Cochlear Melanocytes and MITF Signaling

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Melanocytes occur not only in the skin and eyes but in the cochlea, where they exist as intermediate cells of the stria vascularis. Intermediate cells play an important role for cochlear function: Na^+K^+-ATPase and potassium channels of intermediate cells are essential for production of endocochlear potential and for preparation of ionic milieu in the stria. Consistent with this notion, melanocyte deficiency due to some gene disruptions results in hearing impairment in mice and humans. *Mitf/MITF* is essential for development and maturation of melanocytes, including strial intermediate cells. Disruption of *MITF* causes deafness, heterochromia irides, and leucodermia in Waardenburg syndrome type 2 individuals, whereas that of Mitf causes phenotypes of deafness, microphthalmia, and white coat in mice. Again, all of these phenotypes may be explained by a lack of melanocytes. Many signal transduction pathways target the *Mitf/MITF* gene or Mitf/MITF protein, and disruption of these pathways sometimes results in the phenotype similar to that caused by Mitf/MITF disruption. If not all, certainly many roads lead to MITF in melanocytes. *Key words: GSK3β/hearing/stria vascularis/Waardenburg syndrome.* *Journal of Investigative Dermatology Symposium Proceedings 6:95–98, 2001*

Micrphtahlmia-associated transcription factor (MITF) is a transcription factor with basic-helix-loop-helix-leucine zipper (bHLHZip) structure, and is a melanocyte-inducible transcription factor (Tachibana *et al*, 1994; for review see Tachibana, 1997, 1999, 2000). Whereas loss-of-function mutations of the *MITF* gene cause Waardenburg syndrome type 2 (Tachibana, 1997), a dominant negative mutation of *MITF* causes Tiez syndrome, i.e., albinism–deafness syndrome, whose symptoms are similar to WS2 but more severe (Amiel *et al*, 1998; Smith *et al*, 2000). In both syndromes, *MITF* mutations often cause hearing impairment along with skin and iris pigmentation anomaly. The pigmentation anomaly is caused by the absence of melanocytes in skin and iris; the hearing impairment is also caused by melanocyte absence, although it might sound strange to dermatologists. Indeed, melanocytes exist in the stria vascularis of the cochlea as intermediate cells and play a crucial role in hearing function. In this review, I summarize the history and recent progress of MITF research with emphasis on its function in the cochlea. In addition, I will discuss the current understanding of the signal transduction system targeting the *MITF* gene and MITF protein.

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Abbrevations: ASP, agouti signal protein; cAMP, cyclic adenosine 3′,5′-monophosphate; bHLHZip, basic-helix-loop-helix-leucine zipper: CREB, cAMP response element binding protein; DAG, 1,2-diacylglycerol; EDN, endothelin; EDNRA, endothelin-A receptor; EDNRB, endothelin-B receptor; IGF-I, insulin-like growth factor-I; IGFIR, IGF-I receptor; IR, insulin receptor; HGF, hepatocyte growth factor; H_2R, histamin recetor H2; GSK3β, glycogen synthase kinase 3β; IP3, inositol 1,4,5-trisphosphate; MAP, microtubule-associated protein; MITF, Microphthalmia-associated transcription factor; Lef-1, lymphoid enhancing factor-1; MEK, MAPK/ERK kinase; MSH, melanocyte stimulating hormone; MSHR, MSH receptor; $p70^{S6K}$, ribosomal 70 kDa S6 protein kinase; PKA, protein kinase A; PKC, protein kinase C; RSK-1, ribosomal subunit kinase-1; SF, scatter factor; WS, Waardenburg syndrome.

STRIAL INTERMEDIATE CELLS OF THE COCHLEA ARE MELANOCYTES

Stria vascularis, along with spiral ligament, occupy the lateral wall of the mammalian cochlear duct; it secretes endolymph producing +90 mV endocochlear potential (EP). Stria vascularis consists of marginal, intermediate, and basal cells; the latter two kinds of cells and spiral ligament cells are connected to each other by gap junctions, whereas marginal cells are not. Hence, stria is considered to be composed functionally of two compartments (**Fig 1**). In this scheme, Na^+K^+-ATPase and potassium channels of intermediate cells play a crucial role in the production of EP and ionic milieu bathing marginal cells (Takeuchi and Ando, 1998; for review see Tachibana, 2000), indicating that melanocytes are important for the hearing function. As proof of this, disorders affecting melanocytes are often associated with hearing impairment: these disorders include Waardenburg syndrome (WS) type 1–4, Tietz syndrome, Yemenite deaf–blind syndrome, and Vogt-Koyanagi-Harada syndrome, and they may be classified together as auditory–pigmentary syndrome.

