

SOX10

Downstream Targets

Several studies have demonstrated that **SOX10 activates *Mitf* transcription**, however **contradictory data exists concerning whether PAX3 and SOX10 act synergistically to activate transcription of *Mitf*** (Bondurand et al., 2000, Lang and Epstein, 2003, Lee et al., 2000, Potterf et al., 2000, Verastegui et al., 2000). These discrepancies may in part be due to the use of variably sized *Mitf* promoter constructs.

In cultured cell lines, **SOX10 was shown to strongly activate *Mitf*, and in HeLa cells this activation was enhanced by the presence of PAX3**. Crosses of mice harboring mutations in *Sox10* and *Mitf* demonstrated synergistic spotting, confirming the *in vivo* genetic interaction of *Sox10* and *Mitf*. In vitro transcription assays highlighted one SOX binding site in the *Mitf* promoter (immediately 5' of the PAX3 binding site) to be of primary importance in transcriptional activation, and also suggested that C-terminally truncated *Sox10* mutations that do not affect the N-terminus and HMG domain may act as dominant negatives (Potterf et al., 2000).

Transfection assays confirmed activation of *Mitf* by SOX10, showed the importance of the SOX binding site adjacent to the PAX3 binding site, and demonstrated **PAX3 enhances the activation of *Mitf* by SOX10 in HeLa cells**. Additionally, in situ hybridization on E12.5 *Sox10^{Dom/Dom}* embryos demonstrated the absence of *Mitf*-expressing cells (melanoblasts) and a significant reduction of *cKit*-expressing cells, with only presumed mast cells remaining (Bondurand et al., 2000).

The activation of *Mitf* by SOX10 was also demonstrated in mouse B16 melanoma cells and NIH3T3 cells, however no synergy between SOX10 and PAX3 was observed in either of these cell lines. *SOX10* mutations found in WS4 individuals were shown to be unable to activate *Mitf* transcription, thus giving a molecular explanation for the pigmentary defects and deafness seen in WS4 (Verastegui et al., 2000).

The SOX10 MIC mutant, which is associated with WS4 and which truncates SOX10 protein 3' of the HMG domain, **was shown to act as a dominant negative**. Within the *Mitf* promoter, two SOX10 binding sites were shown to both be critical for SOX10 activation of the promoter. One is located immediately 5' of the PAX3 binding site (the same site identified by Potterf et al., 2000), and the other consists of two overlapping SOX10 binding sites located 3' of the PAX3 site. **Transient transfection assays showed no cooperative binding between SOX10 and PAX3 in numerous cell lines, and showed no synergy between SOX10 and PAX3 activating the *MITF* promoter**(Lee et al., 2000).

Comparative analysis of PAX3-SOX10 co-activation of *MITF* and *c-RET*, which functions in enteric nervous system development, demonstrated differential actions at these two promoters. **SOX10 and PAX3 physically interact and synergistically activate the *MITF* and *c-RET* promoters. SOX10 and PAX3 both must independently bind the *MITF* promoter, while at the *c-RET* promoter protein-protein interactions between SOX10 and PAX3 (via the HMG domain and paired domain, respectively) facilitate promoter binding**. These differences explain why *SOX10* mutations in Yemenite Deaf Blind Syndrome, which abolish DNA binding but allow transactivation to remain, affect melanocyte development but not enteric ganglia development. Specifically, in enteric ganglia, PAX3 recruits SOX10 to the *c-RET* promoter, while in melanocytes PAX3 is unable to recruit SOX10 to the *MITF* promoter, because SOX10 must be able to bind DNA independently (Lang and Epstein, 2003).

cAMP responsive element binding protein (CREB) requires SOX10 to be present in order for CREB to mediate the stimulatory effects of alpha-MSH on *Mitf* (Huber et al., 2003).

Analysis of sequences surrounding the *Tyrp1* locus using BAC transgenics and transient transfections showed that **a transcriptional enhancer located 15kb upstream of the transcriptional start site directed**

melanocyte-specific *Tyrp1* expression. Transient transfection assays showed **SOX10 specifically transactivates a reporter construct under control of this *Tyrp1* enhancer**, with one of 3 potential SOX binding sites within the enhancer showing the strongest effects (Murisier et al., 2006). This enhancer is in a similar location to the SOX10 enhancer of *Tyr* (Murisier et al., 2007).

