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Microphthalmia-Associated Transcription Factor (MITF): Multiplicity in Structure, Function, and Regulation

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Microphthalmia-associated transcription factor (MITF) regulates the differentiation and development of melanocytes and retinal pigment epithelium and is also responsible for pigment cell-specific transcription of the melanogenesis enzyme genes. Heterozygous mutations in the *MITF* gene cause auditory–pigmentary syndromes. MITF consists of at least five isoforms, MITF-A, MITF-B, MITF-C, MITF-H, and MITF-M, differing at their N-termini and expression patterns. Here we show a remarkable similarity between the N-terminal domain of MITF-A and cytoplasmic retinoic acid-binding proteins. To date, four isoform-specific first exons have been identified in the *MITF* gene: exons 1A, 1H, 1B, and 1M in the 5′ to 3′ direction, each of which encodes the unique N-terminus of a given isoform. The 5′-flanking regions of these isoform-specific exons are termed A, H, B, and M promoters, respectively. Among these promoters, the M promoter has received particular attention, because it is functional only in melanocyte-lineage cells and is upregulated by Wnt signaling via the functional LEF-1-binding site. Moreover, the M promoter is upregulated by other transcription factors, PAX3, SOX10, and CREB. The activity and degradation of MITF-M are regulated by extracellular signals via protein phosphorylation, such as c-Kit signaling. Together, multiple signals appear to converge on the M promoter as well as on MITF proteins, leading to the proper regulation of MITF-M in melanocytes and other MITF isoforms in many cell types.* ***Key words: melanocyte/retinal pigment epithelium/retinoic acid/Wnt signaling/Waardenburg syndrome. Journal of Investigative Dermatology Symposium Proceedings 6:99–104, 2001***

Mice with semidominant mutations at the *microphthalmia* (*Mitf*) locus show some of the following defects: loss of pigmentation, reduced eye size, failure of secondary bone resorption, reduced numbers of mast cells, and early onset of deafness (Silvers, 1979). Thus, microphthalmia-associated transcription factor (Mitf), encoded at the mouse *Mitf* locus, plays an essential role in the development and/or survival of several cell lineages, including melanocytes, retinal pigment epithelium (RPE), mast cells, and osteoclasts. Mitf (Hodgkinson *et al*, 1993; Hughes *et al*, 1993) and its human counterpart MITF (Tachibana *et al*, 1994) contain a basic helix-loop-helix-leucine zipper (bHLH-LZ) structure, which is required for DNA binding and dimer formation. The initially identified Mitf, now known as melanocyte-specific Mitf (Mitf-M), consists of 419 amino acid residues and shares 94.4% identity with MITF-M (Tachibana *et al*, 1994). MITF-M mRNA is exclusively expressed in melanocytes and pigmented melanoma cells, but is not detectable in other cell types, including human RPE cell lines (Amae *et al*, 1998; Fuse *et al*, 1999; Vachtenheim and Novotná, 1999). In fact, MITF-M efficiently transactivates the melanogenesis enzyme genes, such as *tyrosinase* and *tyrosinase-related protein 1* (*TRP-1*) in cultured cells (Yasumoto *et al*, 1994, 1997; for review, Shibahara *et al*, 1998, 1999, 2000). Moreover, ectopic expression of MITF-M converted NIH/3T3 fibroblasts into cells expressing the *tyrosinase* and *TRP-1* genes (Tachibana *et al*, 1996). Together, these results indicate MITF-M as a key regulator for melanocyte differentiation.

