

PAX3

Downstream Targets

Transfection assays using constructs of the human *MITF* promoter region showed that PAX3 transactivates the *MITF* promoter. A variety of mutant and synthetic constructs demonstrated that **PAX3 binds to and activates transcription from the PAX3-consensus binding motif located at positions -262 to -240 of the *MITF* promoter**. WS1-associated mutant PAX3 proteins (containing homeodomain or paired domain mutations) were unable to recognize and activate *MITF* promoter constructs, suggesting that the lack of *MITF* activation by PAX3 causes the auditory/pigmentation defects of Waardenburg syndrome 1 (WS1) (Watanabe et al., 1998).

Contradictory data exists concerning whether PAX3 and SOX10 act synergistically or simply additively to activate transcription of *Mitf*, with the majority of the data supporting synergism (Bondurand et al., 2000, Lang and Epstein, 2003, Lee et al., 2000, Potterf et al., 2000, Verastegui et al., 2000).

In HeLa cells, **SOX10 was shown to strongly activate *MITF*, and this activation was enhanced synergistically by the presence of PAX3** (Potterf et al., 2000).

Transfection assays in HeLa cells showed PAX3 synergistically enhances the activation of *MITF* by SOX10. Two PAX3 binding sites are present in the *MITF* promoter, and deletion constructs showed that, in HeLa cells, the PAX3 binding site located at -26 to -40 is of primary importance for transcriptional activation (Bondurand et al., 2000).

Transient transfection assays in mouse B16 melanoma cells and NIH3T3 cells demonstrated SOX10 and PAX3 do not act synergistically to activate *Mitf* (Verastegui et al., 2000).

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. A previous study showed that in enteric ganglia, PAX3 and SOX10 bind and synergistically activate the tyrosine kinase *c-RET*, which is associated with Hirschsprung disease (Lang et al., 2000). This study confirmed that **SOX10 and PAX3 physically interact and synergistically activate the *MITF* and *c-RET* promoters. However, 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**. Specifically, in enteric ganglia, PAX3 can recruit mutant SOX10 to the *c-RET* promoter and allow synergistic activation, while in melanocytes the *MITF* promoter cannot be synergistically activated by PAX3 in combination with mutant SOX10 (Lang and Epstein, 2003).

Transient transfections showed **PAX3 represses *N-CAM*** (Chalepakis et al., 1994b).

PAX3 activates the *c-Met* protooncogene, which encodes the tyrosine kinase receptor for hepatocyte growth factor/scatter factor. *c-Met* is downregulated in Splotch mice, and upregulated in rhabdomyosarcoma cases where the PAX3-FKHR fusion protein is present (Epstein et al., 1996).

In situ hybridization and RT-PCR analyses showed that decreased *Pax3* levels result in increased expression of the cell cycle regulatory gene *Cdc46* along the developing embryonic neural tube. This suggests **PAX3 functions to inhibit *Cdc46***, and that overexpression of *Cdc46* in the context of *Pax3* mutant embryos may contribute to developmental defects (Hill et al., 1998).

Transient transfection assays showed that **PAX3 binds and activates the *Tyrp1* promoter in B16 melanoma cells**. This paper also identified PAX3 as MSF, the previously uncharacterized melanocyte specific factor ([Galibert et al., 1999](#)).

A variety of assays showed that **PAX3 positively and directly regulates transcription of the mRNA encoding the anti-apoptotic protein BCL-XL**, suggesting that PAX3 inhibits apoptosis in part by activation of BCL-XL ([Margue et al., 2000](#)).

PAX3 microarray analysis was performed using cDNA derived from DAOY cell lines (human medullablastoma) that had been stably transfected with mouse *Pax3*. Analysis of microarray results combined with promoter screening for putative paired domain and homeodomain binding sites found 17 new **potential downstream targets of PAX3**. These included **TGF-beta2, GOS2, Amyloid A4 precursor, Pre-proalpha2(I) collagen, Matrix gla, TSC-22, TIMP-3, thyroid transcription factor, and NF-KE**. Targets identified that had previously been postulated included **versican, Dep1, MARCKS, Myelin basic protein, and STX**. Binding assays confirmed PAX3 bound to and activated the STX promoter. **Seventeen additional genes without available promoter sequence but with 5-fold or greater changes in expression were also identified** ([Mayanil et al., 2001](#)).

Using cyclic amplification and selection of targets (CASTing) of PAX3, 28 putative targets from mouse and human were identified. The authors surmised based upon expression pattern, putative function, or altered expression based upon PAX3 expression levels, that the following genes were likely targets: *Itm2A, Cdkn1c, Celsr1, Chrb3, Fath, FLT1, TGFA, and BVES* ([Barber et al., 2002](#)).

