Melanocyte-specific Microphthalmia-associated Transcription Factor Isoform Activates Its Own Gene Promoter through Physical Interaction with Lymphoid-enhancing Factor 1*

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Melanocyte-specific Microphthalmia-associated Transcription Factor Isoform Activates Its Own Gene Promoter through Physical Interaction with Lymphoid-enhancing Factor 1*

Waardenburg syndrome type 2 (WS2) is associated with heterozygous mutations in the gene encoding microphthalmia-associated transcription factor (MITF) and characterized by deafness and hypopigmentation due to lack of melanocytes in the inner ear and skin. Melanocyte-specific MITF isoform (MITF-M) is essential for melanocyte differentiation and is transcriptionally induced by Wnt signaling that is mediated by β-catenin and LEF-1. Here we show that MITF-M transactivates its own promoter (M promoter) by interacting with LEF-1, as judged by transient expression assays and in vitro protein-protein binding assays, whereas no transactivation of the M promoter was detected with MITF-M alone or with the combination of MITF-M and dominant-negative LEF1 that lacks the β-catenin-binding domain. This synergy depends on the three LEF-1-binding sites that are clustered in the proximal M promoter. Importantly, MITF-M recruited on the M promoter could function as a non-DNA-binding cofactor for LEF-1. Thus, MITF-M may function as a self-regulator of its own expression to maintain a threshold level of MITF-M that is required for melanocyte development. We suggest that MITF-M haploinsufficiency may impair the dosage-sensitive role of MITF-M or the correct assembly of multiple transcription factors, involving MITF-M, on the M promoter, which could account for dominant inheritance of WS2.

Transcription factors play critical roles in regulatory networks of many developmental pathways, cell growth, and differentiation, and mutations in the genes coding for transcription factors are associated with various human disorders that are frequently inherited in a dominant manner (1). Dominant inheritance of human disorders provides us with an invaluable opportunity to assess the physiological role of relevant gene products. Waardenburg syndrome (WS)1 is of particular interest among dominantly inherited disorders, because WS is genetically heterogenous but exhibits similar auditory-pigmentary abnormalities that are caused by melanocyte deficiency in the cochlea and skin, leading to sensorineural hearing loss and abnormal pigmentation (2, 3). WS is associated with mutations in the separate genes coding for at least three transcription factors, microphthalmia-associated transcription factor (MITF), PAX3, and SOX10 (4–7). These transcription factors constitute a regulatory network that is responsible for melanocyte development of neural crest origin.

WS2, a subtype of WS, is caused by heterozygous mutations in the MITF gene, exhibiting deafness, hereditary iriditis, and patchy abnormal pigmentation (2, 8). MITF belongs to an evolutionary ancient family of transcription factors, containing a basic helix-loop-helix and leucine-zipper (bHLH-LZ) structure (9). MITF consists of at least seven isoforms, referred to as MITF-M, MITF-H, MITF-A, MITF-B, MITF-C, MITF-D, and MITF-E, which share the entire downstream region, including the bHLH-LZ domain, but possess unique amino termini (10–15). The isoform-specific amino termini are encoded by separate first exons of the MITF gene (13, 16), except for MITF-D and MITF-E, which are identical in the primary structure, and the untranslated regions of their mRNAs are encoded by the separate first exons (14, 15). MITF-M is exclusively expressed in melanocytes and melanoma cells (10, 17) and is under the regulation of the melanocyte-specific promoter (M promoter) (17).

Recently, we have shown that MITF-M interacts with LEF-1, a nuclear mediator of Wnt signaling, to enhance the transcription from the dopachrome tautomerase (DCT) gene promoter, an early melanoblast marker (18). The bHLH-LZ structure of MITF-M is responsible for the interaction with the C-terminal portion of LEF-1, as judged by mammalian and yeast two-hybrid assays and in vitro protein-protein binding assays (18). These results suggest that MITF-M and other MITF isoforms represent a new class of nuclear modulators for LEF-1, which may ensure efficient propagation of Wnt signals in many types of cells. The binding of Wnt signaling molecule to its receptor Frizzled leads to inactivation of glycogen synthase kinase-3β, followed by the accumulation of β-catenin that is then associated with LEF-1. The resulting LEF-1-β-catenin complex trans-activates the target genes (19, 20). The studies in zebrafish have established a crucial role of Wnt signaling in development of pigment cells of the neural crest origin (21) and in expression of nacre, a zebrafish MITF homolog (22). Moreover, direct gene transfer of Wnt1 or β-catenin to mouse neural crest cells re-
sulted in melanocyte expansion and differentiation (23). Exog-
ously added Wnt-3a protein to cultured murine melanocytes
increased the expression of endogenous Mitf mRNA and trans-
activated the M promoter through the LEF-1-binding site (24).
Therefore, MITF-M serves as a target as well as a nuclear
mediator of Wnt signaling.