MITF consists of at least five isoforms with distinct amino-termini, MITF-A, MITF-B, MITF-C, MITF-H, and MITF-M (Amae *et al*, 1998; Fuse *et al*, 1999; Udono *et al*, 2000) (**Fig 1**). The N-terminus of MITF-M, domain M, consists of 11 amino acid residues, and is encoded by the melanocyte-specific exon 1 (exon 1M) (Fuse *et al*, 1996). All isoforms with the extended amino-termini share the entire carboxyl portion with MITF-M. The unique N-terminus of MITF-A, MITF-B, MITF-C, or MITF-H is followed by the common region of 83 amino acid residues (domain B1b), which is significantly similar to the equivalent portion of TFEB (Amae *et al*, 1998) and TFE3 (Yasumoto *et al*, 1998; Rehli *et al*, 1999). Moreover, domain C of MITF-C shares significant amino acid similarity with the putative transactivation segments of the two leukemogenic factors, ENL and AF-9 (Fuse *et al*, 1999; Shibahara *et al*, 2000).

Here we summarize the new findings concerning the regulation of MITF-M expression and discuss the function of MITF isoforms. Comprehensive reviews on the general properties of MITF have been published (Moore, 1995; Shibahara *et al*, 1998, 1999, 2000; Yasumoto *et al*, 1998; Tachibana, 1999; Goding, 2000).

STRUCTURAL ORGANIZATION OF THE *MITF* GENE

The *MITF* gene consists of at least four promoters, their consecutive first exons (exons 1A, 1H, 1B, and 1M), and eight downstream exons that are common to all isoforms (Udono *et al*,

Manuscript received June 14, 2001; accepted for publication June 14, 2001.

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2000) (**Fig 2**). The structural organization of the *MITF* gene is similar to that of the mouse *Mitf* gene (Hallsson *et al*, 2000). The N-termini of the MITF isoforms are encoded by the separate first exons, although the first exon encoding the N-terminus of MITF-C has not been identified. Among the first exons identified, exon 1B is unique because it is also used as a second exon (B1b exon), when the primary transcripts, initiated from exon 1A, exon 1H, or putative exon 1C, are subjected to splicing (Udono *et al*, 2000). Exon 1B therefore encodes the 5′-untranslated region of MITF-B mRNA, the N-terminal domain of MITF-B (domain B1a), and domain B1b (see **Fig 1**).

In contrast to MITF-M, other MITF isoforms are widely expressed in many cell types (Amae *et al*, 1998; Fuse *et al*, 1999; Udono *et al*, 2000). These expression profiles of MITF isoform mRNA suggest that each promoter/exon 1 is under separate control. Especially, the M promoter, the 5′-flanking region of exon 1M, shows the melanocyte-specific promoter function (Fuse *et al*, 1996; Udono *et al*, 2000). In fact, MITF-M protein was detected in all of 76 melanoma surgical specimens examined (King *et al*, 1999). Together, MITF-M has been established as a specific marker for melanocyte-lineage cells.

Consistent in part with the ubiquitous expression patterns of MITF-A and MITF-H, the A and H promoters lack a typical TATA-box at the usual position (Udono *et al*, 2000), which is commonly seen in many house-keeping genes. Transient expression assays suggest that the A and H promoters are functional in RPE, melanoma, and cervical cancer cell lines, but no noticeable B promoter activity was detected in these cell lines (Udono *et al*, 2000). It is of interest to study whether each alternative promoter of the *MITF* gene functions independently or whether some of these alternative promoters are transcribed in a mutually exclusive manner.

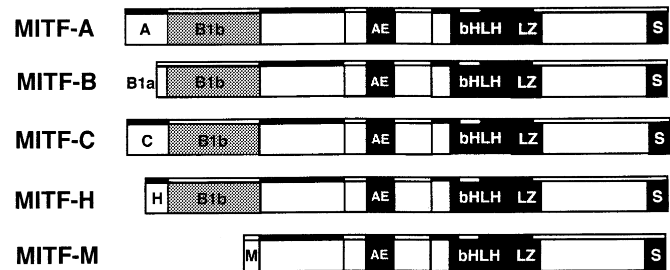

Figure 1. Structures of the MITF isoforms. Shown are the schematic representation of MITF-M and other isoforms, including MITF-A, MITF-B, MITF-C, and MITF-H. All MITF isoforms differ at their N-termini but share the entire carboxyl portion. Domain B1a and domain B1b of MITF-B are encoded by exon 1B (see **Fig 2**). The transcriptional activation domain (AE) (Sato *et al*, 1997), the bHLH-LZ structure, and the serine-rich region (Ser) are indicated. The exons are indicated by horizontal columns (closed or open) above the domains.

