell mass as a nuclear protein, whereas it is cytoplasmic in the trophoblast cells. The inner cell mass consists of the embryonic stem (ES) cells and gives rise to the complete embryo. In the transcriptional regulatory network of ES cells, Sox2 is at the top of the hierarchy. Together with the POU domain transcription factor Oct4, it directly regulates many other essential regulatory network components in ES cells such as *Nanog*, *Utf1*, *Fbx15*, *Fgf4*, *Lefty1* [39–42]. Sox2 and Oct4 additionally autoregulate their own expression [37, 43]. Activation involves cooperative binding of Sox2 and Oct4 to adjacent sites in a composite sox-oct recognition element of the gene's regulatory region, which often is a distal enhancer. For *Sox2* autoregulation, both the upstream SRR1 and the downstream SRR2 contain corresponding composite elements [37]. Whereas these composite elements are sufficient for some target genes to be activated in an ES cell-specific manner, others in addition require Klf4 binding to the target gene promoter [40]. This combination of Sox2, Oct4 and Klf4 is sufficient to induce ES cell-specific gene expression and can therefore reprogram somatic cells into induced pluripotent stem cells (iPS), an ability that is further enhanced by *c-Myc* [44, 45]. This role in stem cells may actually be a very basic and ancient SoxB gene function (Fig. 3A).

With continuing development of the mouse blastocyst, *Sox2* expression becomes restricted to the epiblast (Fig. 3B) [28, 46]. In Sox2-deficient mouse embryos, epiblast cells are no longer present [32]. They thus mark the first stage in mouse embryogenesis that is dependent on embryonic *Sox2* expression rather than the maternal protein. The mouse epiblast expresses both *Sox2* and *Sox3* [28, 46]. Nevertheless, *Sox2* appears to be dominant as *Sox3* deletion in the mouse does not have the same impact on early embryonic development as *Sox2* deletion. In the chicken embryo, however, *Sox3*, but not *Sox2* is expressed prior to gastrulation [47], arguing that subfunctionalization of SoxB1 genes in early embryogenesis may have occurred differently in birds and mammals. In agreement with such an assumption, SoxB1 gene expression is again different in zebrafish, where *Sox3* and the two *Sox19* genes are prominently expressed during the early stages of embryonic development [30].

SoxB genes are also required for early embryogenesis in *Drosophila*. Both *SoxNeuro* and *SoxB2.1* are strongly expressed in the *Drosophila* embryo from the syncytial blastoderm stage onwards, and SoxB2.1 has been shown to function as a non-classical regulator of pair-rule and segment-polarity genes downstream of gap genes [34, 48].

During vertebrate gastrulation, SoxB gene expression becomes confined to ectoderm [32, 46, 49, 50]. Studies on *Strongylocentrotus purpuratus* indicate that sea urchin *SoxB1* is essential for ectoderm formation by antagonizing β-catenin-mediated Wnt signaling in the animal cells (Fig. 3A). Vegetal cells, on the other hand,

require a β-catenin-dependent degradation of the maternal SoxB1 protein to acquire the opposing endomesodermal fate [33]. An antagonizing function of SoxB1 on mesoderm inducing activities has also been described in *Xenopus*, where maternal Sox3 represses the Nodal-related Xnr5 in the animal hemisphere [51].

SoxB genes and neural induction

Soon after ectoderm formation, SoxB1 gene expression is further restricted to the neuroectoderm both in vertebrates and in *Drosophila* [32, 46, 48, 49]. In the chicken, this restriction has been shown to be due to both active down-regulation in the nonneural and dramatic up-regulation in the neural ectoderm [47]. In addition to those SoxB1 proteins that are already expressed, additional SoxB1 genes are turned on in the neuroectoderm. In the neuroectoderm of the mouse, for instance, where *Sox2* and *Sox3* are already present, *Sox1* expression is additionally induced [28, 46].

There is ample evidence that SoxB1 proteins are essential for neuroectoderm formation and function as neural competence factors. In mammals and birds, *Sox2* is probably the essential factor, although Sox3 is expressed as well [46, 47, 52]. Sox2 is also a neural competence factor in *Xenopus*, but is not required for the initial binary decision between neural and epidermal ectoderm [49]. This is probably due to the fact that the primary neuralizing role has been taken over by *SoxD* [53]. *Xenopus SoxD* does not exist in vertebrates other than amphibians. It is furthermore not related with the SoxD genes *Sox5*, *Sox6* and *Sox13* that are present in all vertebrates. Instead, *Xenopus SoxD* is closer to the teleost SoxB1 gene *Sox19* [30]. In line with such an evolutionary relationship, zebrafish *Sox19* genes show a prominent expression during neural induction and have probably a similar function as *Sox2* in birds and mammals [30]. Judged by the same criteria, *SoxB1a* is the best candidate for a neural inductor in amphioxus [26], and in *Drosophila*, *SoxNeuro* acts during neuroectoderm specification. Despite species-specific subfunctionalization, neural induction thus represents another excellent example of the conserved role of SoxB1 proteins (Fig. 3A). SoxB1 proteins may even be part of a wider conserved neuralizing pathway, as SoxNeuro in *Drosophila* and Sox2 in *Xenopus* are regulated by analogous BMP signals and their antagonists [54, 55].

