Review

Sox proteins and neural crest development

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Available online 21 July 2005

Abstract

Among the families of transcription factors expressed at the neural plate border in response to neural crest-inducing signals, Sox proteins have emerged as important players in regulating multiple aspects of neural crest development. Here, we summarize the expression of six Sox genes, namely Sox8, Sox9, Sox10, LSox5, Sox4 and Sox11, in neural crest progenitors and their derivatives, and review some aspects of their function pertaining to neural crest development in several species.

Keywords: Neural crest; Induction; Migration; Sox

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1. Introduction

At the end of gastrulation, the ectoderm of the vertebrate embryo can be divided into three major domains: the non-neural ectoderm, the neural crest and the neural plate. The neural crest is induced at the lateral edge of the neural plate through interactions with surrounding tissues. While the precise nature of the neural crest-inducing signals derived from these tissues is not yet fully understood, there is strong evidence from work in several organisms that Notch signaling and members of the Bmp, Fgf and Wnt families are implicated in this process (reviewed in [1,2]). While non-neural ectoderm and neural plate primarily give rise to epidermis and central nervous system respectively, cells of the neural crest contribute to many different lineages of the embryo. Around the time of neural tube closure and as they initiate their migration into the periphery, neural crest cells progressively adopt specific fates as a result of both intrinsic and extrinsic influences. Among others, the

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doi:10.1016/j.semcdb.2005.06.005
neural crest contributes to spinal ganglia, connective tissue, facial cartilage and bone, pigment cells, and enteric ganglia. With the exception of a region anterior to the diencephalon, neural crest arises from the entire length of the neural tube. However, neural crest from different axial levels gives rise to distinct derivatives. For example, hindbrain-derived neural crest cells migrate into pharyngeal arches to produce specific craniofacial skeletal elements, while neural crest cells in the trunk region form sensory neurons and glia.

One of the immediate consequences of the induction of the neural crest is the activation of a large number of “crest-specific” genes at the neural plate border. These factors belong to multiple families of transcriptional regulators [2–4]. Among these, several belong to the Sox factors which belong to multiple families of transcription factors [5]. Sox genes are part of a larger family of high-mobility group (HMG) proteins, defined by their electrophoretic mobility on SDS-PAGE. Sox proteins bind DNA by means of the HMG domain, allowing them to function as transcription factors. This domain is highly conserved among Sox factors and all Sox proteins appear to recognize a similar motif on the DNA (A/T)(A/T)CAA(A/T)G. According to their sequence homologies within and outside the HMG domain, Soxs have been classified into groups A–H with Sry, the sex determining factor and founding member of this family, being assigned to the SoxA group [6]. Any given Sox factor is expressed in more than one cell type. For instance, Sox9 is expressed in developing chondrocytes and in Sertoli cells of the developing testis [7–10], whereas Sox4 is expressed in the embryonic heart and adult pre-B and pro-T cells [11].

Considering (i) that all Sox proteins recognize a similar DNA binding motif; (ii) that each Sox factor is expressed in a variety of cell types; and (iii) that any given cell may co-express several Sox proteins, it is believed that Sox factors require interaction with cell type specific partner molecules to activate the appropriate set of genes [12,13].

The first evidence for a role of Sox proteins in neural crest development came from the discovery that the neural crest phenotype observed in the Dominant megacolon (Dom) mice was linked to a mutation in the Sox10 gene [14,15]. The Dom mice were discovered as a spontaneous mutation at the Jackson Laboratory and have been, for many years, a mouse model for the Waardenburg-Shah syndrome type IV. This neurocristopathy combines the features of the Waardenburg syndrome [16] and Hirschsprung’s disease [17]. The pathologies associated with this disease include sensorineural deafness and pigmentation defects characteristic of the Waardenburg syndrome and angionlionic megacolon more specific to Hirschsprung’s disease. Subsequently, Sox10 mutations were identified in a number of patients affected by Waardenburg-Shah syndrome type IV (reviewed in [18]). In the last 5 years, a number of studies in fish, frog, chick and mouse have identified several Sox family members expressed in neural crest progenitors and their derivatives. These genes appear to play important functions during the steps leading to neural crest specification, migration and differentiation.

In this review, we present a summary of the most recent findings on the role of Sox proteins during neural crest development.