LESSONS FROM BLACK-EYED WHITE AND RED-EYED WHITE *MITF* MUTANTS

We are interested in the two recessive *Mitf* mutant mice, black-eyed white $Mitf^{mi-bw}$ and red-eyed white $Mitf^{mi-rw}$, because the former may represent the phenotype of Mitf-M deficient mice and the latter may mimic the phenotype of mice lacking all Mitf isoforms with extended N-termini. Essential requirement of Mitf-M for melanocyte development was verified by the molecular lesion of $Mitf^{mi-bw}$ mice (Yajima *et al*, 1999), which are characterized by the complete white coat color, deafness, and normally pigmented RPE without any ocular abnormalities. In $Mitf^{mi-bw}$ mice, the insertion of an L1 retrotransposable element in the intron 3 between exon 3 and exon 4 leads to complete repression of Mitf-M mRNA expression and to reduction of Mitf-A and Mitf-H mRNA expression (Yajima *et al*, 1999). In this context, the M promoter represents the most downstream promoter of the *MITF/Mitf* gene (Tassabehji *et al*, 1994; Fuse *et al*, 1996; Hallsson *et al*, 2000; Udono *et al*, 2000), and may be most susceptible to the transcriptional repression caused by the insertion of the L1 element (**Fig 2**). These results indicate that MITF-M/Mitf-M is a key regulator of the melanocyte development but is dispensable for RPE development. Indeed, *in situ* hybridization analysis revealed that Mitf isoform mRNA containing the B1b exon are expressed in the outer layer of the optic cup, the prospective RPE (Amae *et al*, 1998). These results indicate that MITF isoforms with extended N-termini, such as MITF-A or MITF-H, are important for RPE differentiation.

The homozygous $Mitf^{mi-rw}$ mice exhibit small red eyes and a white coat with some pigmented spots around the head and/or tail (Steingrímsson *et al*, 1994), and its molecular defect is a deletion of the genomic DNA segment containing exon 1H and exon 1B (Hallsson *et al*, 2000) (**Fig 2**). Thus, $Mitf^{mi-rw}$ mice completely lack Mitf-H and Mitf-B but may express aberrant Mitf-A lacking domain B1b, encoded by the 3′-portion of exon 1B; however, deletion of B1b exon results in a frame shift in such aberrant mRNA species. It is therefore conceivable that the phenotype of $Mitf^{mi-rw}$ mice represents the loss of function of all Mitf isoforms containing domain B1b. Moreover, these mutant mice are deficient in melanocytes possibly due to the loss of Mitf-M expression, except for melanocytes located in the head and tail regions, suggesting that the deleted genomic DNA segment may contain the enhancer for the M promoter.

In summary, Mitf-M is essential for melanocyte differentiation, whereas other Mitf isoforms with extended N-termini are responsible for differentiation of RPE and development of normal eye. Transcription from the M promoter is influenced not only by the proximal promoter region but also by the upstream and downstream *cis*-regulatory elements (**Fig 2**), suggesting the existence of the complex regulatory network of transcription factors.

