

SOX10

Cis-Regulatory Elements

Comparative genomic sequence analysis identified 8 regions of highly conserved sequence within the 64.5 kb region upstream of *Sox10*. Three of these regions are deleted in the *Sox10^{Hry}* mouse, which displays reduced *Sox10* expression at E9.5, and subsequent phenotypes of hypopigmentation and megacolon. One of the 3 deleted conserved regions displays enhancer activity in vitro, and contains 2 predicted binding sites for retinoic acid receptor and 3 binding sites for SOX family members. This is the first described enhancer of *Sox10* (Antonellis et al., 2006).

Construction of a transgenic construct containing the 2.8kb of 5' flanking sequence upstream of *Sox10*, followed by exons 1-3 of *Sox10*, followed by an in-frame LacZ/Neo reporter construct demonstrated that **the 2.8kb region upstream of the *Sox10* locus is not sufficient for directing normal *Sox10* expression**, as measured by B-gal expression. Transgenic lines carrying a 218-kb BAC clone (**Sox10^{GeoBAC}**) did confer normal *Sox10* expression patterns, and three spontaneous deletions of some of the transgenic lines showed reduced expression in various tissues, demonstrating the importance of various conserved regions (Deal et al., 2006).

Transgenic B-galactosidase reporter constructs were generated under control of 7 conserved non-coding regions at the *Sox10* genomic locus. Analysis of the resulting B-gal expression patterns during embryonic development showed that some of these regions conferred cell-specific expression in the otic vesicle, oligodendrocytes, developing peripheral nervous system and adrenal gland. **None of the regions conferred melanoblast expression in developing embryos**, even though one region (region U1) had been previously identified (Antonellis et al., 2006) and shown to demonstrate enhancer activity in cultured melanocytes and to be within a deleted region of the *Sox10^{Hry}* mouse, which displays hypopigmentation and megacolon (Werner et al., 2007).

Analyses were performed on eleven multi-species conserved regions of non-coding sequence at murine *Sox10* (*Sox10*-MCSs), which were located 5' of the transcription start site, at the transcriptional start site, and within intron 1. In vitro analyses showed that eight of the eleven regions tested showed enhanced activity in *Sox10*-expressing lines. When expression constructs using the mouse MCS sequence were injected into zebrafish embryos to generate transgenic zebrafish lines, different constructs were able to target *Sox10* expression to specific neural crest- and non-neural crest-derived tissues; six of these targeted expression to developing melanocytes. Notably, this zebrafish transgenesis assay revealed not only differences in expression patterns directed by the various MCS regions but also temporal differences in expression. Two constructs, which directed expression that coincided with most of the endogenous *sox10* expression patterns, were analyzed further by generating transgenic mice harboring LacZ under the control of each of these regions. The fact that the expression patterns in these transgenic mice recapitulated endogenous *Sox10* expression as well as the expression seen in the zebrafish transgenic constructs verified the utility of zebrafish transgenics to analyze expression regulation of *Sox10* during neural crest development. Detailed analyses of these two regions demonstrated that the presence of dimeric, head-to-head SOXE family binding sites were functionally significant to the enhancer capabilities of these regions. Identification and analysis of additional dimeric SOXE binding sites, both upstream of zebrafish *sox10* and throughout the mouse genome, supported the functional relevance of dimeric SOXE binding sites for enhancer activity, and suggest that in the absence of overt sequence conservation across species, cis-regulatory regions may be identified by searching for functional motifs (Antonellis et al., 2008).

Analysis of intron 1 of zebrafish *sox10* demonstrated complex transcriptional regulation of a region within the 3' end of intron 1. Comparative sequence analysis showed conservation of binding sites for Sox9b, Notch, and B-Catenin; transgenesis in zebrafish embryos demonstrated that these three proteins were able to activate reporter constructs containing the putative regulatory region contained in intron 1 of *sox10*.

Additional analyses also showed the presence of numerous Lef1 binding sites and that Lef1 was able to bind within this region and activate transcription. When zebrafish and mammalian sequence were compared, the order and relative positions of these binding sites were conserved ([Dutton et al., 2008](#)).

***Sox10* expression was directly activated in immortalized mammary gland epithelial cells by the TRAP/Drip/mediator complex, which includes Mediator complex subunit 1 (MED1, also known as PBP).** MED1 directs the binding of this complex with nuclear receptors, and the complex subsequently functions to activate gene transcription. **MED1 is recruited to and activates the *Sox10* promoter at two multi-species conserved sequences (MCS4 and MCS7)** that were previously shown by in vivo reporter assays to be capable of directing the majority of endogenous *Sox10* expression patterns. Re-expression of SOX10 in MED1-depleted cells was able to compensate for the growth inhibition caused by MED1 knockdown ([Zhu et al., 2009b](#)).

