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Microphthalmia-associated transcription factor (MITF), a basic-helix-loop-helix (bHLH) and bHLH-leucine zipper transcription factor, is currently an intense focal point in pigment cell biology research. MITF is implicated as the master gene for survival of melanocytes, as well as a key transcription factor regulating the expression of major melanogenic proteins such as tyrosinase and the tyrosinase-related proteins TRP1 and TRP2 (Goding, 2000), via binding to a conserved consensus element, the so-called M-box, in the promoter region of each gene (Yasumoto *et al*, 1997). Mice bearing null alleles of the MITF gene display complete loss of neural crest-derived melanocytes, deafness and a failure of retinal pigment epithelium differentiation (Goding, 2000). At least two separate mechanisms mediate the diverse biological functions of MITF. First, sustained expression of MITF appears to be modulated through a cAMP-dependent pathway (Bertolotto *et al*, 1998; Price *et al*, 1998); and second, MITF function is transiently up-regulated via mitogen-activated protein kinase (MAPK)-dependent phosphorylation of MITF itself (Hemesath *et al*, 1998). Thus,  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) and other cAMP-elevating agents transcriptionally up-regulate the expression of MITF through a cAMP-response element (CRE) in the MITF promoter region (Goding, 2000). In contrast, c-kit transiently increases MITF function by a MAPK-mediated phosphorylation of MITF which recruits p300/CBP (CREB-binding protein), a coactivator family that in turn enhances the transcriptional activity of MITF (Hemesath *et al*, 1998). More recently, MITF was shown to promote survival of pigment cells by up-regulating the expression of the major antiapoptotic protein BCL2 (McGill *et al*, 2002).

In this issue of the JID, two articles further explore the role of MITF in modulation of pigmentation. Lin and colleagues (p. 1330) report that MITF may in part mediate UV-induced pigmentation and that certain antioxidants reduce basal pigmentation as well as UV-induced pigmentation in an MITF-dependent manner. By transfecting an MITF promoter-luciferase construct into murine pigment cells, they were able to demonstrate that MITF promoter activity is enhanced by UVB irradiation (30 mJ/cm<sup>2</sup>), with maximal effect 7–8 h after the irradiation. Their results add MITF to the list of genes whose expression is affected by UV irradiation. The authors hypothesize that UV is not likely to enhance the promoter activity of MITF directly, but rather to act indirectly through  $\alpha$ -MSH and ACTH, which is released from the same propeptide and binds the same receptor as  $\alpha$ -MSH. Given that UV increases production of these hormones by keratinocytes (Wintzen *et al*, 1996) and that  $\alpha$ -MSH is known to induce MITF gene transcription (Bertolotto *et al*, 1998; Price *et al*, 1998), it is indeed likely that UV-induced  $\alpha$ -MSH mediates an increased MITF transcription *in vivo*. However, in the experimental model system employed, a monolayer of pigment cells were transfected with an MITF promoter-luciferase construct. Thus, UV would first need to up-regulate MSH in these cells, which is not yet demonstrated. Further,  $\alpha$ -MSH is known to induce MITF tran-

scription by elevating the intracellular level of cAMP, yet the authors report that UVB irradiation did not increase the intracellular level of cAMP in their experiments. Therefore, one must consider the possibility that UV irradiation up-regulates MITF expression through an alternative pathway yet to be elucidated.

Lin *et al* further found that the antioxidants dihydrolipoic acid (DHLA), lipoic acid (LA) and resveratrol (RES) decreased MITF promoter activity and protein levels, as well as tyrosinase protein level and activity (JID). Other antioxidants, such as ascorbic acid and glutathione, did not have these effects. However, one apparently contradictory recent report, not discussed by the authors, suggests that the level of MITF is down-regulated by oxidative stress (Jimenez-Cervantes *et al*, 2001), and that oxidative stress transiently down-regulates the expression of tyrosinase, TRP-1 and TRP-2 in an MITF-dependent manner (Jimenez-Cervantes *et al*, 2001). These apparent discrepancies will need to be examined in future experiments.

To explore the *in vivo* relevance of their findings, the authors topically applied DHLA, LA and RES on dark-skinned Yucatan swine with or without UV irradiation expected to tan the skin. Topical application of all three agents lightened both basal and UV-induced skin pigmentation. Therefore, the authors suggested that modulation of pigmentation can be achieved through reducing or enhancing MITF activity *in vivo*. Although intriguing, this approach will need to be pursued with caution, given MITF is involved in not only melanogenesis but in the long term is also critical to survival of normal and healthy melanocytes (Goding, 2000).