A LINK BETWEEN MITF AND RETINOID METABOLISM

MITF-A is a predominant isoform expressed in a human RPE line of fetal origin and is also expressed in many cell types (Amae *et al*, 1998; Fuse *et al*, 1999; Udono *et al*, 2000). Domain A of MITF-A shares significant amino acid identity with the N-terminus of TFE3 (Yasumoto *et al*, 1998; Rehli *et al*, 1999). Here we show a similarity between domain A of MITF-A and cytoplasmic retinoic acid-binding protein (CRABP) (**Fig 3**). Two types of CRABP, CRABP-I and CRABP-II, have been extensively characterized but their functions are less well understood (reviewed by Li and Norris, 1996). CRABP-I is expressed in many tissues, whereas CRABP-II expression is localized to the skin. It should be noted that three consecutive portions, covering the entire domain A, are aligned to the equivalent portions of CRABP. Especially, the middle portion of domain A (12 amino acid residues) is remarkably similar to CRABP but is less conserved in TFE3. The implication of such similarity remains unknown, but is reminiscent of the phenotype of a recessive *Mitf* mutant, $Mitf^{vitiligo}$ ($Mitf^{vit}$) (Lerner *et al*, 1986; Steingrímsson *et al*, 1994), which shows late-onset retinal degeneration and abnormalities in retinoid metabolism (Smith *et al*, 1994). When young, the homozygous $Mitf^{vit}$ mice appear normal, with uniformly lighter color, but show aging-dependent melanocyte loss (Lerner *et al*, 1986). In addition, plucking hairs promotes the regrowth of amelanotic hairs due to melanocyte loss in the plucked areas, indicating a crucial role of Mitf-M in postnatal maintenance of follicular melanocytes. The molecular lesion of $Mitf\text{-}M^{vit}$ is the Asp222Asn substitution in the helix 1 of the bHLH-LZ domain (Steingrímsson *et al*, 1994). The $Mitf\text{-}M^{vit}$ protein was shown to bind *in vitro* to DNA as either a homodimer or a heterodimer (Hemesath *et al*, 1994). It is therefore conceivable that the Asp to Asn substitution in the helix 1 of MITF-M, MITF-A, or other MITF isoforms may impair the interaction with a hitherto unidentified protein.

MODULATION OF MITF-M'S FUNCTION BY PHOSPHORYLATION

Recent studies have established that function and stability of MITF-M are regulated by phosphorylation, as summarized in **Fig 4**. The *c-Kit* gene is one of the targets for MITF-M (Tsujimura *et al*, 1996), and the Steel/c-Kit signaling triggers rapid phosphorylation of MITF-M via MAP kinase at Ser 73 (Hemesath *et al*, 1998). The phosphorylation of MITF-M at Ser 73 results in upregulation of the MITF-M function. In this process, CBP/p300 transcription coactivator interacts with MITF-M, further activating the function of MITF-M (Sato *et al*, 1997; Price *et al*, 1998). Moreover, c-Kit signaling was shown to phosphorylate MITF-M at Ser 409 via p90 Rsk, a member of the serine/threonine kinases (Wu *et al*, 2000). These c-Kit-induced phosphorylations caused short-lived activation and destruction of MITF-M. In this context, ubiquitin-conjugating enzyme hUBC9 has been identified as a potential interacting partner for MITF-M (Xu *et al*, 2000). It was shown that phosphorylation of MITF-M at Ser 73 is a prerequisite to the hUBC9-mediated degradation of MITF-M. Moreover, Takeda *et al* (2000a) reported that the Ser298 is phosphorylated by glycogen synthase kinase 3β (GSK3β), thereby enhancing the function of MITF-M. In fact, a Ser298P substitution was found to be associated with WS2 (Tassabehji *et al*, 1995); however, a signal targeting GSK3β, leading to activation of MITF-M, remains to be determined, although GSK3β is known as a negative regulator of the Wnt signaling pathway (reviewed by Eastman and Grosschedl, 1999). It is noteworthy that the phosphorylation sites that have been characterized to date are common to all MITF isoforms.