SoxB genes and early CNS development

After neural induction, SoxB genes remain prominently expressed in neuroectodermal cells and are essential regulators of early central nervous system (CNS) development (Fig. 3A, B). In the early mouse and chicken neural tube, Sox1, Sox2 and Sox3 are expressed widely and in an overlapping manner [28, 46]. As neurogenesis commences, they become restricted to the ventricular zone where the proliferative, self-renewing and multipotent neuroepithelial precursors reside (Fig. 3B). This association of SoxB1 expression with neural stem cells has not only been observed during embryogenesis, but also in the adult CNS, where particularly Sox2, but also Sox3 is found in all remaining neurogenic regions (Fig. 3B), including the subventricular zone of the lateral ventricles, the rostral migratory stream and the subgranular hippocampal layer (reviewed in [56]). Supporting the functional significance of this expression, adult neurogenesis is reduced in mice with *Sox2* mutations [57]. Interestingly, the same *Sox2* gene enhancers that are responsible for ES cell-specific expression (SRR1 and SRR2) also appear to be active in neural stem cells arguing that their activity is associated with the stem cell character [37]. Their activity is not only dependent on the binding and activity of classical transcription factors, but is in addition tightly regulated by chromatin remodeling. SRR1 activity, for instance, is dependent on the Brahma chromatin remodeler, which is always bound to the enhancer, but requires activation through displacement of the interacting HP1α/γ repressors by geminin and Bert in a complex series of events [58].

Chicken *in ovo* electroporation experiments indicate that SoxB1 genes are needed to keep neural progenitors in a pluripotent and proliferating state during early CNS development and prevent premature cell cycle exit and differentiation by counteracting and repressing the activity of proneural genes that code for bHLH transcription factors [59, 60]. SoxB1 genes furthermore appear to be equivalent in this function as all three are capable of maintaining neural progenitor identity and actively prevent neurogenesis after *in ovo* electroporation. Other hints for the existence of functional redundancy between the three SoxB1 genes during early CNS development come from biochemical studies. They indicate that all three SoxB1 proteins activate neural target genes such as *Nestin* comparably, often requiring cooperative interaction with class III POU proteins (*i.e.*, Brn1, Brn2 and Brn4) in a manner highly reminiscent of the interaction between Sox2 and Oct4 in ES cells [30, 61]. Targeted deletion studies in the mouse also support a widespread, but not complete functional redundancy

among the three SoxB1 genes in early CNS development due to largely overlapping expression. Thus, brain and spinal cord are only mildly affected in mice lacking either Sox1 or Sox3, and normal in Sox2 heterozygous mice [57, 62, 63]. If *Sox2* expression is further decreased, then neural progenitors in the retina develop aberrantly. In the absence of *Sox2*, retinal progenitors completely lose their competence to proliferate and differentiate [64]. The main reason for the lack of functional redundancy in this part of the CNS is the absence of Sox1 and Sox3 in the neural retina from the optic cup stage onwards [65, 66]. Unique occurrence of Sox2 in the mammalian retina may also be the basis for anophthalmia or severe microphthalmia in humans with heterozygous *SOX2* mutations [67].

SoxB1 proteins probably also play a role in the maintenance of neural progenitor identity in the early CNS development of teleosts. In zebrafish, *Sox19a* and *Sox19b* are widely expressed. Sox2 and Sox3, in contrast, occur only in restricted parts of the neuroectoderm, and *Sox1a* and *Sox1b* have their major expression sites later in the forebrain [30, 68]. Widespread SoxB1 gene expression has also been detected in the early CNS of non-vertebrate deuterostomes including amphioxus, *Glomeris marginata*, sea squirt, and acorn worm [69–71].

In *Drosophila*, *SoxNeuro* plays a prominent role in neuroectodermal progenitors and neuroblast formation [54, 72]. Despite *SoxNeuro* presence throughout the whole neurogenic region, loss of neuroblasts in *SoxNeuro*-deficient flies is mostly restricted to the lateral and intermediate regions of the *Drosophila* neuroectoderm [54, 72]. Severe defects in the medial neuroectoderm become only visible once *SoxB2.1* is additionally deleted. In this area, *SoxNeuro* expression overlaps with *SoxB2.1*. From the fact that both *SoxNeuro* and *SoxB2.1* single mutants display relatively mild phenotypes in the medial neuroectoderm, whereas the double mutant exhibits severe defects [54, 72], it can be concluded that both *Drosophila* SoxB proteins act in at least a partially redundant manner in the ventral medial neuroectoderm.

This partial functional redundancy is reminiscent of the redundancy seen between SoxB1 genes in early vertebrate CNS development. Similarities even appear to reach down to the molecular level as invertebrate SoxB proteins also exert their function in synergy with class III POU proteins and through their influence on proneural gene expression [54, 72–74]. Interspecies rescue experiments additionally hinted at functional conservation. Thus, it was possible to rescue the CNS phenotypes of *SoxB2.1* and *SoxNeuro* mutants in *Drosophila* with mouse *Sox2* and *Sox1* [25].

