Transgenic mouse analysis demonstrated that the 1.6 kb putative promoter region upstream of Pax3 directs gene expression in the dorsal neural tube, but is not sufficient to direct somite or craniofacial expression (Li et al., 1999, Natoli et al., 1997). Deletion analyses suggested two distinct regions within the 1.6kb promoter were necessary for Pax3 expression in the neural tube (Natoli et al., 1997).

Four sub-regions contained within the previously identified two regions of the -1.6 kb Pax3 promoter were identified by electrophoretic mobility assays using promoter deletion constructs expressed in P19 embryonic carcinoma cells stimulated with retinoic acid. The proteins identified that bound to these four sub-regions were BRN1, BRN2, and members of the PBX and MEIS families, with evidence that PBX members complex with HOX family members at two of these sites, thus demonstrating Pax3 is regulated by both neural and anterior-posterior transcription factors. Specifically, BRN2 together with HOXA1 showed significant activation of the Pax3 promoter. Analysis of transgenic mice carrying Pax3 promoter-gal constructs harboring mutations in the four sub-regions suggested that two of the binding sites function as transcriptional activators, and the other two as repressors (Pruitt et al., 2004).

Further analysis of the 1.6 kb region upstream of Pax3 identified two highly conserved regions, named NCE1 and NCE2. NCE1 overlapped completely with one of the regions previously identified by (Natoli et al., 1997). Deletion of either of these regions abolished dorsal neural tube-directed expression in -gal transgenics and a minimal promoter construct containing both of these regions was sufficient to direct -gal expression in the neural tube and neural crest derivatives. TEAD2, complexed with YAP65, binds directly to NCE2 of the Pax3 promoter. In situ hybridization showed co-localization of Pax3, Tead2, and Yap65 in dorsal neural tube. A variety of assays showed that wild type TEAD2 activates Pax3 transcription by binding to NCE2. Of note, the minimal promoter region containing NCE1 and NCE2 does not fully recapitulate normal Pax3 expression, as cervical and caudal regions showed absent or ectopic expression, and neural tube expression was more dorsally restricted (Milewski et al., 2004).

In B16F1 mouse melanoma cells, the -1.6 kb Pax3 promoter region was shown insufficient to activate transcription, while the larger -14 kb region sufficiently activated transcription. However, mutation of the four previously identified binding regions within the -1.6 kb region affected Pax3 transcription, suggesting these binding elements still regulate transcription, but other upstream elements are also important for Pax3 expression in melanocytes. Comparative sequence analysis between mouse and human identified a 1.1 kb region of conserved sequence in the Pax3 promoter at -6.9 to -5.8, and the authors suggest additional Pax3 transcriptional regulation occurs via this region. OCT1 and BRN2 binding was confirmed at two of the previously described binding sites within the -1.6 kb region of the Pax3 promoter, with both activating transcription. PBX and PREP1 were shown to complex together at the remaining two sites, where they repressed Pax3 expression (Zhu and Pruitt, 2005).

Transgenic mice expressing Cre recombinase under control of the 1.6 kb Pax3 promoter directed Cre expression to neural crest tissues, but not somites, in a pattern similar to that of endogenous Pax3. These mice can facilitate specific gene deletions in Pax3-expressing neural crest cells (Li et al., 2000).

Construction of a transgenic mouse line, Tg(Pax3-tv-a)HPvn, in which the avian RCAS vector TVA is expressed under control of the 1.6kb Pax3 promoter, demonstrated that this construct allowed targeting of genes to the melanocyte lineage via RCAS infection of neural crest cultures. This transgenic can be used to test the roles of various genes in melanocyte development (Hou et al., 2004).

Comparative genomic sequence analysis of the -15 to -6 kb region upstream of Pax3 in human and mouse identified 3 highly conserved regions. Subsequent creation of a panel of transgenic mice harboring
B-gal under control of various combinations of these regions showed the presence of a 291-bp element (located at approximately -6kb) that specifically directed Pax3 expression in muscle precursor cells that arise from the ventrolateral lip of the somite and migrate to the limbs, tongue, and skeletal muscle. This expression began at E9.0, later in development than initial Pax3 expression elsewhere in the embryo, and appeared independent of Pax3 function, as β-gal expression in hypaxial muscle was still seen in transgenic mice on a Splotch background (Brown et al., 2005).

Regulatory sequences located at positions -135 to -98 in human PAX3 are directly bound and regulated by a SMAD4-SKI complex. This complex becomes activated by TGF-beta signaling that originates from keratinocytes, resulting in inhibition of Pax3 mRNA transcription in melanocytes. These regulatory sequences are conserved across human, monkey, rat, and mouse (Yang et al., 2008).

Targeted deletion of a 674bp region upstream of the mouse Pax3 locus (named NCE) which contains previously identified upstream enhancer regions (see Milewski et al., 2004) did not impair Pax3 expression in neural crest, suggesting the NCE was not solely responsible for Pax3 expression. Of note, the mice retaining the floxed PGK-neomycin cassette showed disrupted Pax3 expression, exhibiting phenotypes identical to those of Pax3Splotch mice. Crossing of these mice retaining the floxed cassette with neural crest-specific or hypaxial somite-specific CRE constructs allowed tissue-specific rescue of Pax3 expression, and complete phenotypic rescue of the CRE-expressing tissue types in these mice suggested that the PAX3-dependent developmental pathways in neural crest and somites do not regulate one another. Analysis of intronic and downstream evolutionarily conserved regions at the Pax3 locus identified an enhancer located within the 4th intron (named ECR2), which, along with a minimal Pax3 promoter, could direct reporter gene expression even in the absence of the NCE. ECR2 also directed neural crest expression of a reporter construct in transient transgenic zebrafish, and this expression required functional Lef/TCF sites within ECR2 (Degenhardt et al., 2010).