2. Sox genes expression in neural crest progenitors and their derivatives

Four major Sox genes are expressed at the neural plate border: Sox8, Sox9 and Sox10, which belong to the SoxE group, and LSox5, which belongs to the SoxD group. While they all show expression in neural crest progenitors at some point following neural crest induction, there are also some differences in the onset and the sequence of induction of these genes across species. Fig. 1 summarizes the onset of expression of several Sox genes in zebrafish, Xenopus, chick and mouse neural crest progenitors. In Xenopus, Sox8 is the first Sox family member detected in the presumptive neural crest around stage 11.5 (M. O’Donnell, X. Huang, C.-S. H and J.-P.-S.J., unpublished) and is immediately followed by Sox9 at stage 12. Sox10 is not detected prior to the early neurula stage (stage 14, [20,21]). Sox8 and Sox9 are among the earliest genes activated in neural crest in the frog preceding the well-established crest markers Slug [22] and FoxD3 [23]. In contrast, in zebrafish, Sox8 does not appear to be expressed in neural crest progenitors or their derivatives and is only detected later in embryogenesis after the hatching stage [24]. Zebrafish has two orthologs of the tetrapod Sox9 that presumably arose through genome duplication: Sox9a and Sox9b [25]. Sox9b is first detected in neural crest progenitors as early as most early crest markers, including FoxD3 [24,26], while Sox10 is expressed later in neural crest progenitors [27]. In chick and mouse embryos, the expression of Sox9 and Sox10 is initiated before Sox8 expression [15,28–33] and, at least in the chick, Sox9 is the first SoxE gene expressed in the neural crest progenitors largely overlapping with FoxD3 [28]. Sox10 is also expressed in neural crest precursors in humans [34]. In the chick embryo, the non-SoxE group gene LSox5 is activated in neural crest progenitors around the same time as Sox10 [35].

There are also some differences in the relative importance of the factors promoting the neural crest expression of these genes. While the canonical Wnt pathway can activate Sox9 and Sox10 expression in Xenopus [19–21,36], in chick embryos, Wnt3a overexpression does not affect the levels of Sox9 or any other neural crest markers [28]. Sox9 misexpression upregulates Wnt3a in neural plate explants suggesting that Sox9 is acting upstream of Wnt3a during neural crest formation in the chick [28]. Alternatively, this observation may also suggest that Wnt3a alone is not sufficient to activate Sox9 in this explant system. In the chick, BMP4 induces Sox9 expression in neural plate explants [28], however, this is not the case in Xenopus [21,36]. These differences, while significant, may reflect species-specific variations in the morphogenetic and inductive processes involved in the generation of the neural crest [2].
Fig. 1. Comparative analysis of the onset of expression of Sox genes in neural crest progenitors in several species. The timing of Sox gene expression in zebrafish (A); Xenopus (B); chick (C) and mouse (D) embryos is represented. For each organism, the stages of development are shown and the beginning of gastrulation (G) and neurulation (N) are indicated by an arrow. Dotted lines indicate that the precise onset of expression has not been described for this gene. The expression of the winged-helix gene FoxD3 is provided as a reference point for neural crest induction. This diagram does not take into account quantitative differences in the levels of expression of these genes at various stages.

Following their initial expression in neural crest progenitors, and as neural crest cells initiate their migration, the same Sox genes show differential expression in neural crest derivatives. Table 1 summarizes the expression pattern of these genes in the different lineages of the neural crest. In Xenopus, prior to their emigration in the periphery, neural crest cells coexpress all three SoxE genes (Sox8, 9 and 10) at the neural plate border (Fig. 2), and these genes are maintained in a similar expression pattern in the premigratory and migratory neural crest cells up to the tailbud stage. Around that time, Sox9 and Sox10 exhibit a complementary expression pattern where Sox9 is maintained in the migrating cranial neural crest cells and downregulated in premigratory trunk neural crest cells, while Sox10 persists in the trunk and rapidly fades in the branchial arches [19,20]. Interestingly, the Sox8 expression pattern at this stage appears to be sum of Sox9 and Sox10 as it is maintained in both cranial and trunk neural crest lineages (M. O’Donnell, X. Huang, C-S H and J-P S-J, unpublished).