A combination of in vivo analyses in mice harboring pigment gene mutations revealed that ***Dct* transcription in melanoblasts is controlled by SOX10**. In *Sox10^{Dom}* heterozygous embryos, *Dct* shows a unique pattern of transiently low expression at E11.5-E12.5 followed by recovery of expression at later developmental time points and recovery of near-normal pigmentation postnatally, suggesting a specific requirement for wildtype SOX10 protein levels at this time period in the melanoblasts. In vitro transient transfections confirmed that SOX10 is able to activate transcription at the *Dct* promoter (Potterf et al., 2001). Correlating data was found from analysis of *Dct*-expressing cells in *Sox10^{LacZ}* transgenic mice. In comparison to wild type embryos, while *Sox10^{LacZ}/Sox10^{LacZ}* homozygotes showed a complete absence of either *Dct*- or *Mitf*-positive cells, *Sox10^{LacZ}/+* heterozygotes showed only 12% *Dct*-positive cells, as compared to 50% *Mitf*-positive cells (Britsch et al., 2001). Two subsequent studies used a variety of assays to **confirm the interaction of SOX10 with the *Dct* promoter, and also demonstrated this binding was synergistic with MITF** (Jiao et al., 2004, Ludwig et al., 2004a). Out of 6 potential SOX10 binding sites, one dimeric and two monomeric SOX10 binding sites were identified and shown to be functional (Ludwig et al., 2004a). Conversely, Jiao et al. found a different SOX10 binding site located farther upstream of the *Dct* promoter, consisting of two SOX10-binding sites in inverse orientation, and showed it to be essential for transcriptional activation in melanocyte/melanoma cells. Dimeric binding was not demonstrated. This site appeared cell-type specific, as the effect of removing this site is minimal in 293T cells (this perhaps explains the differing results from Ludwig et al, who performed an initial analysis in neuroblastoma cells) (Jiao et al., 2004).

***Ednrb* expression was downregulated in *Sox10^{Dom}* homozygous embryos**, suggesting SOX10 regulates *Ednrb*. However this lowered expression may result from increased apoptosis of melanoblasts, therefore reflecting fewer *Ednrb*-expressing melanoblasts, rather than downregulation of *Ednrb* expression levels (Southard-Smith et al., 1998).

Transient transfection of human melanoma cell lines and melanoblasts with ***SOX10 siRNA* caused downregulation of SOX10 and EDNRB** expression at both the RNA and protein levels. Interestingly, SOX10 downregulation by siRNA resulted in increased BRN2 expression in melanoblasts, but decreased BRN2 expression in melanoma cell lines (Cook et al., 2005).

Human SOX10 activates EDNRB in human melanoma cell lines, either alone or together with the transcription factor SP1. This was demonstrated by downregulation of *EDNRB* transcript following *SOX10* siRNA application in human melanoma cells. Also, a variety of binding assays showed that, in melanocyte cell lines, SOX10 does not utilize the 3 potential binding sites within a previously identified enteric nervous system enhancer for *EDNRB*, but binds to two CA-rich regions and the GC box within the -12 to -105 *EDNRB* promoter region. This binding is in combination with binding of the transcription factor SP1. In addition, expression of SOX10 in HeLa cells can induce *EDNRB* expression after application of a demethylating agent to remove methylation present at the *EDNRB* locus in HeLa cells (Yokoyama et al., 2006).

Detailed analysis of mice harboring mutations in *Sox10* and *Ednrb* or *Sox10* and *Edn3* revealed interactions between these genes during melanocyte development. Increased white spotting occurred in *Sox10^{Dom+};Ednrb^{s/s}* double mutants and in the majority of *Sox10^{Dom+};Edn3^{ls/ls}* pups. Melanocytes of the inner ear were at wildtype levels in *Sox10^{Dom+}*, *Ednrb^{s/+}*, and *Sox10^{Dom+};Ednrb^{s/+}* mice, were reduced in 4 out of 6 *Ednrb^{s/s}* mutants, and were either severely reduced or completely absent in *Sox10^{Dom+};Ednrb^{s/s}* mutants. In addition, double mutants of *Sox10/Ednrb* and *Sox10/Edn3* showed increased lethality and more severe enteric nervous system defects relative to single mutants (Stanchina et al., 2006).