Despite the fact that *SoxNeuro* is a SoxB1 gene and *SoxB2.1* a SoxB2 gene, these two subgroups exhibit partial functional redundancy in *Drosophila* CNS development. In contrast, vertebrate SoxB2 genes do not appear to be functionally redundant to SoxB1 genes during CNS development, although *Sox21* is also widely expressed in the developing CNS of many vertebrates including mice and zebrafish [36, 75]. Vertebrate SoxB2 genes rather appear to counteract SoxB1 function in neural progenitors as evident from the fact that their overexpression in the chicken neural tube caused loss of progenitor cell identity and premature neuronal differentiation [76]. Mechanistically, vertebrate SoxB2 proteins have been proposed to target the same genes as SoxB1 proteins in the early developing CNS, but to influence their expression as transcriptional repressors in the opposite direction as the activating SoxB1 proteins [75]. Target gene expression is thus probably the result of a fine counterbalance between SoxB1 and SoxB2. In this respect, up-regulation of *Sox21* expression by proneural proteins is noteworthy [76]. By increasing SoxB2 protein amounts, proneural genes have been postulated to interfere with SoxB1 function and thereby tip the balance in a neural progenitor towards neuronal differentiation. Repressor activity has also been described for *Sox21a* in zebrafish [36]. Taking the different roles of SoxB2 proteins in vertebrates and *Drosophila* into account, it is very difficult to predict SoxB2 function in the CNS of non-vertebrate species such as amphioxus, where no experimental data are available and *SoxB2* is expressed in addition to *SoxB1a* and *SoxB1c* [26].

SoxB genes and late CNS development

During further CNS development, expression of SoxB genes diverges so that each SoxB protein becomes restricted to particular CNS regions and to distinct subsets of mature neurons. In some of these neurons, SoxB genes remain expressed throughout adulthood, arguing that SoxB genes may also function in neuronal maturation or maintenance. Sox2, for instance, is found in cortical, striatal and many thalamic GABAergic neurons, whereas *Sox1* expression is particularly strong in select areas of the ventral striatum and Sox3 occurs primarily in mature neurons of the ventral hypothalamus [63, 77, 78]. Recent studies in genetically modified mice have shown that *Sox2* is required for neuronal maturation, in particular for maturation of GABAergic neurons in cortex and olfactory bulb so that their numbers were greatly diminished and their morphology and migration patterns severely abnormal in *Sox2*-mutant mice [77]. These defects are

sufficient to explain the CNS deficits found in patients with heterozygous *SOX2* mutations, including the frequent occurrence of epileptic seizures [67]. Other SoxB1 gene deletions and mutations similarly present with neurological phenotypes that can be attributed to neuroanatomical abnormalities, neurodegeneration and disruption of neuronal circuits in the area of their preferred expression. Developmental alterations in the striatum of *Sox1*-deficient mice for instance lead to severe hyperexcitability of the piriform cortex and epilepsy [78]. *Sox3* deletion in mice or *SOX3* mutation in humans, on the other hand, affect hypothalamic development and thus lead to disruption of the hypothalamic-pituitary axis and hypopituitarism [63, 79]. This late SoxB function in neuronal maturation again appears to be conserved in protostomes, as SoxB genes also occur in distinct subsets of mature *Drosophila* neurons and as regulatory mutations of the *SoxB2.1* gene cause select neurons to disappear from the fly's tritocerebrum [48, 80].

SoxB genes and placodal development

SoxB genes are also prominent factors in the development of placodes (Fig. 3A). These vertebrate-specific structures are derived from the ectoderm and are essential for the development of sensory organs and distal cranial ganglia in these deuterostomes. Studies in medaka have shown, for instance, that *Sox3* expression precedes the actual placode formation. It occurs in all placodes, including lens and otic placodes, and continues to be expressed in placode-derived structures [68]. The importance of SoxB genes for placode development has also been confirmed in gain-of-function experiments as *Sox3* overexpression led to formation of ectopic lens and otic vesicles in the fish. These studies also showed that SoxB expression levels are critical and need to be tightly controlled as dysgenesis of the endogenous sensory organs was simultaneously observed [68]. Dose dependence of SoxB function has also been seen in the CNS in overexpression studies and may be an important aspect of SoxB action [77].

Further evidence for a functional role of SoxB genes in placode development has been obtained in mammals. *Sox2* mutations, for instance, cause severe inner ear malformations and hearing impairment in mice, as well as anophthalmia in humans [67, 81]. *Sox1*-deficient mice, on the other hand, exhibit cataracts because of a severe lens phenotype [62].

In particular, lens development has been intensely investigated (Fig. 3C). Depending on the species, either Sox2 (mouse), Sox3 (*Xenopus*) or a combination of both (chicken) are induced from basal levels in the forming lens placode when the optic vesicle comes into contact with the overlying head ectoderm [65, 82]. Sox1 follows with a significant delay so that it plays no role in the early lens placode, but rather in the differentiating lens. This role in late lens development is particularly evident in mammals where, concomitant with *Sox1* induction, *Sox2* expression is down-regulated [65], making Sox1 the only remaining SoxB protein (Fig. 3C). This also explains the development of cataract and microphthalmia in *Sox1*-deficient mice [62].