The mutations identified in WS2 individuals include splicing
mutations, nonsense mutations, and missense mutations (5,
25), most of which are likely to cause a loss of function (26).
These facts support the notion that haploinsufficiency (half
normal levels) of MITF-M could account for WS2 (25). It is
therefore of clinical importance to explore the regulatory mech-
anism of MITF-M expression. Here we show a novel mecha-
nism by which MITF-M regulates its own promoter through
physical interaction with LEF-1, suggesting that MITF-M
could function as a component of the transcription factor net-
work that regulates transcription from the M promoter. The
implication of the present study is discussed in relevance to
the haploinsufficiency of MITF-M as a molecular mechanism for
WS2.

EXPERIMENTAL PROCEDURES

Plasmid Construction—MITF expression plasmids, pRC/CMV-
MITF-M, pRC/CMV-MITF-A, pRC/CMV-MITF-H, and pRC/CMV-
MITF-D, were described previously (10, 14, 27). FL9B, a mammalian
eexpression plasmid, contains the full-length human LEF-1 cDNA (28).
Dominant-negative LEF-1 (DNLEF1) that lacks the β-catenin-binding
domain (amino acid residues 2-37) (29, 30) was prepared (18). Reporter
plasmids contain the firefly luciferase gene, linked to the 5′-flanking
region of the human MITF gene (17, 31) or the human DCT gene (32).
A mutant construct pGL3-MITF/M(m195) carrying base changes at the
MITF region of the human Ink promoter was observed when LEF-1 and MITF-M were coex-
pressed in HeLa cervical cancer cells that lack endogenous expression of
MITF and MITF-M, which is consistent with our previous findings
(10, 18, 38). Interestingly, synergistic transactivation of the M pro-
moter was observed when LEF-1 and MITF-M were coex-
pessed (Fig. 1A), whereas no transactivation was detected
with MITF-M alone or with the combination of MITF-M and
DNLEF1 that lacks the β-catenin-binding domain. Thus,
β-catenin is involved in the observed synergism between LEF-1
and MITF-M, which is consistent with our previous findings
that β-catenin alone or its combination with LEF-1 transacti-
vated the M promoter (24).

To localize the cis-acting region of the M promoter that is
required for the synergistic activation by LEF-1 and MITF-M,
we screened various reporter constructs, including a construct
that contains the MITF-M-distal enhancer for the M promoter
(31). MITF-M distal enhancer contains a functional SOX10-
binding site that also matches with the consensus sequence
CCCTG(A/T)(A/T) of LEF-1-binding sites (39). However, the
degree of activation of this construct is not significantly differ-
ent from the values obtained with short constructs (Fig. 2A).
Further deletion studies have localized the proximal region
(positions −258 to −46) that is involved in the transactivation

FIG. 1. Functional synergy between LEF-1 and MITF-M on the
M promoter. A, schematic representation of the human MITF gene.
Open and closed boxes indicate the untranslated regions and the pro-
coding regions, respectively. Arrows represent the transcriptional
initiation sites of isoform-specific first exons. Exon 1M is under
the regulation of the melanocyte-specific M promoter. Also shown is
the equivalent position of the insertion identified in the recessive black-
eyed white Mitf<sup>−/−</sup> mouse (51). B, effect of MITF-M on the LEF-1-
mediated activation of the M promoter. HeLa cervical cancer cells were
cotransfected with the M promoter-reporter plasmid (pGL3-MITF/M) and
the indicated effector plasmid(s). The degree of activation is pre-
sented as the ratio of normalized luciferase activity obtained with each
effector to that with vector DNA (pRC/CMV). The results of at least five
independent experiments are shown with standard deviations.

Extracts of COS-7 cells expressing DNLEF-1 (300 µg of protein) were
added to 30 µl of GST-MITF resin suspension and diluted with buffer C
(20 mM HEPES, pH 7.9, 133 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM
dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 20% glycerol, 0.1%
Nonidet P-40) to adjust the protein concentration to 1 µg/ml. The sample
was then incubated at 4 °C for 90 min. The resin was washed with 700 µl of
buffer C for four times, and a final suspension of 10 µl was applied to
SDS-PAGE. c-Myc-tagged DNLEF-1 was detected by Western blot
analysis with anti c-Myc antibody (Santa Cruz Biotechnology).