Generation of a *Sox10* transgenic mouse with *Sox10* expressed under the control of the *Dct* promoter demonstrated that **overexpression of *Sox10* was unable to rescue *Ednrb* mutant hypopigmentation**. No increase in hypopigmentation was seen in *Sox10^{Dom+};Ednrb^{s-/+}* double heterozygous mutants, suggesting no

direct genetic interaction between *Sox10* and *Ednrb*. In situ hybridization and neural crest culture analyses also showed that **EDNRB does not activate *Sox10*, and that *Ednrb* expression persists in migrating neural crest cells in *Sox10* mutant embryos** at E10.5 ([Hakami et al., 2006](#)).

In mouse melanocytes, **SOX10 activates the *Tyrosinase (Tyr)* enhancer**. This was demonstrated indirectly by lineage-directed gene transfer of *Mitf* into cells lacking functional SOX10. In these cells, *Mitf* expression in the absence of SOX10 allowed melanoblasts to survive and to express several pigmentation genes with the exception of *Tyr*, showing that SOX10 itself is required for *Tyr* activation ([Hou et al., 2006](#)).

Analysis of a *Tyr* distal regulatory element, located approximately 15kb from the transcription start site and containing a central enhancer region, showed that **SOX10 directly binds to and activates this *Tyr* enhancer region**. SOX10 binding motifs in the *Tyr* enhancer are evolutionarily conserved across mammals, and transient transgenics demonstrated that the SOX10 binding motifs are required for *Tyr* enhancer activity ([Murisier et al., 2007](#)).

Comparative genomics combined with differential expression profiling and chromatin immunoprecipitation led to the identification of **SOX10 itself as a direct target of SOX10 in human Schwannoma cells (*PLP*, *SOD3*, and *PTN* were also identified)** ([Lee et al., 2008](#)).

SOX5 competitively binds to the promoters of *Mitf* and *Dct* in Neuro2a cells and in B16 mouse melanoma cells, thus functioning to inhibit SOX10's ability to activate transcription of these genes. Similar modulation of SOX10 and SOX9 function by SOX5 had been previously shown in oligodendrocytes. SOX5 was shown to recruit the transcriptional co-repressors CtBP2 and HDAC1 to the promoters of *Mitf* and *Dct*. The long isoform of SOX5 performs this modulation of SOX10 function, and does not complex with SOX10 at any promoters. SOX5 is expressed in early melanocytes (E10.5-E12.5), but then expression is downregulated by E16.5. SOX5 and SOX10 do not influence each other's transcription ([Stolt et al., 2008](#)).

SOX10 was shown to activate the *Jagged1 (Jag1)* promoter in luciferase assays. JAG1 activates the Notch signaling pathway and is necessary for development of the inner ear prosensory domain ([Breuskin et al., 2009](#)).

***In vitro* luciferase assays showed that SOX10 could synergize with SF1 to activate transcriptional targets normally found in testis in a similar manner to SOX9**. These targets were promoter and enhancer sequences found at the Anti-Muellerian hormone (*Amh*) promoter and upstream of *Sox9*, respectively ([Polanco et al., 2010](#)).

In melanoma, SOX10 was shown to act synergistically in two apparently independent pathways: 1, with PAX3 and 2, with MITF to activate expression of *MET*. SOX10 alone did not activate transcription of *MET* through binding at a predicted SOX site in the *MET* promoter, but PAX3 was shown to recruit SOX10 to the *MET* promoter ([Mascarenhas et al., 2010](#)).

Analysis of conserved enhancer regions at the human *ERBB3* locus demonstrated that **an enhancer, termed ERBB3-MCS6 and located within Intron 1 of ERBB3, is directly bound by SOX10**. SOX10 binding to a single site in ERBB3-MCS6 is necessary and sufficient for *ERBB3* expression *in vitro*. *In vivo*, zebrafish assays demonstrated that ERBB3-MCS6 directs reporter gene expression in a neural crest pattern consistent with endogenous *ErbB3* expression, and SOX10 binding to ERBB3-MCS6 is necessary for *ErbB3* expression ([Prasad et al., 2011](#)).

Expression of the MADS domain transcription factor MEF2C in the neural crest/ melanocyte lineage is regulated by direct binding of SOX10 to an intronic enhancer (*Mef2c-F1*). Further analyses showed that MEF2C also binds to *Mef2c-F1*, thus exhibiting autoregulation, and **MEF2C and SOX10 heterodimerize at *Mef2c-F1* and synergistically activate transcription**. MEF2C appears to play a role in regulation of melanocyte development, as MEF2C neural crest knockout mice have melanoblasts/melanocytes defects, including reduced melanocyte number and reduced melanosomes ([Agarwal et al., 2011](#)).