How species-specific differences in SoxB gene expression come about became evident when the regulatory regions of the *Sox2* gene were analyzed in chicken [83]. A whole series of enhancers with distinct spatiotemporal activities was identified with three enhancers being responsible for *Sox2* expression in the lens: one for early basal expression, a second one for up-regulation in the forming lens and a third one for expression during late lens development. Analogously, the combined activity of five enhancers generates the panneural expression of Sox2 in early chicken embryos. Many of the enhancers are well conserved between chicken and mammals, some even between chicken and zebrafish [30, 83]. Others, however, are poorly conserved. The enhancer important for Sox2 expression in the late developing lens of chicken is, for instance, not present in the mouse genome, thus explaining the lack of *Sox2* expression in the mouse at the corresponding stage [83, 84].

One of the transcription factors that induces *Sox2* expression in the forming lens placode of chicken is the Paired domain protein Pax6 [85]. Once present, Sox2 in turn maintains high-level Pax6 expression [86]. Interestingly, Sox2 cooperates on the corresponding *Pax6* enhancer with the POU protein Oct1, indicating that partnership between Sox and POU proteins is also relevant in the context of placode development [87].

Sox2 and Pax6, however, do not only activate each other's expression, they are also important for the cooperative induction of many lens-specific genes including the *δ-crystallins* at early phases of lens development and the *γ-* and *α-crystallins* at later phases (reviewed in [84]). As shown paradigmatically for the *δ1-crystallin* enhancer, binding of either Sox2 or Pax6 to this enhancer is only possible *in vivo* in the presence of the other and occurs at a composite sox-paired site [66]. Once a ternary complex has formed on this site, the transactivation domains appear to undergo conformational changes and organize a compound activation domain [66]. The synergy between Sox2 and Pax6 thus not only involves cooperativity between the DNA-binding domains, but also between the transactivation domains. Interestingly,

the combination of Sox2 and Pax6 protein is also important for the development of other compartments of the eye such as the retina [64], arguing that similar to the Sox-POU interaction, the Sox-Pax interaction may be relevant in many developmental contexts.

The role of SoxB genes in the vertebrate-specific placodes are a typical example of neofunctionalization (Fig. 3A). Nevertheless, evidence for related functions has been obtained in other organisms. Although placodes do not exist in cephalochordates, amphioxus *SoxB1c* is expressed in a region of the non-neural ectoderm from which sensory cells arise in this organism [26]. SoxB1 genes may thus have played a role in sensory organ formation already in the common ancestor of all chordates.

Conserved aspects of SoxB1 function during sensory organ development have even been detected in *Drosophila.* Despite great differences between the vertebrate and the *Drosophila* eye, introduction of the chicken *δ1-crystallin* enhancer into *Drosophila* led to a specific activation in the crystallin-secreting cone cells of the insect compound eye [88]. As this activation was mediated by SoxNeuro and Shaven, the *Drosophila* Pax2 ortholog, the regulatory circuits again appear remarkably conserved.

SoxB genes and development of non-ectodermal derivatives

Despite the strong ectodermal occurrence, SoxB gene expression is not completely restricted to this germ layer. SoxB genes are additionally expressed in derivatives of endoderm and mesoderm. This seems to be the case in *Drosophila* [25, 80], in amphioxus [26] and in vertebrates [89–91]. Expression in these two germ layers is, however, not contiguous with the earlier expression in the epiblast, as SoxB genes are first down-regulated during endoderm and mesoderm formation and then reappear at a later time [32]. Sox2, for instance, plays a role in the developing foregut endoderm of mammals, birds and amphibians during dorsoventral patterning and in boundary formation [89, 91]. In the mesoderm-derived osteoblasts, Sox2 inhibits differentiation by antagonizing β-catenin-mediated Wnt signaling [90].

Phylogeny of SoxE genes

How duplication, gene loss and diversification events shaped the Sox gene family is also evident in the SoxE group. In *Drosophila, Sox100B* is the only SoxE gene (Fig. 1A) [92]. Early chordates probably also possessed a single ancestral SoxE gene [93]. After vertebrate divergence, however, gene duplication events must have occurred early so that all vertebrates, be they jawless (*i.e.*, agnathans) or jawed (gnathostomes) have several SoxE genes [4, 5, 94].

In the jawless sea lamprey, three SoxE genes (*SoxE1*, *SoxE2* and *SoxE3*) have been identified so far [94], and at least two SoxE genes (SoxEa and Sox9) exist in hagfish [95]. All gnathostomes, on the other hand, possess three SoxE genes [4, 5]. Each of these *Sox8*, *Sox9* and *Sox10* genes can exist in two paralogs in teleosts, as shown for *Fugu rubripes* [20]. In other teleosts including zebrafish and medaka, two paralogs have so far only been detected for *Sox9* [96–99].

The relationship between SoxE genes in agnathans and gnathostomes appears as complicated as the previously mentioned phylogenetic relationship between SoxB genes in vertebrates and other deuterostomes. Whereas lamprey SoxE3 shows at least a limited homology to Sox9 in gnathostomes outside the HMG domain, SoxE1 and SoxE2 are not orthologs of either Sox8 or Sox10 [94, 95]. Agnathans and gnathostomes thus seem to share a common SoxE ancestor gene, whose duplicated copies, however, diversified along different routes in both groups. *Sox8* and *Sox10* therefore exclusively occur in gnathostomes and even the *Sox9* genes of agnathans and gnathostomes are only distantly related.