$Mitf^{mi\text{-}rw}$ $Mitf^{mi\text{-}bw}$

MITF gene 1A 1C 1H 1B (B1a,B1b) 1M 2 3 4 5 6 7 8 9 2.0 kb

5' SOX10 PAX3 LEF-1 CREB Exon 1 3' +1

SOX10: TGAAAGAGAAATACCATTGTC (-282 to -262)

PAX3: ATTAATACTACTGGAAC (-260 to -244)

LEF-1: CTTTGAT (-199 to -193)

CREB: TGACGTCA (-147 to -140)

Figure 2. Structural organization of the *MITF* gene. The direction of transcription is from left to right, indicated by *arrows*. The 5′ and 3′-untranslated exons are indicated by open boxes, and the protein-coding exons are indicated by closed boxes. The numbers show exons. Domains A, C, and H are encoded by exon 1A, putative exon 1C, and exon 1H, respectively. Note that exon 1C remains to be determined. The 3′-portion of exon 1B (exon B1b) is used as a second exon for generation of MITF-A, MITF-C, and MITF-H mRNA. The M promoter region is highlighted to show multiple *cis*-acting elements, which are bound by the indicated transcription factors. Exons 2–9 are common to all isoforms. Also indicated are the equivalent positions of the deletion identified in red-eyed white $Mitf^{mi\text{-}rw}$ mice (Hallsson *et al*, 2000) and the insertion seen in black-eyed white $Mitf^{mi\text{-}bw}$ mice (Yajima *et al*, 1999).

MITF-M PROMOTER AS A TARGET OF WNT SIGNALING

Recent studies from other investigators (Dorsky *et al*, 2000) and our group (Takeda *et al*, 2000b) have demonstrated that Wnt signaling upregulates MITF-M expression. Wnt proteins, secreted cysteine-rich glycoproteins, have been established as developmentally important signaling molecules (reviewed by Cadigan and Nusse, 1997). Wnt-1 and Wnt-3a are especially important in the expansion of neural crest precursors and in determining the fates of neural crest cells (Dorsky *et al*, 1998). Targeted disruption of the *Wnt-1* and *Wnt-3* genes in the mouse causes deficiency of neural crest derivatives, including melanocytes (Ikeya *et al*, 1997). Moreover, the onset of Wnt-3a expression is detected at 7.5 embryonic days (Takada *et al*, 1994), which precedes the onset of Mitf expression in neural crest cells (9.5–10.5 embryonic days)

```
                                 +   +    +   +   ++ ++  +              + + ++
hCRABP-I       1' MPNFAGTWKMRSSENFDELLKALGVNAMLRKVAVAAASKPHVEIRQDGDQFYIKTSTTVR
hCRABP-II      1" MPNFSGNWKIIRSENFEELLKVLGVNVMLRKIAVAAASKPAVEIKQEGDTFYIKTSTTVR
                  *.. **
MITF-A         1" MQSESGIV----------------------------------------------------
hTFE3         43" LLPESGIV----------------------------------------------------

                                  ++
hCRABP-I      61' TTEINFKVGEGFEEETVDGRKCRSLATWENENKIHCTQTLLEGD--GPKTYWTRELAND-
hCRABP-II     61" TTEINFKVGEEFEEQTVDGRPCKSLVKWESENKMVCEQKLLKGE--GPKTSWTRELTNDG
                      .*.*****.*.                                ***.
MITF-A         9" ---PDFEVGEEFHEE--------------------------------PKTYY--------
hTFE3         51" ---ADIELENVLD----------------------------------PDSFY--------

                                                          **..    *.*
MITF-A        26" ---------------------------------------ELKSQ--PLKSS
hTFE3         66" ---------------------------------------ELKSQPLPLRSSLPISLQATP

                     + +        + +
hCRABP-I     118' ELILTFGADDVVCTRIYVRE
hCRABP-II    119" ELILTMTADDVVCTRVYVRE

hTFE3         87" ATPATL
```

hCRABP-II

MITF-A

Exon 1A Exon B1b

hTFE3

Exon 1 Exon 2

Figure 3. Sequence alignment of CRABP and the N-terminus of MITF-A. Domain A and the N-terminal portion of human TFE3 are aligned to human CRABP-1 and CRABP-II. Gaps (–) are introduced to obtain better alignment. Residues of CRABP within 3.5 Å of bound ligand are indicated (+) (Li and Norris, 1996). The positions of aligned regions are schematically shown as stippled boxes at the bottom. The entire structure of CRABP is shown over the N-termini of MITF-A and TFE3.