RESULTS

Transactivation of the M Promoter by MITF-M and LEF-
1—A newly recognized interaction between MITF-M and
LEF-1 prompted us to analyze the effect of their combination
on the M promoter activity in HeLa cervical cancer cells that
lack endogenous expression of LEF-1 and MITF-M mRNAs (10,
18, 38).

Electrophoretic Mobility Shift Assays—Nuclear extracts were pre-
pared from HMV-II melanoma cells by the method of Schreiber et al.
(36). EMSA was performed with nuclear extracts or GST-LEF-1 fusion
protein, as described previously (18, 37).

In Vitro Protein-Protein Interactions—Dominant-negative LEF-1
(DNLEF1) was prepared as a fusion protein containing α-cMyC epitope
tag at its C terminus (18). COS-7 cells (5 × 10<sup>5</sup>) were transfected with
8 µg of a DNLEF-1 expression vector and harvested 42 h post-trans-
fection. GST-MITF-M fusion proteins (wild-type and mutant) were pre-
pared as previously described (37) and purified on GST-Sepharose 4B
resin (Amersham Biosciences), according to the manufacturer’s instruc-
tions. The resin was preincubated with untransfected COS-7 nuclear
extracts at 4 °C for 1 h to reduce the nonspecific binding. Nuclear
by MITF-M and LEF-1 (Fig. 2B). Note that the degree of activation of pHMIL1 was 10-fold higher than that of pGL3-MITF/M (Fig. 2A), despite the fact that the two constructs contain the same promoter region of 2.2 kb. This difference was due to the separate vector systems used for constructions of the pHMIL series (17) and pGL3 series (31). Under the conditions used, basal luciferase activity was always lower with each construct of the pHMIL series, giving rise to higher relative luciferase activity.

Clustered LEF-1-binding Sites Required for the Activation by MITF-M and LEF-1—The localized cis-acting region (−258 to −46) contains a functional LEF-1-binding site, CTTTGAT (positions −199 to −193), that is responsible for Wnt signaling (24). This site was termed LBS195 for LEF-1-binding site at position −195. In addition, two potential LEF-1-binding sites, LBS218 (CCTTGAT: −222 to −216) and LBS201 (GTGGCAC: −205 to −199), are located immediately upstream from the functional LBS195 (Fig. 3). Consequently, the base changes were introduced into each of the putative LEF-1-binding sites, and their effects on the M promoter activity were assessed. As reported previously (24), the activation level of the M promoter was significantly reduced when the functional LBS195 was altered (Fig. 3). Unexpectedly, the base change at either LBS218 or LBS201 abolished the activation of the M promoter by the combination of LEF-1 and MITF-M, suggesting the functional importance of the newly recognized LEF-1-binding sites. In this context, these three LEF-1-binding sites are well conserved in the mouse M promoter at the equivalent positions, except that the T residue at position −204 of LBS201 is changed to C residue in the mouse counterpart (GenBank™ accession number AC021060). Incidentally, the zebrafish nacre promoter also contains the three LEF-1-binding sites (22).

We then performed EMSA to analyze whether a nuclear protein of melanoma cells binds these putative LEF-1-binding sites (Fig. 4A). A synthetic probe (−226 to −185) containing the three LEF-1-binding sites was bound by nuclear extracts prepared from HMV-II melanoma cells that endogenously express LEF-1 and MITF-M (lanes 2 and 17). The formation of the protein-DNA complex was competed for by an unlabeled probe (lanes 3 and 4) or a consensus LEF-1-binding site (lanes 5 and 6) but not by an MITF-M-binding site (lanes 7 and 8) or a competitor containing the base changes at the three LEF-1 sites (lanes 9 and 10). The formation of the complex was reduced when the binding assays were performed in the presence of a competitor carrying the base changes at each LEF-1-binding site (lanes 11–16). Thus, it is likely that the detected protein-DNA complex contained LEF-1 or its related proteins.