SoxE genes and gonadal development

Similar to SoxB genes, members of the SoxE group have probably retained a number of ancestral functions during evolution as judged from their expression in gonad, kidney and intestinal epithelium of both protostomes and deuterostomes [92, 100, 101]. Most data exist for the gonad, where SoxE genes are expressed in the somatic component (Fig. 4A). In *Drosophila*, Sox100B occurs in two male-specific cell populations that are important for testis formation and/or function [102, 103]. The Sox100B-expressing somatic gonadal precursors are originally present outside the gonad in both male and female sexes, but only survive and coalesce with other cells in the male sex to form the gonad [102]. The Sox100B-expressing pigment cell precursors, in contrast, are induced from fat body mesoderm exclusively in the male sex and enter the already existing testis at a later time [103]. A causal role of *Sox100B* in male sex determination has not been proven, but it is the earliest known sexually dimorphic marker in the *Drosophila* gonad [102].

Vertebrate SoxE genes are also found in the somatic tissue of the developing gonad in a sexually dimorphic pattern with predominant occurrence in the male sex

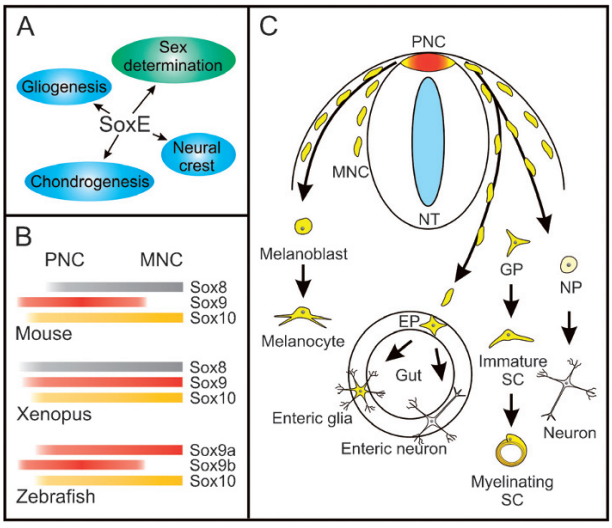

Figure 4. Developmental expression and function of SoxE genes. (*A*) Overview of ancient (green) and newly acquired, vertebrate-specific (blue) SoxE functions. (*B*) SoxE gene expression in the early neural crest of various vertebrate species from the premigratory (PNC) to the early migratory (MNC) stage. Differences in the expression pattern translate into different roles of the SoxE genes in mouse, *Xenopus* and zebrafish, and present an example of species-specific subfunctionalization. (*C*) SoxE gene expression in the mammalian neural crest and its derivatives. At the trunk level, premigratory neural crest cells (PNC) express *Sox9* (red), before they turn on *Sox10* expression (yellow) immediately prior to epithelial-to-mesenchymal transition. Migrating neural crest cells (MNC) express *Sox10* and require this transcription factor for specification to melanoblasts, precursors of the enteric nervous system (EP), glial precursors (GP) and probably neuronal precursors (NP). Even after specification, Sox10 continues to be expressed in several neural crest derived lineages and drives their differentiation to melanocytes, enteric glia and Schwann cells (SC).

[100, 104–106]. Sox9, in particular, is essential for testis cord formation, Sertoli cell differentiation and thus for overall testis development. As a consequence, male to female sex reversal is observed both in humans with heterozygous *SOX9* mutations and in mice in which gonadal *Sox9* expression is abolished [107–110]. *Sox9* overexpression in genetically female mice, on the other hand, induces testis development [111].

Interestingly, *Sox8* and *Sox10*, the other two vertebrate SoxE genes are also expressed in the developing male gonad [110, 112–114]. Although the phenotype of mice deficient for either *Sox10* or *Sox8* excludes an essential role in male gonad development, accessory functions are possible. Thus, it has been shown that even residual levels of Sox9 in the mouse gonad are sufficient to prevent male to female sex reversal. However, when Sox8 levels were additionally reduced, sex cord formation was completely blocked and sex reversal was achieved, suggesting that Sox8 reinforces Sox9 function in testis differentiation [110]. Progressive infertility in Sox8-deficient male mice furthermore points to an additional role of Sox8 in adult Sertoli cells [115].

In mammals, Sox9 functions downstream of Sry in the male sex determination pathway [116]. Despite the fact that there is no Sry in other vertebrates and that the choice of sex is triggered differently, the role of Sox9 in male sex determination appears to be conserved as Sox9 is strongly expressed in the developing testis of mammals, birds and reptiles [100, 104, 106]. One of the two teleost Sox9 paralogs is also found in the testis [96, 98, 99, 105]. Interestingly, it is Sox9a in zebrafish, whereas it is Sox9b in medaka. This argues that subfunctionalization has occurred differently in these teleost species. Even more intriguing is the fact that the second paralog occurs in the ovary. Despite their role in male sex determination, SoxE proteins are thus not completely confined to the male gonad. *Sox8*, for instance, is sex-specifically expressed only in mammals, but occurs both in the developing male and female gonads of birds and reptiles [113]. Even mammalian Sox9 is not completely restricted to the male gonad, as low Sox9 levels appear to be present in the indifferent gonad [117] before the onset of Sry-dependent up-regulation of *Sox9* in the male sex [100, 116]. This Sox9 is soon removed from the nuclei in the female gonad [118]. Export from the nuclear compartment is thought to function as a rapid means of inactivating existing Sox9 protein and is possible because SoxE proteins possess a nuclear export signal [118, 119].