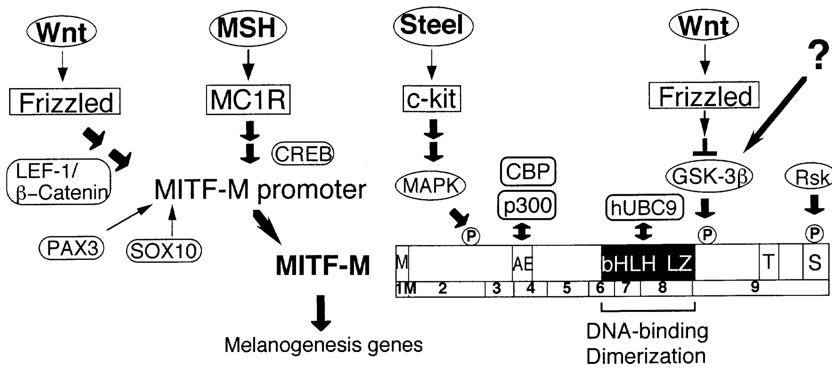

Figure 4. Regulation of MITF-M by multiple signals. Extracellular signaling molecules and their receptors are shown. Three phosphorylation sites indicated are the Ser residues at 73, 298, and 409. Interacting proteins, CBP/p300 and hUBC9, are also indicated. Also shown is the threonine-rich domain (T), which possesses modest transcriptional activation activity (Takeda *et al*, 2000a). A signal targeting GSK3β, leading to activation of MITF-M, remains to be determined. c-Kit triggers dual phosphorylations at Ser73 via MAPK and Ser409 via Rsk, leading to activation and degradation of MITF-M, respectively (Wu *et al*, 2000).

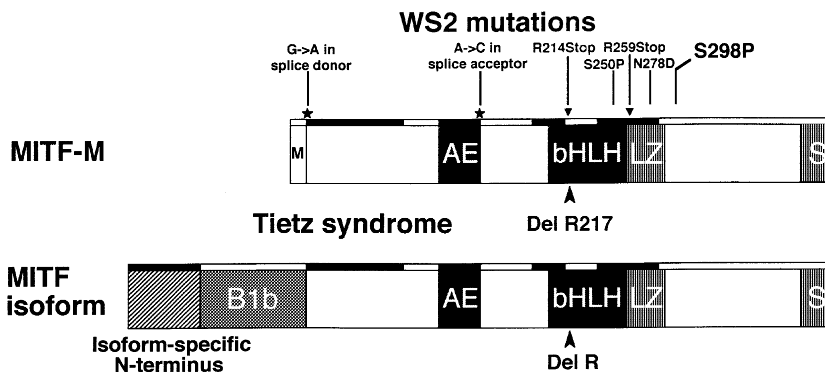

Figure 5. *MITF* mutations associated with WS2 or Tietz syndrome. The mutations found in individuals affected with WS2 syndrome (Tassabehji *et al*, 1994, 1995; Nobukuni *et al*, 1996) are shown above the functional domains. The del R217 is responsible for Tietz syndrome (Amiel *et al*, 1998). Asterisks and triangles indicate the splicing mutations and the nonsense mutations, respectively. The S298P substitution and the del R217 are enlarged.

(Nakayama *et al*, 1998). These results suggest that Wnt-3a is a good candidate that may regulate the differentiation of neural crest cells toward melanocytes.