We therefore examined whether the clustered LEF-1-binding sites are bound by LEF-1, using recombinant LEF-1 and MITF-M, each of which was fused to GST (Fig. 4B). A nonspecific band, indicated by a small arrow, was consistently detected with either GST, GST-LEF-1, or GST-MITF-M (lanes 2–4 and lanes 11–13), and appeared to be competed by wild-type (lanes 7 and 15) and by some of mutant oligonucleotides (lanes 17 and 21). These results suggest that a certain protein present in bacterial extracts was copurified with GST and GST fusion proteins and did bind the probe oligonucleotide. The
probe was also bound by LEF-1 but not by MITF-M (lanes 1–4 and 10–13). The specificity of the DNA-LEF-1 complex was confirmed by the competition studies (lanes 5–9 and 14–21). It should be noted that the mobility of the LEF-1-DNA complex was not changed in the presence of MITF-M. These results confirm that LEF-1 is able to bind the three LEF-1-binding sites, which is consistent with the functional analysis of the LEF-1-binding sites. However, the complex involving LEF-1 and MITF-M was not detectable by EMSA probably due to the unstable complex involving MITF-M or the inaccessibility of MITF-M to the LEF-1-DNA complexes under the conditions used. Taken together, these results suggest that the binding of LEF-1 to the three consecutive sites is essential for the LEF-1-mediated activation, which in turn may recruit MITF-M on the M promoter.

MITF-M as a Non-DNA Binding Coactivator for LEF-1 on the M Promoter—We next examined the effects of two types of mutations in the bHLH-LZ region of MITF-M on the interaction with LEF-1 by pull-down assays. The Asp-222 → Asn substitution in the helix 1 and the Gly-244 → Glu substitution in the helix 2 represent molecular lesions of the recessive Mitf−/− (33) and semidominant Mitf(ab) (34), respectively (Fig. 5). The Mitf−/− protein is able to bind in vitro to DNA (40), whereas Mitf(ab) protein lacks the DNA-binding activity (34). In this experiment, DNLEF-1 was used instead of LEF-1, because the β-catenin-binding domain is dispensable for the interaction with MITF-M (18) and the signal of c-Myc-tagged LEF-1 overlapped with the nonspecific signal seen in all lanes (shown as a closed circle in Fig. 5). The vit and b mutations did not noticeably impair the in vitro interaction of MITF proteins with DNLEF-1 under the conditions used. Unexpectedly, the bound fraction contained large amounts of smaller fragments of c-Myc-tagged DNLEF-1 that may represent partial degradation products retaining the c-Myc-tag at their C termini. The presence of these small LEF-1 fragments supports the notion that the C terminus of LEF-1 is involved in the interaction with MITF-M (18). Perhaps, overexpressed DNLEF-1 protein was rapidly degraded in COS-7 cells. In this context, we were unable to detect endogenous LEF-1 in HMV-II melanoma cells by Western blot analysis of the c-Myc-tagged DNLEF-1 that bound to MITF-M or mutant MITF-M immobilized on the resin. Tagged DNLEF-1 is shown as an arrowhead. Lanes 2 and 3 contain nuclear extracts (NE) from untransfected and transfected COS-7 cells, respectively. The specific signals are indicated with open and closed circles. Partial degradation fragments of DNLEF-1 are shown as an asterisk. Lane 1 contains size markers and serves as a negative control for the Western blot analysis.

Fig. 4. Identification of the clustered LEF-1-binding sites in the M promoter. A, electrophoretic mobility shift assay (EMSA), showing three LEF-1-binding sites in the M promoter. Nuclear extracts of HMV-II melanoma cells were incubated with a 32P-end-labeled probe in the absence (lanes 2 and 17) or presence of an indicated competitor (200- and 500-fold excesses, shown as triangles). The competitors used are the probe itself (WT), LEF-1-binding site (LEFB5), GGTTAAGATCAGAAGGGGTA (54), and tyrosinase distal enhancer (TDE), tcgaGAGATCATGTGATGACTTCg (27). Other competitors carry base changes at one or three LEF-1-binding sites, as shown in Fig. 3A. Lane 1 represents a buffer control lacking nuclear extracts. The arrowhead indicates the specific protein-DNA complex. Unbound probes are indicated with a small arrow. B, EMSA with recombinant LEF-1 and MITF-M. Lanes 1 and 10 represent a buffer control lacking fusion proteins. The probe was incubated with GST (lanes 2 and 11), GST-LEF-1 (lanes 3 and 12), or GST-MITF-M (lanes 4 and 13). In lanes 5–9 and 14–21, the probe was incubated with both GST-LEF-1 and GST-MITF-M. The LEF-1-DNA complex is indicated by an arrowhead. Small arrows indicate the unspecific protein-DNA complex and unbound probes.
Western blot analysis. Moreover, the trials of coimmunoprecipitation of endogenous MITF-M and LEF-1 were unsuccessful, probably due to the low expression levels of LEF-1 in melanocytes and melanoma cells.