Sox10 is primarily detected in the peripheral nervous system in several species [15,27,30,31,34]. Sox10 is also expressed in neural crest-derived melanoblasts and persists in some melanoblasts [15,20,27]. In frog, chick and human embryos, Sox10 is also transiently expressed in cranial neural crest as they populate the pharyngeal arches [20,31,34]; however, Sox10 expression is not maintained in the ectomesenchymal derivatives of the cranial crest [20,31,34]. In mouse [15,30] and zebrafish [27] embryos, Sox10 is not expressed in the migrating cranial crest and appears to be confined at all times to the trunk lineage. During development of the peripheral nervous system, Sox10 expression persists in differentiating glia, but not in cells that are committed to the neuronal lineage [28,30].
Table 1
Sox genes expression in neural crest progenitors and their derivatives

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>Cloning</th>
<th>Expression in NC progenitors</th>
<th>Expression in NC derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sox8</td>
<td>Fish</td>
<td>[24]</td>
<td>−</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>Frog</td>
<td>AY324658</td>
<td>α6</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Chick</td>
<td>[33]</td>
<td>+ [33]</td>
<td>+ [33]</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>[30]</td>
<td>+ [32]</td>
<td>+ [32]</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>[70]</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sox9</td>
<td>Fish (a)</td>
<td>[25]</td>
<td>+ [24]</td>
<td>+ [24,25]</td>
</tr>
<tr>
<td></td>
<td>Fish (b)</td>
<td>[25]</td>
<td>+ [24,26]</td>
<td>+ [24,25]</td>
</tr>
<tr>
<td></td>
<td>Frog</td>
<td>[19]</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Chick</td>
<td>[80]</td>
<td>+ [28]</td>
<td>+ [28]</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>[7]</td>
<td>+ [29]</td>
<td>+ [7]</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>[57]</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sox10</td>
<td>Fish</td>
<td>[27]</td>
<td>+ [27]</td>
<td>+ [27]</td>
</tr>
<tr>
<td></td>
<td>Frog</td>
<td>[20,21]</td>
<td>+ [20,21]</td>
<td>+ [20]</td>
</tr>
<tr>
<td></td>
<td>Chick</td>
<td>[31]</td>
<td>+ [31]</td>
<td>+ [31]</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>[81]</td>
<td>+ [15,30]</td>
<td>+ [15,30]</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>[64]</td>
<td>+ [34]</td>
<td>+ [34]</td>
</tr>
<tr>
<td>Sox11</td>
<td>Fish</td>
<td>[44,45]</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Frog</td>
<td>[46,47]</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Chick</td>
<td>[49]</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>[42]</td>
<td>+ [42,43]</td>
<td>+ [42,43]</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>[50]</td>
<td>+ [50]</td>
<td>+ [50]</td>
</tr>
</tbody>
</table>

The numbers are the references describing the expression pattern of the listed genes in various species. “+” and “−” indicate whether the gene is expressed or not expressed in a specific tissue, respectively. A blank space indicates that the expression has not been examined or reported for that gene and species. PNS, peripheral nervous system; NC, neural crest.

<table>
<thead>
<tr>
<th>Gene</th>
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<th>Expression in NC progenitors</th>
<th>Expression in NC derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sox9</td>
<td>Fish (a)</td>
<td>[25]</td>
<td>+ [24]</td>
<td>+ [24,25]</td>
</tr>
<tr>
<td></td>
<td>Fish (b)</td>
<td>[25]</td>
<td>+ [24,26]</td>
<td>+ [24,25]</td>
</tr>
<tr>
<td></td>
<td>Frog</td>
<td>[19]</td>
<td>+ [19]</td>
<td>+ [19]</td>
</tr>
<tr>
<td></td>
<td>Chick</td>
<td>[80]</td>
<td>+ [28]</td>
<td>+ [28]</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>[7]</td>
<td>+ [29]</td>
<td>+ [7]</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>[57]</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sox10</td>
<td>Fish</td>
<td>[27]</td>
<td>+ [27]</td>
<td>+ [27]</td>
</tr>
<tr>
<td></td>
<td>Frog</td>
<td>[20,21]</td>
<td>+ [20,21]</td>
<td>+ [20]</td>
</tr>
<tr>
<td></td>
<td>Chick</td>
<td>[31]</td>
<td>+ [31]</td>
<td>+ [31]</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>[81]</td>
<td>+ [15,30]</td>
<td>+ [15,30]</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>[64]</td>
<td>+ [34]</td>
<td>+ [34]</td>
</tr>
</tbody>
</table>