A TRANSCRIPTION FACTOR GENE *Mitf/MITF* IS MUTATED IN SOME HEREDITARY DEAF MICE and HUMANS

We previously examined microphthalmic and deaf white transgenic mice with an insertional mutation at the *microphthalmia* (*mi*) locus (Tachibana *et al*, 1992). We found that these phenotypes are caused by a lack of melanocyte in eye, skin, and cochlea, and proposed these mice as a mouse model for WS2. Stria vascularis of these mice was thin due to a lack of intermediate cells and showed severe degeneration. Successively, sensory hair cells degenerated and startle response to sound was lost. Further molecular analysis of these mutant mice led to the cloning of the *Mitf* gene, a mouse homolog of *MITF* (Hodgkinson *et al*, 1993). Soon after this

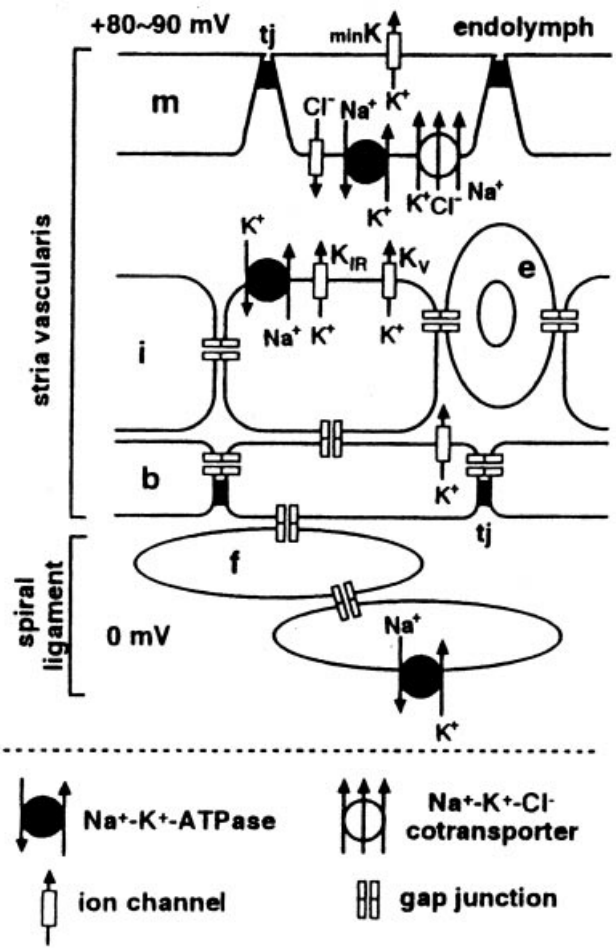

Figure 1. Extended the "two cell model" for generation of endocochlear potential. In this model, K^+ channels in the intermediate cells and speculative K^+ channels in the basal cell are the major source of endocochlear potential. minK, slowly activating K^+ channel; K_{IR}, inward rectifier K^+ channel; K_v, deploarization-activated K^+ channel; b, basal cell; e, endothelial cell and pericyte of capillary; f, fibrocyte; i, intermediate cell; m, marginal cell; tj, tight junction. Adapted from Takeuchi and Ando, 1998 with permission.

observation, *MITF* was found to be mutated in WS2 individuals (Tassabehji *et al*, 1994; Tachibana, 1997). Cochleae of WS2 individuals lack melanocytes (Nakashima *et al*, 1992), and thus all three symptoms of WS2, i.e., hearing impairment, hetreochromia irides, and leucodermia, may be accounted for by melanocyte anomaly as in the case of the *mi* mutant mice. These observations, along with the notion that bHLHZip proteins are often transcription factors involved in cell differentiation, led us to believe that MITF/Mitf is a Melanocyte Inducible Transcription Factor, and we found that ectopic expression of *MITF* converted fibroblasts into cells with melanocyte characteristics (Tachibana *et al*, 1996). Recently, a mutation of *MITF*, which encodes dominant-negative mutant MITF (Takebayashi *et al*, 1996), was found in Tietz syndrome (Amiel *et al*, 1998; Smith *et al*, 2000), another auditory–pigmentary syndrome.