We next analyzed the functional consequences of the \( \text{vit} \) and \( \text{b} \) mutations in the synergistic activation of the \( M \) promoter, because these mutations profoundly impaired the functional cooperation between MITF-M and LEF-1 on the \( DCT \) promoter (Fig. 6), as already reported (18). Particularly, the \( \text{vit} \) mutation almost abolished the transactivation of the \( DCT \) promoter. In contrast, either MITF-M\( \text{vit} \) protein or MITF-M\( \text{b} \) protein showed the synergism with LEF-1 on the \( M \) promoter, as did wild-type MITF-M, suggesting that both mutant proteins could act on the \( M \) promoter through LEF-1. Importantly, MITF-M\( \text{b} \) protein may function as a non-DNA-binding cofactor for LEF-1 on the \( M \) promoter. Such differential effects of the mutations may be related to the fact that the \( DCT \) promoter contains the binding site for MITF-M (M box) (35), unlike the proximal \( M \) promoter.

Taken together, these results suggest that functional consequences of these mutations vary depending on the gene promoters and the interacting partners.

**Dosage-sensitive Effects of MITF Isoforms on the Synergism with LEF-1**—To explore whether the synergism between MITF-M and LEF-1 represents a general feature of MITF isoforms, we examined the effects of MITF-A, MITF-D, or MITF-H on the LEF-1-mediated transactivation of the \( M \) promoter (Fig. 7). MITF-A and MITF-H possess the extended N termini that are different from the N terminus of MITF-M but share the entire C-terminal portion, including the bHLH-LZ region. The initiation Met of MITF-D is located in the downstream domain (B1b domain) that is shared by other MITF isoforms (14). MITF-A and MITF-H are widely expressed in many cell types (10, 16). In contrast, MITF-D is preferentially expressed in retinal pigment epithelium, macrophages, and osteoclasts that are affected in the \( \\
'\text{Mitf} \) mutant mice but not expressed in other \( \\
'\text{Mitf} \)-target cells, including melanocyte-lineage cells and natural killer cells (14). We used various concentrations of each MITF plasmid for transfection, because the transiently expressed levels of MITF isoform proteins vary depending on the isoforms (12, 14). Like the case with MITF-M, a combination of LEF-1 with MITF-A, MITF-H, or MITF-D activated the \( M \) promoter. These results are consistent with the finding that the bHLH-LZ region is responsible for the interaction with LEF-1 (18) and suggest a potential role for MITF isoforms in the transcriptional regulation of hitherto unknown target genes in certain cell types. Moreover, the dose-response study has revealed the remarkable reduction in the degree of synergistic activation by excess amount of each MITF isoform (Fig. 7), which may account for the results that the synergistic activation of the \( M \) promoter by LEF-1 and MITF-M was not detectable in HMV-II melanoma cells (data not shown). Under the conditions used (total amount of DNA kept at 10 \( \mu \)g), lower doses of a given MITF isoform tend to enhance the LEF-1-mediated activation of the reporter gene more efficiently. It should be noted that the degree of activation by LEF-1 alone unchanged even with the highest dose of MITF isoform protein used.

**FIG. 6.** MITF-M functions as a non-DNA-binding cofactor for LEF-1. HeLa cells were cotransfected with pGL3-MITF/M, LEF-1, and each of the indicated MITF-M constructs. Likewise, the effect on the \( DCT \) promoter (pHDTL8) was analyzed for comparison, and its structure is schematically shown. The degree of activation is presented as the ratio of normalized luciferase activity obtained with each effector to that with vector DNA. The results of five independent experiments are shown with standard deviations.
DISCUSSION

Here we provide evidence for a novel mechanism by which MITF-M transactivates its own promoter through physical interaction with LEF-1, which may ensure efficient transcription from the M promotor at the sensitive stage of melanocyte development. Thus, MITF-M by itself serves as a component of the transcription factor network that directs transcription from the M promoter. Importantly, an excess amount of MITF-M appears to impair the functional cooperation with LEF-1 but does not affect the LEF-1-mediated activation of the M promoter (Fig. 7), suggesting that MITF-M may not enhance the effects of Wnt signal on the M promotor when MITF-M content is above a certain threshold level. Taken together with the in vivo observations of other investigators (22, 23), these results suggest that initiation of MITF-M expression is triggered by Wnt signaling through LEF-1 and is temporally facilitated by the functional cooperation of LEF-1 with MITF-M. Such a proposal is also consistent with the expression profiles of Lef-1 and Mitf mRNAs in developing mouse embryos: the onset of Lef-1 mRNA expression is detected at embryonic day 7.5 (41), which precedes the onset of Mitf-M expression (9.5–10.5 days) (31, 42).