The signals evoked by Wnt proteins lead to intracellular accumulation of β-catenin, a key downstream component of the Wnt signaling pathway (reviewed by Cadigan and Nusse, 1997; Eastman and Grosschedl, 1999). β-Catenin then activates the target genes through the interaction with a member of LEF-1/TCF transcription factors, containing a high-mobility group domain. Thus, LEF-1/TCF transcription factors mediate a nuclear response to Wnt signals. We have shown that exogenously added Wnt-3a protein induces endogenous Mitf-M mRNA in cultured melanocytes (Takeda *et al*, 2000b). In addition, we identified the functional LEF-1-binding site in the M promoter (see **Fig 2**), and provided evidence that Wnt-3a signaling recruits β-catenin and LEF-1 to the M promoter, leading to the increased transcription from the M promoter. These results indicate a direct link between Wnt signaling and Mitf-M/MITF-M expression; however, the effect of Wnt-3a signaling observed may mimic that of other *Wnt* molecules. Further studies, such as extensive *in situ* hybridization analysis and gene knock-out experiments, will be required to reveal the bona fida *Wnt* gene, which regulates the expression of Mitf-M mRNA *in vivo*.

REGULATION OF MITF-M PROMOTER BY MULTIPLE TRANSCRIPTION FACTORS

The M promoter is upregulated via the separate *cis*-acting elements by CREB (Bertolotto *et al*, 1998; Price *et al*, 1998), PAX3 (Watanabe *et al*, 1998), and SOX10 (Bondurand *et al*, 2000; Lee *et al*, 2000; Potterf *et al*, 2000; Verastegui *et al*, 2000) (see **Fig 2**). CREB is phosphorylated and functionally activated via cAMP in response to MSH signaling (**Fig 4**), suggesting an important role of MITF-M in sun tanning (reviewed by Buscà and Ballotti, 2000). Both PAX3 and SOX10 are directly involved in the pathogenesis of auditory-pigmentary disorders. PAX3 is a transcription factor with a paired-homeodomain, and is responsible for Waardenburg syndrome (WS) type 1 and 3 (Tassabehji *et al*, 1995). SOX10 is a new member of the SOX family transcription factors with a high-mobility group box as a DNA-binding motif (Pusch *et al*, 1998). A truncation mutation of the *Sox10* gene is associated with the mouse mutant *Dominant megacolon*, a model for human congenital megacolon (Hirschsprung disease) (Southard-Smith *et al*, 1998). SOX10 is defective in some cases of Shah–Waardenburg syndrome, also known as Waardenburg-Hirschsprung disease or WS4, which is characterized by aganglionic megacolon, sensorineural deafness, and pigmentation abnormalities (Pingault *et al*, 1998). Thus, SOX10 is responsible for differentiation of the two types of neural crest-derived cells, melanocytes and intestinal ganglia cells. Moreover, a single heterozygous mutation of *SOX10* was found in a patient affected with a mild form of the Yemenite deaf–blind hypopigmentation syndrome (Bondurand *et al*, 1999). It is therefore conceivable that PAX3 and SOX10 may affect the development of melanocytes by regulating transcription of the M promoter.

Recently, by *in situ* hybridization analysis we showed coexpression of Sox10 and Mitf (most likely Mitf-M) mRNA in migrating melanoblasts at mouse embryonic day (E) 11.5 (Watanabe *et al*, 2000). By E 13.5, Sox10 mRNA expression became undetectable in migrating melanoblasts, in which Mitf expression, however, continues to be detectable. In this context, *Mitf*-positive cells were undetectable in the homozygous *Dominant megacolon* embryos of E 12.5 (Bondurand *et al*, 2000). In postnatal day 8 and adult cochleas, *Sox10* expression was detected only in the supporting cells of the organ of Corti (Watanabe *et al*, 2000). Taken together, these results support the notion that Sox10 is required for transcription from the M promoter of the *MITF/Mitf* gene during early stage of melanoblast development.