Although the function of Sox9 in the development of the male gonad is well conserved among vertebrates, there are differences in its exact role. Expression of the anti-Müllerian hormone *Amh*, for instance, follows Sox9 expression in zebrafish and mammals [100, 105, 117]. In mouse, *Amh* has even been shown to be a direct target gene of Sox9 [120, 121]. Nevertheless, *Amh* expression precedes that of Sox9 in chicken and reptiles [104, 122]. It is thus unlikely that *Amh* is the only or even the major target gene for Sox9 in the male gonad and Sertoli cell differentiation. So far, however, only few other targets have been identified in the male gonad. Mammalian target genes include Vanin-1 [123], prostaglandin D synthase [124] as well as *Sox8* as a factor that reinforces Sox9 function [110] and the orphan nuclear receptor *Sf1* [125] which, once induced, cooperates with Sox9 in the synergistic activation of *Amh* [121].

SoxE genes and skeletal development

Vertebrate SoxE genes have not only preserved ancestral expression patterns and functions, they have additionally acquired novel expression sites,

particularly in those cell types and tissues that are vertebrate-specific inventions. From the fact that SoxE functions are comparable in all vertebrates studied so far, it can be inferred that the acquisition of novel functions must have occurred early during vertebrate evolution.

One prominent example of such a neofunctionalization is the endoskeleton (Fig. 4A). Sox9 plays such an essential role, especially in chondrocyte development and cartilage formation, that even haploinsufficiency leads to severe skeletal malformations, which are known as Campomelic Dysplasia in humans [100, 101, 107, 108, 126–128]. Sox9 is expressed in early mesodermal precursors from which all osteo-chondroprogenitor cells are derived. Consequently, Sox9 is already essential for the formation of mesenchymal condensations, which represent the first step in chondrogenesis [129]. However, there are additional functions for Sox9 in later steps of cartilage development, including chondrocyte differentiation, chondrocyte proliferation and prevention of their conversion into hypertrophic chondrocytes [128, 130]. In mammals, Sox9 also induces *Sox5* and *Sox6* expression in chondrocytes [130]. These two SoxD proteins then cooperate with Sox9 during the later phases of chondrocyte development [131] as evident from the fact that *Sox5/Sox6*-double deficient mice display a similar chondrodysplasia as mice in which *Sox9* was deleted in chondrocytes after mesenchymal condensation had occurred [130, 132].

In addition to *Sox5* and *Sox6*, a plethora of additional target genes for Sox9 has been identified in chondrocytes. These include the genes for the two calcium binding proteins S100A1 and S100B [133], but also the collagen genes *Col2a1*, *Col4a2, Col9a1*, *Col11a2*, and *Col27a1* [134–138], *aggrecan* and the *link protein* gene [139, 140], thus revealing a strong link between Sox9 and extracellular matrix formation in these cells. For most of these genes, the Sox9-responsive regulatory regions have been identified. They usually bind several Sox9 molecules in a cooperative manner [141, 142]. Sox9 function on these regulatory regions furthermore appears to be enhanced by Sox5 and Sox6 [131, 133] and requires functional interaction of Sox9 with different coactivators including Trap230, p300 and PGC1α [143–147]. During chondrocyte differentiation, Sox9 furthermore functions by counteracting β-catenin-dependent Wnt signaling in a manner that involves a direct interaction between Sox9 and β-catenin [148]. Such an interaction has also been noted for numerous other Sox proteins [149].

Although *Sox9* is the dominant SoxE gene in chondrocytes, other SoxE genes are also expressed. *Sox8*, for instance, exhibits strong chondrocytic expression during mouse embryogenesis, but is dispensable for their generation and differentiation [112]. Interestingly, however, *Sox8* is additionally expressed in osteoblasts as the second cell type involved in skeleton formation. In the absence of *Sox8*, osteoblasts exhibit reduced proliferation rates and differentiate prematurely, leading to osteopenia in *Sox8*-deficient mice [150].

SoxE genes and the neural crest

SoxE genes are not only important for those parts of the skeleton that are derived from mesoderm, but also for the neural-crest derived cranial parts, thus pointing to a general role of SoxE genes in vertebrate skeletogenesis [93, 151].

Cranial cartilage and bones are, however, not the only neural crest derivates that depend on SoxE genes. In fact, neural crest development provides a second paradigm of SoxE gene neofunctionalization (Fig. 4A). SoxE function in the neural crest is strongly conserved among vertebrates with SoxE genes being essential for the generation of neural crest cells, their survival and the maintenance of pluripotency (reviewed in [10]). SoxE genes thus exert functions in the neural crest that are analogous to those of SoxB1 genes in the early vertebrate CNS. Expression of these two Sox groups is, however, mutually exclusive in the early stem cell populations of neural crest and CNS, as SoxE overexpression in a preferentially SoxB-expressing precursor switches its identity from a pluripotent neuroepithelial precursor to a neural crest stem cell and vice versa [152, 153]. This has led to the assumption that SoxB and SoxE genes may function antagonistically under certain conditions.