Recruitment of MITF-M on the M promotor is an essential step for the self-activation of MITF-M expression and depends on the binding of LEF-1 to the three adjacent binding sites. It is therefore likely that transcription from the M promotor is relatively sensitive to the concentration of LEF-1 and MITF-M, although the exact stoichiometry involving LEF-1 and MITF-M remains to be elucidated. Such a notion also supports the haploinsufficiency of MITF-M as a molecular mechanism of WS2. Conversely, the requirement of three adjacent LEF-1-binding sites for the synergism between MITF-M and LEF-1 may represent an important mechanism that prevents MITF-M to function as a coactivator on many gene promoters containing a single LEF-1-binding site.

Two lines of evidence suggest that MITF-M is able to transactivate the M promotor by interacting with LEF-1 but without binding to the M promotor. First, the cis-regulatory region of the M promotor does not contain the CATGTG motif, a well-established binding site for MITF-M, and is not bound by MITF-M in vitro. Second, the MITF-M protein lacking the DNA-binding activity enhances the LEF-1-mediated transactivation of the M promotor. Likewise, Mitf-M was shown to interact with c-Jun to transactivate the mouse mast cell protease 7 gene promoter that lacks a typical MITF-binding element (43). It is therefore conceivable that MITF-M functions as a non-DNA-binding cofactor for LEF-1 on the M promotor. This notion is of physiological significance to understand the phenotypic consequences of various MITF and Mitf mutations that alter the DNA-binding activity. On the other hand, Mitf-M is expected to regulate the expression of a certain target gene by directly binding to its promoter sequence that is required for melanoblast survival, because homozygous Mitf-/- mice are completely white and lack melanocytes in the skin and eye (34).

Transcription from the M promotor is up-regulated via the separate cis-acting elements by two other transcription factors, PAX3 (44) and SOX10 (45–48). PAX3, containing a paired homeodomain, is responsible for WS1 and WS3 (4, 6) that are characterized by dystopia canthorum without or with limb abnormalities, respectively. SOX10, containing a high mobility group box as a DNA-binding motif, is responsible for WS4, also known as Waardenburg-Hirschsprung syndrome (7), which is
characterized by aganiblial colonies. SOX10 activated transcription from the M promoter through a proximal region (~260 to ~244) (46–48), and the SOX10-mediated transactivation of the M promoter was further stimulated by PAX3. Thus, those transcription factors constitute the regulatory network that directs the temporal and spatial transcription from the M promoter through multiple protein-protein interactions. In fact, Sox10 and Mitf-M mRNAs are coexpressed in migrating melanoblasts by about embryonic day 12, and then Sox10 expression became undetectable in melanoblasts while Mitf-M is continuously expressed in melanoblasts in the stria vascularis of the cochlea (31).

The synergism between LEF-1 and MITF-M is also responsible for the transcriptional regulation of the DCT gene but through the different mechanism from that of the M promoter. DCT, a melanoblast marker, has been implicated in detoxification of melanin precursors (49) and may be important for the survival of melanocytes. The finding that the vit mutation impairs the synergism with LEF-1 on the DCT promoter but not on the M promoter is of particular interest in view of the phenotype of homozygous Mitf<sup>vit</sup> mice that appear normal at the sensitive stage of melanocyte development. Therefore, haploinsufficiency of Mitf-A or other isoforms may not be expressed at sufficient levels for melanoblasts at sensitive stage (probably around day 12) and may account for the pathogenesis of WS2 (1992) Nature 355, 637–640.

Sox10 and Mitf-M transcription factors constitute the regulatory network that controls survival of melanocytes. The finding that the Sox10 expression is reduced in melanoblasts during fetal development in the intron between exon 3 and exon 4 that leads to the dosage-sensitive role of Mitf-M in the wild-type mouse (31). The dosage-sensitive role of Mitf-M may account for the pathogenesis of WS2 (31).

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