By contrast, in all species examined, Sox9 expression in the peripheral nervous system has not been reported. Sox9 is primarily restricted to the pharyngeal arches that will develop into a number of cranial skeletal elements [7,19,24,25,28] and in cardiac neural crest derivatives, at least in chick and mouse embryos [8,37,38]. In the chick embryo, both Sox8 and Sox9 are expressed in regions of the heart, some of which receive contribution from the cardiac neural crest, in addition to other regions derived from the cardiac mesoderm [37]. On the other hand, Sox10 does not appear to be expressed in the neural crest component of the heart [31]. In the mouse, while Sox9 is expressed in the endocardial cushions [8,39], the entire heart tissue is negative for Sox9 expression throughout development [32,39]. However, Sox9 is strongly detected in the pharyngeal arches, similar to Sox9 [32,33,39]. Sox9 expression is also associated with enteric neurons of the gut and dorsal root ganglia in the chick and mouse embryo [32,33,39], following a pattern similar to Sox10 in these tissues.

Regarding the expression of non-SoxE group genes, in the chick, LSox5 mRNA is found in the premigratory cranial neural crest and at lower levels in the trunk neural crest. Although LSox5 is maintained in the peripheral glia in the head region [35], it is not clear whether the peripheral glial expression of LSox5 extends to more caudal regions of the embryo to include sensory and enteric ganglia. In both chick and mouse embryos, Sox4, a member of the SoxC group, is not expressed in neural crest progenitors but is detected in the neural crest component of the pharyngeal arches and in the endocardial cushions and ridges of the heart that receive a contribution from the cardiac neural crest [40,41]. In the chick embryo, Sox4 is also detected in dorsal root ganglia [41]. Finally, Sox11, another member of the SoxC group, is expressed ubiquitously in the early mouse embryo excluding the heart primordium [42,43]. However, later in embryogenesis Sox11 is detected in the heart, primarily in the outflow tract [43]. In zebrafish and Xenopus, two orthologs of Sox11 have been isolated [44–47]. In these species, Sox11 does not appear to be expressed in neural crest precursors, but is restricted to the neural plate and the pre-placodal region [44,45,48]. In all species examined so far, Sox11 is later expressed in the peripheral nervous system [42,43,49,50] in both glial and neuronal lineages [42,43,49] and the pharyngeal arches [42,43,45].

The sequential expression of Sox genes, first in neural crest progenitors and later in specific and distinct lineages of the neural crest, indicates that Sox genes may carry out distinct functions during the multiple steps of neural crest development. Defining the precise timing of expression of these genes
Fig. 2. Expression of Sox8, Sox9 and Sox10 in Xenopus neural crest progenitors. (A) Schematic representation of a transverse section through a neurula stage embryo. Dorsal is at the top; the dotted lines mark the midline of the embryo. ep, presumptive epidermis; en, endoderm; nc, neural crest (arrow); no, notochord; np, neural plate; so, somite. (B) Overlapping expression domains of Sox8, Sox9 and Sox10 in neural crest progenitors (arrows) detected by in situ hybridization on section. In each case, only the left side of the stained embryo is shown and is equivalent to the portion of the embryo represented on the left side of the dotted lines in panel (A).

and their relationship to one another in neural crest progenitors and their derivatives is therefore critical to understanding their function in the neural crest. For instance, Sox9 and Sox10 appear to have largely non-overlapping domains of expression in various neural crest derivatives, whereas Sox8 shares substantial overlap with both genes in several neural crest domains. This predicts possible functional redundancy between Sox8 and other SoxE group genes.

3. Sox function in neural crest development

Combinations of knockout, knockdown and gain of function approaches have been developed to address Sox function in neural crest development. Table 2 is an attempt to summarize these studies in different species. In the next sections, we briefly describe some of these findings for each individual Sox gene.