In the second paper, Lei and colleagues (p. 1341) report induction of pigmentation by 8-methoxypsoralen (8MOP) in the absence of UV and examine possible mechanisms. Employing an immortalized murine melanocyte line as a model system, the authors ask whether MITF, the cAMP-dependent protein kinase (PKA) and/or protein kinase C (PKC) pathways, as well as proteasome-mediated degradation of tyrosinase might play a role in 8MOP-induced pigmentation. They treated cells with 8MOP, the PKA activator forskolin, or the PKC activator 1-oleoyl-2-acetyl-glycerol (OAG) for 6 days and observed an increase in tyrosinase activity and melanin in all except in OAG-treated cells. Then presumptively specific inhibitors for PKA and for PKC were employed to determine if the 8MOP-induced increase in pigmentation utilizes one or both of these pathways. The authors conclude that while PKA inhibition blocks 8MOP-induced increases in tyrosinase protein and activity, as well as in total melanin content, PKC inhibition had no effect. However, their Fig 4 shows that the PKC inhibitor clearly blocks the 8MOP-induced increase in melanin content to the same extent as the PKA inhibitor, although by day 6 (when measurements were first made) there was no effect on tyrosinase activity. Since changes in melanin level are subsequent to changes in tyrosinase activity, these results are compatible with the PKC pathway playing an important role prior to day 6, for example by activating pre-existing

tyrosinase protein, as reported by our group (Park *et al*, 1999). Time course studies would be of interest in further exploring the role of the PKC-dependent pathway in 8MOP-induced pigmentation. A final caveat: Despite the efforts of these authors and the others whose work they cite to examine 8MOP in isolation, it is extremely difficult to exclude the possibility that minute amounts of incidental UVA irradiation did photoactivate this exquisitely sensitive compound. Their findings would then pertain to pigmentation-induced by PUVA rather than by 8MOP.

Much is yet to be learned about MITF, the presumptive master gene for pigment cells. The fact that MITF is now recognized to be a family of at least five isoforms (Shibahara *et al*, 2001) suggests considerable complexity for the biological function of this transcription factor. The two articles in this JID issue provide further insight into how MITF mediates pigmentation as the regulatory protein for many different agents.

## REFERENCES

- Bertolotto C, Abbe P, Hemesath TJ, Bille K, Fisher DE, Ortonne JP, Ballotti R: Microphthalmia gene product as a signal transducer in cAMP-induced differentiation of melanocytes. *J Cell Biol* 142:827–835, 1998
- Goding CR: Mitf from neural crest to melanoma. Signal transduction and transcription in the melanocyte lineage. *Genes Dev* 14:1712–1728, 2000
- Hemesath TJ, Price ER, Takemoto CM, Badalian T, Fisher DE: MAP kinase links the transcription factor microphthalmia to c-Kit signaling in melanocytes. *Nature* 391:298–301, 1998
- Jimenez-Cervantes C, Martinez-Esparza M, Perez C, Daum N, Solano F, Garcia-Borron JC: Inhibition of melanogenesis in response to oxidative stress: Transient downregulation of melanocyte differentiation markers and possible involvement of microphthalmia transcription factor. *J Cell Sci* 114:2335–2344, 2001
- McGill GG, Hortsman M, Widlund HR, *et al*: Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. *Cell* 109:707–718, 2002
- Park HY, Perez JM, Laursen R, Hara M, Gilchrist BA: Protein kinase C- $\beta$  activates tyrosinase by phosphorylating serine residues in its cytoplasmic domain. *J Biol Chem* 274:16470–16478, 1999
- Price ER, Hortsman MA, Wells AG, Weilbaecher KN, Takemoto CM, Landis MW, Fisher DE:  $\alpha$ -melanocyte-stimulating hormone signaling regulates expression of microphthalmia, a gene deficient in Waardenburg Syndrome. *J Biol Chem* 273:33042–33047, 1998
- Shibahara S, Takeda K, Yasumoto K, Udono T, Watanabe K, Saito H, Takahashi K: Microphthalmia-associated transcription factor (MITF). Multiplicity in structure, function, and regulation. *J Invest Dermatol* 6:99–104, 2001
- Wintzen M, Yaar M, Burbach JPH, Gilchrist BA: Proopiomelanocortin gene product regulation in keratinocytes. *J Invest Dermatol* 106:673–678, 1996
- Yasumoto KY, Kouji Y, Kazuhiro T, Yasushi T, Shigeki S: Functional analysis of microphthalmia-associated transcription factor in pigment cell-specific transcription of the human tyrosinase family genes. *J Biol Chem* 272:503–509, 1997