In accord with the essential role of SoxE genes in neural crest development, at least one SoxE gene is already expressed when the early neural crest arises at the border of neuroectoderm and epidermal ectoderm. In mammals and birds, the first expressed SoxE protein is Sox9 (Fig. 4B) [154, 155]. In zebrafish, it is Sox9b with Sox9a following suit [93, 97, 98]. The neural crest expression of *Sox9b* furthermore appears to be conserved in other teleosts such as medaka [96]. Sox9 is thus the essential SoxE gene for the generation of neural crest cells in birds and mammals and for the survival and the maintenance of pluripotency in the premigratory neural crest (Fig. 4C) [152, 154]. Sox9 furthermore provides the competence for neural crest cells to undergo an epithelial-mesenchymal transition, the implementation of which additionally requires the Slug/Snail and FoxD3 proteins [154, 155]. All three transcription factors thus form a regulatory network by which neural crest cells obtain their migratory properties.

Coinciding with the transition from premigratory to migratory neural crest cells, Sox9 induces *Sox10* expression in the neural crest of birds and mammals [154]. Whereas Sox9 expression subsides in many migratory neural crest cells except those that belong to the cranial neural crest, *Sox10* expression persists throughout migration (Fig. 4B, C) [156–161]. In migratory neural crest cells, Sox10 takes over some of the functions earlier ascribed to Sox9. It keeps migratory neural crest cells in a proliferative state and helps them to survive and maintain their pluripotency [160]. As a consequence there is extensive apoptosis in the migratory neural crest of *Sox10*-deficient embryos [157, 158, 160–162]. Certain subpopulations of neural crest cells appear to be affected more strongly than others. Thus, apoptosis is particularly high in the vagal neural crest and the later wave of trunk neural crest that migrate along a dorsolateral pathway [156, 157, 161–163]. As these neural crest cells give rise to the enteric nervous system and melanocytes (Fig. 4C), respectively, these cell types are present in insufficient amounts in the presence of only one functional *Sox10* allele, thus leading to aganglionosis in the distal colon and partial pigmentation defects, a condition known as Waardenburg-Hirschsprung disease in humans [164].

In birds and mammals, the essential functions of Sox9 and Sox10 in neural crest stem cells contrast strongly with the role of Sox8. In these vertebrate classes, *Sox8* expression is the last to be induced in the neural crest [10] (Fig. 4B). There are also no obvious neural crest defects in a *Sox8*-deficient mouse model, arguing that Sox8 is dispensable for neural crest development in mammals [112]. This also appears to be the case in teleosts, as *Sox8* is not even expressed in the neural crest of zebrafish [93]. Nevertheless, all three SoxE proteins including Sox8 are capable of supporting early neural crest development with only minor differences when ectopically expressed in the chicken and *Xenopus* embryo [152, 165]. Similarly, replacement of *Sox10* by *Sox8* in the mouse does not affect early neural crest development [166].

Thus, it is likely that the differential activity of the three vertebrate SoxE genes in early neural crest development of birds and mammals is mainly caused by differences in their onset and level of expression. The latter is also supported by recent studies on the role of SoxE genes in neural crest development of *Xenopus* [167, 168]. In the amphibian, the order of SoxE gene expression is altered compared to other vertebrates (Fig. 4B). Here, *Sox8* expression precedes that of *Sox9* and *Sox10* [167, 169]. Under these altered conditions, *Sox8* now has a key role in initiating neural crest formation. This study nicely illustrates that, although the role of SoxE proteins in neural crest formation is conserved among vertebrates, the exact distribution of this function among the different SoxE proteins can vary.

SoxE proteins are, however, not only required for the generation and survival of neural crest stem cells as well as for the maintenance of their pluripotency, they also drive cell fate decisions in this population. *Sox9*, for instance, is required in cranial neural crest for the adoption of a chondrogenic fate [93, 151, 168]. *Sox10*, on the other hand, is essential for the development of neural crest cells into melanocytes and cells of the peripheral nervous system, in particular glial cells (Fig. 4C). These specification functions are conserved from teleosts and amphibians to mammals as shown both in loss-of-function and gain-of-function studies [156–159, 161, 163, 170–172]. In these cell fate decisions, SoxE genes are no longer as interchangeable as they were in early neural crest development, as evident from replacement of *Sox10* by *Sox8*. Severe melanocyte and enteric nervous system defects were observed in the corresponding mouse model despite a normal development of the early neural crest [166]. After specification, SoxE proteins continue to play essential roles in several neural crest-derived cell lineages (Fig. 4C). The best studied example is the role of Sox10 in the melanocyte lineage. Not only is Sox10 required for the induction of the transcription factor Mitf during the specification event [170, 171, 173–175], it also cooperates with Mitf during further pigment cell development in the mouse [171, 176, 177]. Certain pigmentation genes such as tyrosinase require the presence of both proteins. As a consequence, retroviral *Mitf* expression in *Sox10*-deficient mouse melanoblast cannot fully rescue melanocyte development [171]. In zebrafish, however, ectopic *Mitf* expression is able to fully rescue pigment cell development in the absence of Sox10, arguing that the only essential function of Sox10 during zebrafish melanocyte development is the induction of *Mitf* [170]. The regulatory circuits between Sox10, Mitf and downstream pigmentation genes are thus similar, but not exactly identical between zebrafish and mouse.