### 3.1. Sox8

Mouse embryos carrying a mutation in the Sox8 gene are characterized by idiopathic weight reduction. Homozygous Sox8 mutant mouse are viable and do not exhibit any neural crest phenotype [32]. In these mutant embryos, the lack of a neural crest phenotype has been explained by the possible redundant function of Sox9 and/or Sox10 because their expression overlaps substantially with Sox8 in neural crest progenitors [32]. Later in embryogenesis, Sox8 and Sox10 are both expressed in the developing enteric nervous system. While inactivation of one Sox10 allele is sufficient to elicit agangliogenesis of the gut (a pathology characteristic of Hirschsprung disease), homozygous Sox8 mutant mouse embryos develop normal enteric ganglia. What is the basis for this unilateral compensatory activity of Sox8 by Sox10 in the enteric nervous system? It has been proposed that this difference could be due to unequal amounts of Sox8 and Sox10 proteins in the developing embryo [32,51]. In the central nervous system, Sox8 and Sox10 are both expressed in the oligodendrocyte lineage and a similar argument has been raised to explain the milder impact on oligodendrocyte development observed in Sox8 deficient embryos as compared to Sox10 heterozygous embryos [52].

Recently, the same group provided evidence that Sox8 acts as a modifier gene in Sox10 mutant embryos [51]. Mice carrying a mutation in the Sox10 gene exhibit agangliogenesis of the distal portion of the colon. Loss of Sox8 increases the penetrance and severity of the gut phenotype in Sox10 heterozygous mice. These animals have impaired colonization...
of the gut due to increased cell death in the migrating vagal neural crest before it is able to enter the gut, suggesting that Sox8 and Sox10 are both required to maintain this neural crest population [51]. Aganglionic megacolon is also observed as a result of a mutation in the gene encoding endothelin-3 [53] or its receptor, endothelin receptor B [54]; however, in this case, endothelin-3-mediated signaling is required for the terminal migration of enteric neuron precursors rather than for their survival [55]. A remaining question is whether Sox8 has similar modifier activity in these backgrounds. Recently, interaction between another SoxE gene, Sox10, and endothelin receptor B mutant embryos. In other species, the precise function of Sox8 remains largely unknown. In misexpression experiments in the chick embryos, Sox8 can promote neural crest formation in the chick neural tube [28], a property also shared by the two other SoxE genes, Sox9 and Sox10 [28], and LSox5 [35].

3.2. Sox9

Mutations in one allele of Sox9 are found in humans affected by a condition known as Campomelic dysplasia, a skeletal dysmorphology syndrome characterized by sex reversal and skeletal malformation of endochondral bones often associated with a number of craniofacial defects [57,58]. Sox9 heterozygous mutant mice present major skeletal defects, including a shortened jaw and cleft palate reminiscent of Campomelic dysplasia [59]. Since Sox9 homozygous mutant embryos die midway through gestation, it has been difficult to determine whether specific subsets of cranial crest derivatives are affected in embryos lacking Sox9 function. Using the Cre-loxP recombination system, conditional inactivation of Sox9 in the Wnt1 expression domain has been generated, resulting in a neural crest specific inactivation of Sox9 [60]. Sox9-deficient embryos using Wnt1-Cre lack endochondral skeletal elements derived from the cranial neural crest, while neural crest cells in the trunk appear to develop normally. These defects result not from abnormal migration or increased apoptosis in the cranial neural crest compartment, but from an inability of the postmigrating cranial neural crest cells to differentiate into chondrocytes [60].

In Xenopus, morpholino-mediated knockdown of Sox9 generated embryos exhibiting a specific loss of early neural crest markers, including Slug, Snail, Pax3, FoxD3 and Twist [19]. Further, induction of neural crest progenitors in Xenopus is regulated by Wnt signaling and this process appears to be largely dependent on Sox9 function as Wnt-mediated neural crest induction is inhibited in the context of Sox9-depleted embryos [36]. Altogether, these observations argue for an early function of Sox9 in neural crest specification. Later in embryogenesis, the loss of neural crest progenitors observed in morpholino-injected embryos correlated with a number of defects in skeletal elements derived from the cranial neural crest without affecting the development of melanocytes, a trunk crest derivative [19]. However, because the antisense morpholinos block the function of Sox9 early on, it is unclear whether the craniofacial phenotype arises exclusively as a direct consequence of depleting these embryos from neural crest progenitors or if it reflects a later function for Sox9 in the differentiation of cranial neural crest derivatives.