MOLECULAR BASES OF WAARDENBURG SYNDROME TYPE 2 AND TIETZ SYNDROME

Mutations in the MITF gene have been identified in some patients with WS2, a typical auditory–pigmentary syndrome (Farrer *et al*, 1994; Tassabehji *et al*, 1994, 1995; Nobukuni *et al*, 1996). It is

noteworthy that the missense mutations are clustered in the bHLH-LZ structure, which is required for DNA-binding and dimer formation, except for the Ser298P substitution (**Fig 5**). WS2 is an autosomal dominant disorder characterized by varying combinations of sensorineural hearing loss, heterochromia iridis, and patchy abnormal pigmentation of the hair and skin (reviewed by Liu *et al*, 1995). The exhibited hypopigmentation and hearing impairment are caused by the lack of melanocytes in skin and inner ears. The identified MITF mutations include splicing mutations, nonsense mutations, and missense mutations (**Fig 5**), but there is no particular correlation between the molecular defects of mutant MITF proteins and clinical severity. These results support the notion that haploinsufficiency of MITF-M is a molecular basis for WS2 (Nobukuni *et al*, 1996). On the other hand, most of the MITF mutations may also affect the functions of MITF isoforms with extended N-termini, such as MITF-A and MITF-H, although phenotypic consequences of altered function of MITF-A and other isoforms are not apparent in WS2. It is therefore conceivable that melanocytes are most sensitive to the reduced levels of functional MITF and that altered functions of MITF isoforms may be phenotypically detectable only in the homozygous state (reviewed by Shibahara *et al*, 1999), as seen in the homozygous *Mitf*$^{mi-rw}$ mice.

Recently, a mutation responsible for Tietz syndrome (albinism–deafness syndrome) was identified as an in-frame deletion of the MITF gene, removing one of four consecutive Arg residues (del R217) in the basic region of MITF-M (Amiel *et al*, 1998) (**Fig 5**). Tietz syndrome is characterized by profound deafness, generalized albinism with blue eyes, and hypoplasia of the eyebrows (Tietz, 1963). Unlike WS2, Tietz syndrome shows dominant inheritance with complete penetrance and does not involve the eyes. In contrast to Tietz syndrome, patchy depigmentation is characteristic of WS2, and deafness is seen in 77% of WS2 individuals (Liu *et al*, 1995). Thus, the del R217 mutation causes more severe dysfunction of melanocytes than do other MITF mutations. It is unknown whether melanocytes are present in the skin of patients affected with Tietz syndrome.

Surprisingly, the del R217 mutation found in Tietz syndrome is equivalent to the mouse semidominant *microphthalmia* (*mi*) mutation (Hodgkinson *et al*, 1993). The heterozygote *mi* mice show white spotting of the coat and reduced pigment in the iris, and the homozygotes present with totally white, deaf, small eyes, and osteopetrosis. The functional consequence of the del R217 mutation was shown to be a loss of DNA-binding activity and to function as a dominant negative form of MITF-M (Hemesath *et al*, 1994). Moreover, MITF-M protein, containing the del R217, is unable to translocate to the nucleus and inhibits nuclear localization of wild-type MITF-M (Takebayashi *et al*, 1996). These results together with the phenotypic differences between Tietz syndrome and WS2 suggest that a dominant-negative function of the del-R217 MITF-M is responsible for Tietz syndrome.

PROSPECTS

The *MITF* gene consists of widely spaced multiple promoters, which generate not only the diversity in the transcriptional regulation of these promoters but also the structurally different isoforms. The *MITF* gene will be a good model to study the mechanism of promoter selectivity during development or under certain metabolic conditions, perhaps related to retinoid metabolism. The isoform multiplicity of MITF also provides the functional diversity and redundancy. Future analysis of MITF isoforms *in vivo* will clarify the role of each MITF isoform in differentiation and development of the pigment cell and other cell types. It is also of significance to identify the interacting partners with the unique N-terminal domains as well as the bHLH-LZ domain that modulates the function of MITF isoforms.

This work was supported in part by Grants-in-Aid for Encouragement of Young Scientists (to K. Y.), and for Scientific Research (B) from the Ministry of Education, Science, Sports, and Culture of Japan. This work was also supported in part by the Nakatomi Foundation and the Kao Foundation for Arts and Sciences.

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