MITF IN THE MATURED COCHLEA

Whether MITF is essential for maintenance of the matured cochlea remains to be examined. The possibility remains that *Mitf* is expressed postnatally at a low level, which may not be detected by *in situ* hybridization technique, and that it may have some physiologic or pathophysiologic function. Strial intermediate cells show continuous basic mitosis at a rate comparable with that of melanocytes in the skin (Conlee *et al*, 1994), and noxious stimuli such as noise increase melanogenesis in these intermediate cells (Gratton and Wright, 1992). Skin melanocytes also show basic mitosis (Jimbow *et al*, 1975), and noxious stimuli, such as UV stimulation (Jimbow and Uesugi, 1982) and wounding (Hirobe, 1983), enhances the mitosis. Mitf/MITF may be involved in these mitotic events in skin and in stria.

MITF IS REGULATED BY VARIOUS SIGNALING PATHWAYS IN MELANOCYTES

A plethora of signaling pathways have been implicated in differentiation, proliferation, and maturation of melanocytes, including cochlear melanocytes. Disruption of some genes related to these pathways in mice results in the phenotype resembling that of *mi* mutant mice, suggesting a linkage between these pathways and Mitf. Indeed, recent studies have revealed that some pathways post-translationally modulate the MITF protein by phoshorylation, whereas other pathways modulate it at the transcription level by stimulating the *MITF* promoter. Among these pathways, the cyclic AMP (cAMP) pathway plays a key role in the regulation of melanogenesis by regulating MITF at both the promoter and the protein levels (Bertolotto *et al*, 1996, 1998; for review see Busca and Balloti, 2000) (**Fig 2**); the increased level of cAMP in melanocytes activates protein kinase A (PKA) and Raf oncoprotein; activated PKA phosphorylates and activates cAMP responsive element (CRE) binding protein (CREB), which then binds to CRE in the *MITF* promoter; at the same time, activated cytoplasmic kinase Raf phosphorylates and activates MAPK/ERK kinase (MEK); activated MEK then pshosphorylates and activates MAP kinase (MAPK); activated MAPK phsohorylates and activates MITF protein on Ser73 (Hemesath *et al*, 1998) and ribosomal subunit kinase-1 (Rsk-1); finally activated Rsk-1 phoshorylates and activates MITF protein on Ser409 (Wu *et al*, 2000).

We recently identified the third endogenous phoshorylation site of MITF (Takeda *et al*, 2000). This site, Ser 298, may be phosphorylated by glycogen synthase kinase 3β (GSK3β), but the signaling pathway for this phosphorylation remains to be elucidated. Because GSK3β is shown to be inhibited by ribosomal 70 kDa S6 protein kinase ($p70^{S6K.}$) *in vitro* (Southerland and Cohen, 1994), we postulated that $p70^{S6K.}$ might be involved in the pathway for GSK3β-mediated phosphorylation of MITF. To test this, rapamycin, a potent inhibitor of $p70^{S6K.}$, was included in melanocyte culture: consistent with our hypothesis, melanogenesis was enhanced by rapamycin in a concentration-dependent manner (unpublished data). As cAMP is shown to inhibit $p70^{S6K.}$ in some cells (Cass and Meinkoth, 1998), it is conceivable that an increased level of cAMP results in phosphorylation and activation of MITF via inhibition of $p70^{S6K.}$ and GSK3β in melanocytes (**Fig 2**).

It is well established that α-melanocyte stimulating hormone (α-MSH) activates MSH receptor (MSHR), i.e., melanocortin receptor 1, which is a G-coupled seven transmembrane domain receptor; this activation induces melanogenesis. When this receptor is activated upon ligand binding, G-protein activates adenylate cyclase, resulting in elevation of cAMP in melanocytes (for review see Busca and Ballotti, 2000). Histamine is an inducer of melanogenesis; this cytokine also increases cAMP through interaction with H2 receptor, which is also a G-coupled seven transmembrane domain receptor, and elevates cAMP level in melanocytes (Yoshida *et al*, 2000). Thus, MSH and histamine may regulate MITF protein by phoshorylation on Ser73, Ser298, and Ser 409.

MITF IN COCHLEAR DEVELOPMENT

In situ hybridization study of mouse embryo revealed that *Mitf*-positive cells appeared in cephalic neural crest on E~10, and then migrated to a location between the otic vesicle and neuroepitheliun of the hindbrain on E~10.5 (Nakayama *et al*, 1998). Subsequently, these cells increased in number and became intimately associated