A transgenic mouse line was generated that contained Cre expressed under the control of the MCS4 regulatory region located upstream of *Sox10*. Subsequent crosses with Cre-inducible R26R:LacZ reporter mice showed Cre expression was consistent with previously known SOX-expressing neural crest-derived tissues (forming craniofacial skeleton, dorsal root ganglia, sympathetic ganglia, enteric nervous system, aortae, and melanoblasts), as well as in oligodendrocytes and the ventral neural tube ([Stine et al., 2009](#)).

Analysis of the *Sox10* genomic region in chick identified two distinct enhancer regions located approximately 1kb downstream of *Sox10*. One, termed Sox10E1, regulates *Sox10* expression in later migrating vagal and trunk neural crest cells in chick. Interestingly, the other region, termed Sox10E2, regulates *Sox10* expression exclusively in chick early cranial neural crest, and a variety of assays showed that this region was directly bound and activated by SOX9, ETS1, and cMYB, at binding sites showing relatively high levels of cross-species conservation ([Betancur et al., 2010](#)). Further analyses of SOX10E2 regulation in the otic placode showed that **while the same binding motifs within SOX10E2 are used in neural crest and otic placode, different SOX and ETS transcription family members are used in each tissue**, with SOX9, ETS1, and cMYB in neural crest, and SOX8, PEA3, and cMYB in otic placode ([Betancur et al., 2011](#)).

A new BAC-transgenic mouse was generated, where the Venus fluorescent protein was inserted at the *Sox10* locus, thus providing the complete regulatory machinery required for mimicking *Sox10* expression with fluorescent signal. **The transgene-directed fluorescence recapitulates endogenous embryonic *Sox10* expression, including melanoblasts**, and the fluorescent marker allows visualization of *Sox10* expression in live cells ([Shibata et al., 2010](#)).

By generating mice susceptible to melanoma (*Nras*(Q61K)::*Ink4a*^{-/-}) as well as deficient for Activating Transcription Factor 2 (*Atf2*) in melanocytes, it was shown that **lack of ATF2 function resulted in decreased melanoma susceptibility. Further analysis showed that ATF2 directly binds upstream of *Sox10*, regulating *Sox10* expression and that of downstream target genes, including *Mitf*. In normal mouse and human melanocytes as well as approximately 50% of melanomas, ATF2 inhibits *Sox10* expression, while in a subset of melanomas, ATF2 activates *Sox10* expression.** Further analyses showed that ATF2 heterodimerizes with JunB to inhibit *Sox10* expression, while ATF2 activation of *Sox10* expression in melanoma occurs when ATF2 and JunB can no longer heterodimerize ([Shah et al., 2010](#)).

An 8268bp deletion along with a 10bp insertion occurring 14kb upstream of the chicken *Sox10* locus was shown to be the cause of the Dark brown (DB) plumage color, in which eumelanin is reduced and pheomelanin increased in distinct plumage pigmentation patterns. This region partially overlaps with the region homologous to the murine *Hry* deletion, including the evolutionarily conserved region *Sox10*-MCS7 ([Antonellis et al., 2008](#)). However, in contrast to *Hry* mice, which show widespread neural crest defects, *DB* only affects melanocyte eumelanin levels, even in the homozygous state ([Gunnarsson et al., 2011](#)).

A neural crest specific enhancer that regulated *Sox10* expression during later stages of chick neural crest development was identified in a study that created expression constructs for persistent expression

in neural crest. This 3571bp enhancer was located at -10,762 bp to -7192 bp upstream of the chicken *Sox10* locus, and was active during later stages of neural crest development (Yokota et al., 2011).

A new BAC transgenic mouse was generated, *Sox10*-H2BVenus, in which the fluorescent Venus protein, fused with Histone 2B, was inserted under control of the *Sox10* genomic regulatory regions. This construct allowed live cell visualization of the nuclei of cells that normally express *Sox10*, including identification of putative melanoblasts, mitotic cells, and cells undergoing cell death. This mouse model differs from the *Sox10*-Venus construct generated by Shibata et al., 2010, which labels cell cytoplasm (Corpening et al., 2011).

A *Sox10*-IRES-Venus transgenic mouse was generated, in which the gene encoding Venus protein was inserted at the *Sox10* locus downstream of the *Sox10* stop codon, thus not interfering with normal levels of *Sox10* expression. **Subsequent analyses of neural crest cells from these transgenic animals in vitro determined that SOX10+; KIT+ as well as SOX10+; KIT- cells, were multipotent, giving rise to neuronal, glia, or melanocyte cell types** (Motohashi et al., 2011).