Comparison of wild type and mutant PAX3 protein binding to *MITF* and *TRP1* promoter sequences revealed that PAX3 regulates these two target genes through different modes of binding. PAX3 utilizes both the paired domain and homeodomain to bind to the *MITF* promoter with high affinity, even though the binding sites for both domains are non-canonical and independently show low affinity (ie. the two domains must be within the same protein to bind). In contrast PAX3 utilizes only the paired domain to bind the *TRP1* promoter with moderate affinity. PAX3 mutations associated with WS showed disparity in their actions on the two promoters, reflecting the differential recognition employed by PAX3; for example, the G48A mutation showed no change in *TRP1* binding but an increase in *MITF* binding, while the R53G mutation showed completely absent *TRP1* binding but unchanged *MITF* binding ([Corry and Underhill, 2005](#)).

Analysis of PAX3 regulation of *Mitf* in melanocytes and cardiomyocytes showed that **PAX3 regulates *Mitf* via the melanocyte-specific M-promoter in melanocytes, but not via the heart-specific H-promoter in cardiomyocytes**. This specificity of PAX3 for melanocyte-expressed *Mitf* transcripts was shown by transfections of PAX3 with M- and H-promoter *Mitf* constructs into NIH3T3 cells, and also by transfected PAX3 activating endogenous *Mitf* in melanocytes, but not activating endogenous *Mitf* in cardiomyocytes ([Tshori et al., 2006](#)).

PAX3 directly activates *Tgf2b* transcription, and in situ hybridization showed reduced levels of *Tgf2b* transcript in the developing neural crest of *Pax3* E10.0 mutant embryos ([Mayanil et al., 2006](#)).

PAX3 binds and negatively regulates expression of the tumor suppressor *Pten* ([Li et al., 2007](#)).

Microarray analysis with a mouse oligo gene array, using melan-a cells transfected with three *PAX3* isoforms with differential activity in vitro (*PAX3c*, *PAX3e*, and *PAX3g*) identified 109 genes with greater than two-fold differential expression. Only 11 of these genes showed similar expression patterns with all three *PAX3* isoforms, and the rest showed variable induction or repression among the 3 different *PAX3* isoforms, suggesting that these ***PAX3* isoforms have different downstream targets**. Potential *PAX3* downstream targets newly identified and confirmed by RT-PCR and Western blotting were *Kitl, Dhh, Msx1, Fgf17, and Rac1*, and previously identified targets that were seen included *Met, MyoD, and Muc18* ([Wang et al., 2007](#)).

PAX3 directly regulates the secretory signaling glycoprotein *Wnt1*, as shown by a 40% reduction in *Wnt1* transcript levels in *Pax3* null mouse embryos, the presence of a functional PAX3 binding site in the *Wnt1* promoter starting at position -130, and the direct binding of PAX3 to the *Wnt1* promoter in vivo ([Fenby et al., 2008](#)).

Neural crest cultures from E10.0 *Pax3^{Sp/Sp}* embryos exhibited impaired migration of neural crest cells and also showed increased expression of *Brn3a*, indicating premature neurogenesis. Immunohistochemical analysis of *Pax3^{Sp/Sp}* embryos also displayed increased *Brn3a* expression. In situ hybridization and quantitative RT-PCR showed that *Pax3^{Sp/Sp}* embryos showed reduced expression of the basic helix-loop-helix transcription factors Hairy and enhancer of split homolog-1 (*Hes1*) and Neurogenin2 (*Ngn2*) during neural crest development. *Hes1* functions to maintain neural stem cells, while *Ngn2* regulates differentiation of neuronal subtypes from neural crest. In vitro reporter constructs and gel shift assays along with in vivo chromatin immunoprecipitation experiments showed that PAX3 directly binds to the promoters of *Hes1* and *Ngn2*. These data suggest that **PAX3 acts during at least two developmental timepoints during neural crest development: first, to maintain neural stem cell properties via its activation of *Hes1*, which inhibits differentiation; and later, to promote differentiation of the neuronal lineage via activation of *Ngn2*** ([Nakazaki et al., 2008](#)).

Generation of mice that were doubly mutant for *Pax3* and *Tgfb*, as well as in vitro transcription assays using reporter constructs, indicated that **the genetic regulatory pathways of PAX3 and TGF β interact in developing neural crest, and have opposing effects on downstream molecules that include *Hes1*, *Ngn2*, and *Sox9*** ([Nakazaki et al., 2009](#)).

Mice harboring both *Kit* and *Pax3* ENU-induced mutations showed synergistic spotting, suggesting interaction between PAX3 and KIT genetic pathways. A variety of transfection assays showed that PAX3 activates the *Kit* promoter. However, this activation appears to be indirect, as no direct binding of PAX3 was shown at the *Kit* promoter ([Guo et al., 2010](#)).

To identify different PAX3-regulated pathways in normal skin melanocytes and melanoma cells, **PAX3 downstream targets were compared in HEM1455 cells (human primary melanocytes) and A2058 cells (metastatic melanoma) using ChIP and quantitative RT-PCR. Melanoma-specific PAX3 targets were identified, including NES, TPD52, BCL2L1, and PTEN.** In addition, **PAX3 binding to target genes utilized in both HEM1455 and A2058 was stronger in A2058 (melanoma cells) as compared to HEM1455**, with significantly higher PAX3 binding seen at SOX9, MCAM, and CSPG4 in A2058 cells ([Medic et al., 2011](#)).