In zebrafish, two orthologs of the Sox9 have been isolated: Sox9a and Sox9b [25]. In Sox9a mutant zebrafish, neural crest specification and migration is normal. Furthermore, Sox9a has been shown to play a later role in chondrocyte differentiation by regulating the expression of col2a1 and the morphogenetic process involved in the stacking of chondrocytes [61]. Sox9b is expressed earlier than Sox9a in the neural crest [26]. A recent analysis of Sox9b mutants revealed distinct roles of Sox9a and Sox9b in neural crest and cartilage development [24]. This illustrates how subfunction partitioning of zebrafish co-orthologs of the tetrapod Sox9 gene can expose both an early and a late requirement for Sox9 function in neural crest development [24,61]. In overexpression studies, Sox9a and Sox9b have a positive regulatory influence on each other’s expression and cause ectopic expression of several of early neural crest markers, consistent with an early function of Sox9 in neural crest formation [24].

Recently, Sox9 was conditionally inactivated in male and female germ lines using the Cre-loxP recombination system to generate embryos completely lacking Sox9. These Sox9-null mutants showed massive cell death in the trunk neural crest population prior to or shortly after delamination [62]. However, increased apoptosis has not been observed in the context of Sox9-deficient embryos using Wnt1-Cre [60], in Xenopus that received injection of a Sox9 morpholino or an inhibitory mutant of Sox9 [19,36] and in zebrafish Sox9 mutants [24,61]. Based on these conflicting results a role for Sox9 in survival of neural crest progenitors remains unclear. In mouse, Sox9 mutant embryos maintain a normal expression of FoxD3 [62], which is in contrast to what was reported in Xenopus and zebrafish where Sox9 and Sox9b morpholino-treated embryos, respectively, show reduced FoxD3 expression [19,24]. These differences across species may reflect differences in the timing of expression of these genes in neural crest progenitors (Fig. 1). In zebrafish, loss of Sox9b, Sox9a or both did not interfere with the expression of another crest-specific transcription factor, ifap2 [24]. Similarly, in mouse embryos lacking Sox9, Ap2 was unaffected [60], thereby positioning Ap2 upstream of Sox9 in the cascade leading to neural crest specification. Consistent with this view, Sox9 has been shown to be downregulated in Xenopus embryos that received injection of Ap2a morpholino [63].

In Xenopus, an inducible inhibitory mutant of Sox9 [36] activated at the neurula stage therefore bypassing the early requirement for Sox9 in neural crest specification, showed no effect on cranial neural crest migration providing evidence that neural crest migration does not require Sox9 function in Xenopus [36]. Similarly, experiments in the zebrafish indicate that Sox9 activity is not required for cranial neural crest induction.
crest migration as dlx2a expression in the branchial arches is only marginally affected in Sox9a, Sox9b or double mutant embryos [24]. Gain of function experiments in the chick embryo indicate that while Sox9 can promote crest-like behaviors in trunk neural plate cells, Sox9 is not sufficient to induce delamination of these ectopically generated neural crest cells [28].

Finally, conditional inactivation of Sox9 using Wnt1-Cre has shown that Sox9 is required for the development of some cardiac neural crest derivatives in the heart, as Sox9 mutant embryos have defective endocardial cushions [38].

3.3. Sox10

Humans, mice and zebrafish carrying mutations in Sox10 [14,15,27,64] suffer from defects in vagal/trunk neural crest derivatives (enteric ganglia, pigment cells, and dorsal root ganglia), but not in cranial (craniofacial cartilages and bones) or cardiac (outflow tract of the heart) neural crest derivatives [65], suggesting a strict requirement for Sox10 in the vagal and trunk neural crest. Neural crest appears to form normally in the absence of Sox10 function. For example, neural crest-derived embryonic structures are present in Dom homozygous embryos [14,15,66,67] suggesting that Sox10 is probably not required for initial neural crest specification. Further, the appearance of neural crest-derived embryonic structures in Sox10 mutant embryos indicates that the initial migration of at least some neural crest cells is unperturbed in the absence of Sox10. This suggests that Sox10 is not required for neural crest cells migration either. However, the postmigratory neural crest cells that form and migrate in the absence of Sox10 function undergo apoptosis before they can differentiate [15,65,68], pointing towards a role of Sox10 as a survival factor.