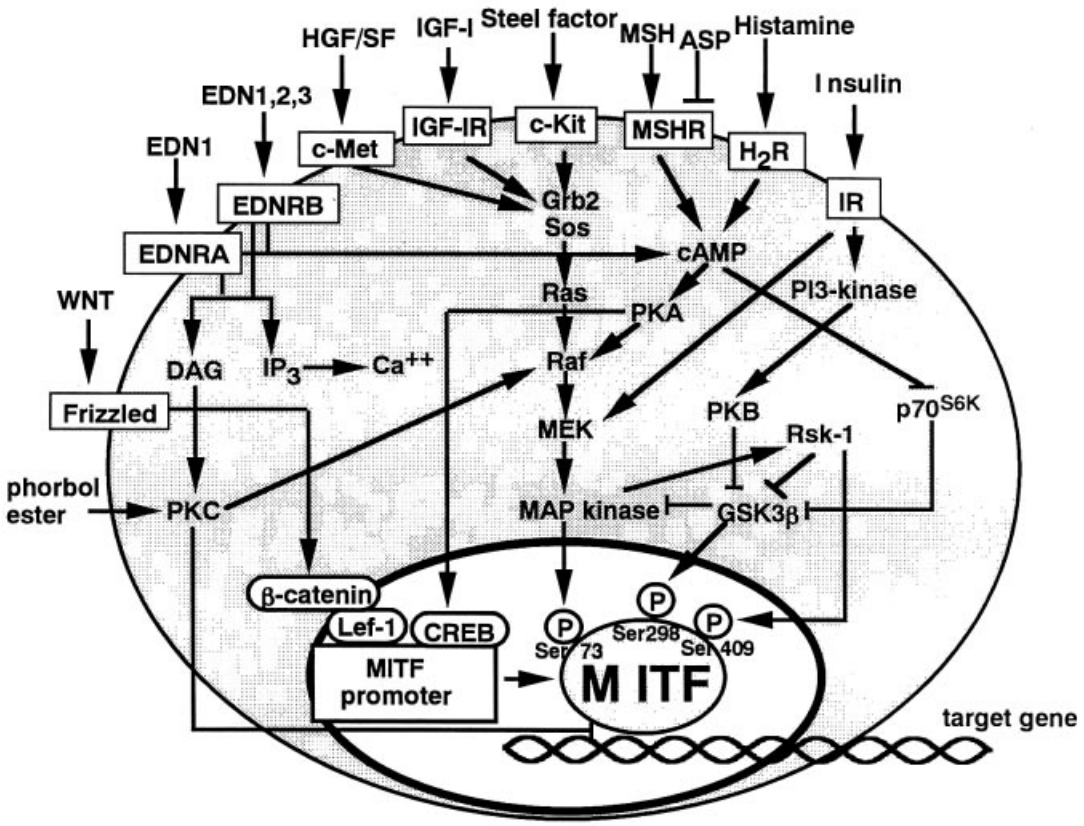

Figure 2. Signaling pathways related to MITF regulation at the promoter level and protein level. Although many of these pathways are established, some remain hypothetical. ASP, agouti signal protein; cAMP, cyclic adenosine 3′,5′-monophosphate; CREB, cAMP response element binding protein; DAG, 1,2-diacylglycerol; EDN, endothelin; EDNRA, endothelin-A receptor; EDNRB, endothelin-B receptor; IGF-I, insulin-like growth factor-I; IGF-IR; IGFIR, IGF-I receptor; IR, insulin receptor; HGF, hepatocyte growth factor; H_2R, histamin recetor H2; GSK3β, glycogen synthase kinase 3β; IP3, inositol 1,4,5-trisphosphate; MAP, microtubule-associated protein; Lef-1, lymphoid enhancing factor-1; MEK, MAPK/ERK kinase; MSH, melanocyte stimulating hormone; MSHR, MSH receptor; $p70^{S6K}$, ribosomal 70 kDa S6 protein kinase; PKA, protein kinase A; PKC, protein kinase C; RSK-1, ribosomal subunit kinase-1; SF, scatter factor.

with the otic vesicle. On E16.5, they were concentrated in an area of future stria vascularis. Later, the *Mitf* signal in the stria vascularis was less intense – and at birth was undetectable – whereas *Mitf* in the hair follicles was expressed after birth.

We previously examine microphthalmic and deaf white transgenic mice by insertional mutation and proposed them as a mouse model for WS2. We found that these phenotypes are caused by a lack of melanocyte in eye, skin, and cochlea (Tachibana *et al*, 1992). Stria vascularis of these mice was thin due to a lack of intermediate cells and showed severe degeneration with endolymphatic space collapsed. Sensory hair cells degenerated and response to sound was lost. *Mitf* is grossly mutated in these mice (Hodgkinson *et al*, 1993), and cochlea of deaf WS2 syndrome individuals shows a similar pathologic finding (Nakashima *et al*, 1992).

These observations indicate that MITF/Mitf is essential for the development and maturation of the cochlea, dependent on normal development of stria vascularis. Thus, the growth factor signaling pathways targeting MITF/Mitf are also involved in hearing function.

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