SoxE genes and myelinating glia

Myelinating glia present a final good example of a cell type that has specifically evolved in vertebrates and relies on SoxE proteins for its development (Fig. 4A). They consist of Schwann cells that myelinate axons in the peripheral nervous system, and oligodendrocytes that perform a comparable function in the CNS.

Schwann cells as neural crest derivatives require Sox10 for their specification in mammals as well as in zebrafish [157,158]. Recent studies on hypomorphic

Sox10 mouse mutants have furthermore shown that Schwann cells continue to rely on Sox10 for lineage progression and for terminal differentiation [178]. During these processes, Sox10 appears to exert its function by interacting with other transcription factors that are expressed stage-specifically during Schwann cell development. Thus, interaction of Sox10 with the class III POU protein Oct6 during a phase immediately prior to myelination drives the induction of the transcription factor Krox20 [179]. Interaction with Krox20 is in turn needed for the synergistic activation of myelin genes during the final phase of terminal differentiation and myelin formation as evidenced for *connexin-32* and *myelin protein zero* as major myelin components [180–182].

Oligodendrocytes of the CNS on the other hand rely on *Sox9* for their generation and specification [183], on *Sox9* and *Sox10* for their progression through the oligodendrocyte precursor state [184] and finally on *Sox10* alone for their terminal differentiation [185]. During terminal differentiation, many components of the myelin sheath including *MBP*, *PLP*, *connexin-32* and *connexin-47* are again under direct transcriptional control of Sox10 [180, 185, 186]. At least for activation of the *MBP* gene, a bHLH transcription factor of the Olig family is additionally required as interaction partner [187]. Sox10 furthermore appears to be modulated in its activity by Sox5 and Sox6, thus providing a second example in addition to chondrocytes in which these SoxD proteins modulate SoxE activity [188]. Although most of the functional studies have so far been performed in mammals, the presence of Sox10 in oligodendroglia is widely observed in many different vertebrate species [187]. In mammals, *Sox8* is also expressed in the oligodendrocyte lineage, but contributes only little to its development as evident from the fact that only a slight transient delay in terminal oligodendrocyte differentiation is observed in *Sox8*-deficient mice [189]. Replacement of *Sox10* by *Sox8*, on the other hand, is not compatible with efficient myelination, arguing that different SoxE proteins are not equivalent in this function [166].

Conclusions

Sox proteins are important regulators in many developmental processes and influence self-renewal and pluripotency of stem cell populations, but also specification and differentiation of many cell types throughout the animal kingdom. Our phylogenetic approach highlights the fact that some of these functions are highly conserved and probably ancestral, whereas others have been more recently acquired, in particular in the vertebrate lineage (Figs 3 and 4). Neofunctionalization was paralleled by an expansion of the Sox gene family in vertebrates that also required partitioning of ancestral functions.

In general, SoxB gene functions appear fairly well conserved between protostomes and deuterostomes, and even more so among vertebrates (Fig. 3A). However, a given SoxB gene in one class of vertebrates is frequently replaced in its function by another SoxB gene in another class as a consequence of different subfunctionalization as exemplified in Figure 3C. This is possible because related SoxB proteins are similar or even equivalent with regards to the ancestral functions. The one prominent exception are the vertebrate SoxB2 proteins whose function as transcriptional repressors appears poorly conserved, thus likely representing a case of neofunctionalization in the SoxB group.

SoxE genes, on the other hand, have retained several ancestral functions, but have also acquired many novel functions, particularly in vertebrate-specific cell types such as chondrocytes, neural crest cells and myelinating glia (Fig. 4A). Most of the ancestral functions are performed in vertebrates by *Sox9*, whereas *Sox8* and *Sox10* obtained novel roles. This association of *Sox9* with ancestral functions is also reflected by the fact that many regulatory regions of the *Sox9* gene are highly conserved among vertebrates [190]. In contrast, there is little sequence conservation for regulatory regions of the mammalian *Sox10* gene beyond birds [191, 192].

Although Sox proteins participate in highly diverse processes, a number of common functional aspects become apparent. Thus, it has been shown again and again that Sox proteins perform their function in synergy with partner transcription factors. In particular POU and Pax proteins are frequently employed, but other transcription factors are also used. Evidence is also accumulating that Sox proteins may additionally be involved in numerous interactions with other components of the transcriptional and chromatin modifying machineries.

Among target genes of Sox proteins, signaling molecules including transcriptional regulators are highly represented. Many Sox proteins also antagonize β-catenin-dependent Wnt signaling as part of their function. It may actually be time to analyze how stringent this link is and whether Sox proteins may even be part of the Wnt signaling system.

In addition to signaling molecules, Sox proteins also regulate many target genes that code for extracellular matrix components or cell adhesion molecules of which the myelin proteins are a very specialized form. In evolutionary terms, this influence of Sox proteins on extracellular matrix deposition and cell adhesion may have been of similar importance for the gener-

ation of multicellularity as their impact on cell signaling thus offering an explanation for the timing of their appearance on the evolutionary stage.

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