The zebrafish colourless (cls) mutant that carries a mutation in Sox10 shows a specific loss of non-ectomesenchymal derivatives [27]. By labeling premigratory neural crest cells in cls mutants, it was shown that differentiation into non-ectomesenchymal fates was rarely observed in these mutants. Instead, most labeled cells failed to migrate and underwent cell death, also supporting a role for Sox10 in trunk/vagal neural crest survival [27].

In Xenopus, Sox10 morpholinio knockdown blocks Slug, Sox9 and FoxD3 expression suggesting that Sox10 is involved in neural crest specification [21]. However, considering that in the embryo Slug, Sox9 and FoxD3 precede Sox10 expression in neural crest progenitors [20], this result may suggest that Sox10 is more likely to be involved in maintaining the expression of these genes in neural crest progenitors. Misexpression of Sox10 in Xenopus can upregulate Slug expression and at later stages these embryos have ectopic expression of the melanocyte marker Trp2 [20], suggesting that Sox10 is sufficient to activate the regulatory cascade involved in melanocyte formation.

Ectopic expression of Sox10 in the chick neural tube at the trunk level induced expression of HNK-1 in neuroepithelial cells [69]. This was followed by extensive emigration from all levels of the dorsoventral neuraxis, including from the floor plate [69], which is in contrast to what has been reported for Sox9 misexpression [28]. Since these neuroectodermal cells misexpressing Sox10 failed to express neuronal, Schwann, or melanocyte markers, it has been proposed that these cells were maintained in an undifferentiated state [69].

In vitro studies using rat neural crest cells also support the view that Sox10 may function in maintaining neural crest cell multipotency [70]. Bmp2 promotes the differentiation of rat neural crest cells into neurons and, at the same time, extinguishes glial potential. On the other hand, Tgf-β causes rat neural crest cells to differentiate as smooth muscle and represses neuronal fate. In rat neural crest cells expressing Sox10, Bmp2 and Tgf-β could no longer extinguish glial and neuronal potential in these cells, respectively [70]. These results indicate that Sox10 has the ability to sustain both neurogenic and glial potential, thereby, maintaining multipotency in rat neural crest cells. This is a feature shared by another family member, Sox2, which has been shown to be required for stem cell maintenance in the early mouse embryo [71].

3.4. LSox5

In the chick embryo, the long form of Sox5 (LSox5) was isolated in a screen for genes activated at embryonic stages 8–11, corresponding to the onset of migration of the newly formed cephalic neural crest. As such, LSox5 is expressed in premigratory and migratory cephalic neural crest, and also in the trunk neural crest [35]. Similarly to Sox8, Sox9 and Sox10 [28], LSox5 misexpression in the dorsal neural tube induces ectopic HNK1-positive cells in the chick dorsal neural tube [35]. Further characterization of these cells indicate that they also express a broad range of neural crest markers, including Slug, Foxd3, Sox10, Pax7 and RhoB. RhoB is a small GTPase implicated in cell shape remodeling associated with neural crest delamination [72]. Consistent with this observation, LSox5 electroporated cells down-regulate N-cadherin and adopt a mesenchymal phenotype. Interestingly, in the cephalic lateral and ventral neural tube electroporated cells expressing RhoB were able to exit the neural tube, but these cells failed to express neural crest markers, indicating that they did not fully acquire neural crest traits [35]. This observation suggests that LSox5 is not sufficient to generate neural crest fate in the lateral neural plate. This is in contrast to Sox9, which has the ability to induce the neural crest marker HNK1 even when misexpressed in the lateral neural tube [28]. However, Sox9 expressing cells were rarely able to delaminate from these lateral regions. Consistent with this observation, Sox9 is not sufficient to activate RhoB when misexpressed in the lateral region of the neural tube [28]. This difference between Sox9 and LSox5 activity could be explained by the fact that upon L-Sox5 misexpression in the dorsal neural tube, where Sox9 is already expressed, the combination of both factors promotes neural crest fate.
and their delamination. In the lateral region of the neural tube, where Sox9 is not expressed, LSox5 alone fails to activate neural crest markers, but is sufficient to upregulate RhoB and promote migration of these cells. Conversely, in the lateral neural tube, Sox9 misexpression is sufficient to promote neural crest fate but not their migration, an activity that requires LSox5 and is only observed when Sox9 is expressed dorsally, within the LSox5 expression domain. It has been previously shown that LSox5 cooperates with Sox9 and Sox6 to promote chondrogenesis [73]. LSox5 and Sox9 may also cooperate in the context of the neural crest to promote neural crest specification and its delamination.

3.5. Sox4

In amniotes, the cardiac neural crest contributes to the conotruncal cushions and to the aorticopulmonary septum, that divides the truncus arteriosus into the pulmonary artery and the aorta [74]. Sox4 is expressed in the neural crest derived ventricular outflow tract, and in Sox4-deficient mice, the endocardial ridges fail to fuse to generate a septum. As a consequence, semilunar valves never form [40]. These mutant mice have been proposed as an animal model for “common arterial trunk”, a rare congenital heart anomaly defined by defects in the outlet portion of the interventricular septum [75]. Because Sox4 is also expressed in the mesenchyme of the branchial arches and as the ductus arteriosus and the distal portion of the aortic arch are derived from the fourth and sixth branchial arch, respectively, variable defects of the arterial duct have also been described in Sox4-deficient mouse embryos [75]. However, since Sox4-deficient mice die early in embryogenesis, it is not known whether Sox4 is also required for craniofacial development.

3.6. Sox11

Mouse embryos with targeted deletion of Sox11 have a broad range of defects reflecting Sox11 expression in multiple tissues [43]. Sox11 deficient embryos die shortly after birth with defects at sites where extensive tissue remodeling is taking place during embryogenesis. Affected neural crest-derived tissues include craniofacial skeleton, which presents extensive clefting of the upper jaw and lips, associated with cleft palate, consistent with the expression of Sox11 in the cranial neural crest [43]. Ventricular septation defects and malformation of the outflow tract of the heart, similar to those observed in Sox4-deficient embryos [40,75], have also been described in these mutant embryos [43]. However, it is unclear whether these defects result from disruption of endocardial cells or cardiac neural crest cells, which both contribute to the outflow tract tissue.

3.7. Sox2

In the chick, Sox2 is expressed throughout the prospective neural plate. Misexpression of Sox2 in the neural fold inhibits neural crest formation [76]. This observation suggests that the neural plate-specific SoxB1 gene, Sox2, may function in repressing neural crest fate within the neural plate. These results are somewhat in conflict with a previous report showing that Xenopus animal explants expressing Sox2 and Fgf were able to produce neural crest along with neural plate markers [77,78]. However, since interaction between the neural plate and non-neural ectoderm have been shown to promote neural crest fate, the expression of neural crest markers in these explants is likely to be secondary to the induction of neural plate tissue by Sox2 and Fgf. This work in the chick [76] suggests that contiguous and non-overlapping expression domains of Sox genes might be involved in setting up the neural crest-neural plate boundary in the developing embryo.

4. Perspectives

Despite the large body of work generated in the last few years on the role of Sox proteins in neural crest development, still a great deal of work is needed to fully understand the function of this important class of transcription factors in the multiple steps leading to neural crest induction and diversification. Common themes in Sox function during neural crest development include survival, migration, differentiation and maintenance of multipotency. Several of the Sox genes expressed in the premigratory neural crest appear to share some of these features; however, it is still unclear whether these genes are functionally equivalent during embryogenesis.

One major step in further understanding Sox function in the development of the neural crest requires identifying downstream target genes and defining how they are regulated in a cell type specific manner. It is now well established that the presence of a consensus of Sox binding sites in the regulatory sequence of a given gene is not sufficient criterion to define it as a potential target gene [12,13]. The search for partner molecules that cooperate with individual Sox proteins in a cell-specific manner is an essential step in this direction.

Acknowledgements

We thank Dr. Trish Labosky and Christine Credidio for comments on the manuscript and Dr. Young-Hoon Lee for help in the preparation of Fig. 2B. We apologize to colleagues whose work is not cited due to space limitations.

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suggests the reciprocal loss of ancestral gene expression patterns in sex determination.


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