SPECIAL SECTION: REVIEW

MITF—the first 25 years

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All transcription factors are equal, but some are more equal than others. In the 25 yr since the gene encoding the microphthalmia-associated transcription factor (MITF) was first isolated, MITF has emerged as a key coordinator of many aspects of melanocyte and melanoma biology. Like all transcription factors, MITF binds to specific DNA sequences and up-regulates or down-regulates its target genes. What marks MITF as being remarkable among its peers is the sheer range of biological processes that it appears to coordinate. These include cell survival, differentiation, proliferation, invasion, senescence, metabolism, and DNA damage repair. In this article we present our current understanding of MITF's role and regulation in development and disease, as well as those of the MITF-related factors TFEB and TFE3, and highlight key areas where our knowledge of MITF regulation and function is limited.

Supplemental material is available for this article.

Microphthalmia-associated transcription factor (MITF) genetics

For a cat, it may not matter whether a mouse is black or white, but for a mouse, it makes a world of difference, especially when whiteness is associated with mutations at a locus called "*mi*" (short for "*microphthalmia*"). As originally discovered by Paula Hertwig in 1942 (Hertwig 1942), homozygosity for a mutation at this locus causes mice to lack neural crest-derived melanocytes and have small (microphthalmic) eyes due to abnormalities in their retinal pigment epithelium (RPE) (Müller 1950). It was later found that *microphthalmia* mice are also deaf because of the absence of inner ear melanocytes (Tachibana et al. 1992). Remarkably, mice with the original *mi* mutation are still available, and >40 additional forward mutations at this locus have since been found or generated in mice (Mouse Genome Informatics, http://www.informatics.jax.org). They typically display the major trias of whiteness, microphthalmia (Fig. 1A), and deafness and, depending on the allele, may show auxiliary symptoms such as osteopetrosis, mast cell deficiencies, heart hypotrophy, or altered nephron numbers. In some cases only a minor reduction in the levels of the pigment enzyme tyrosinase is observed, as seen with homozygosity for *mi-spotted*, an allele that was found only because it renders mice spotted when combined with other *mi* alleles. Because the phenotypes associated with specific alleles or allele combinations reveal a high degree of complexity, it was originally thought that the full phenotypic spectrum associated with the *mi* locus might be due to mutations in two or more linked genes (Hollander 1968). A molecular understanding of this complexity had to wait until two chance transgenic insertional mutations led to the discovery of the *Mitf* gene (Hodgkinson et al. 1993; Krakowsky et al. 1993), and it was shown that all mice with mutations at *mi* in fact had mutations in this single gene (for reviews, see Steingrímsson et al. 2004; Arnheiter 2010).

The *Mitf* gene, which has homologs all the way down to primitive metazoans, including trichoplax (Gyoja 2014) and sponges (Simionato et al. 2007), encodes a transcription factor of the basic domain helix–loop–helix leucine zipper (bHLH-LZ) class that binds DNA as dimers (Figs. 2, 3). It belongs to the MiT subfamily of factors that in vertebrates also includes TFEB, TFE3, and TFEC with which it can form heterodimers (Hemesath et al. 1994; Pogenberg et al. 2012). As outlined below in more detail, in mammals the gene is spread over many exons and sports a number of distinct transcriptional start sites, and its RNA is subject to a multitude of alternative splicing events and regulation by microRNAs (Fig. 2; annotated human and mouse genomic sequences are shown in Supplemental Figs. S1, S2, respectively). This arrangement allows for the generation of many isoforms that differ in primary sequence, undergo a considerable diversity of posttranslational modifications, and can be finely tuned in their expression. Hence, the original idea of an "*mi*" locus comprised of more than one gene can perhaps be rescued: *Mitf* might theoretically give rise to hundreds of

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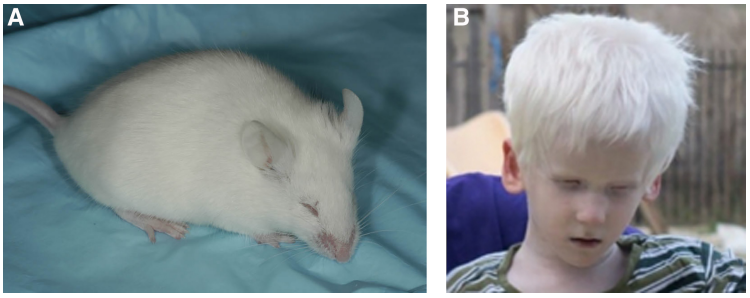

Figure 1. Phenotypes associated with *MITF* mutations in mice and humans. (*A*) Microphthalmia and white coat seen in a mouse homozygous for the $Mitf^{mi\text{-}vga9}$ mutation (due to the insertion of a transgene). (*B*) COMMAD (coloboma, osteopetrosis, microphthalmia, macrocephaly, albinism, and deafness) syndrome, here due to compound heterozygosity for K206N/R217Del based on the (+) MITF-M sequence or K307N/R318Del based on the (–) MITF-A sequence as published by George et al. (2016). (Note, however, that based on the deletion of one of three AGA codons in a row, it is impossible to determine which of the three corresponding arginines R215-R217 is deleted.) (Photograph courtesy of the Withrow family.)

distinct proteins, each potentially with tissue-preferential expression and activity levels. Not surprisingly, indels and nucleotide substitutions can selectively affect distinct promoters, splicing events, and functional protein domains, including those regulating intracellular distribution, stability, dimerization, and sequence-specific DNA binding. The inheritance mode of the different alleles may be semidominant or recessive, but notably, functional null alleles are haploid sufficient in mice, although not in humans.

The consequences for coat color phenotypes of the different alleles in mice are often intriguing. Some alleles or allele combinations, for instance, can lead to a black head spot on an otherwise completely white mouse, while others can lead to a white head spot on an otherwise completely black mouse; some allele combinations can even yield tricolored (white/tan/black) mice (Debbache et al. 2012).

Given that *Mitf* is evolutionarily conserved, it is not surprising that its roles in pigmentation can be seen in other species. In domestic dogs (Baranowska Körberg et al. 2014) and horses (Hauswirth et al. 2012), for instance, alterations in the promoter giving rise to the major melanocyte isoform of MITF are responsible for white spotting. Nevertheless, pigmentation need not always be the major target for *Mitf* homologs. In *Drosophila melanogaster*, *Mitf* functions primarily in the gut (Zhang et al. 2014) and expression of a dominant-negative mutant in the eye disc expands the neuronal field (Hallsson et al. 2004) in contrast to mice, where *Mitf* mutations lead to retinal hypoplasia (Müller 1950; Bharti et al. 2006).

Naturally, of special interest to us is human *MITF*. As in mice (Fig. 4, top panel), human germline mutations (Fig. 4, bottom panel) cluster in the functionally important bHLH-LZ domain and are largely associated with pigmentary disturbances and deafness (Waardenburg

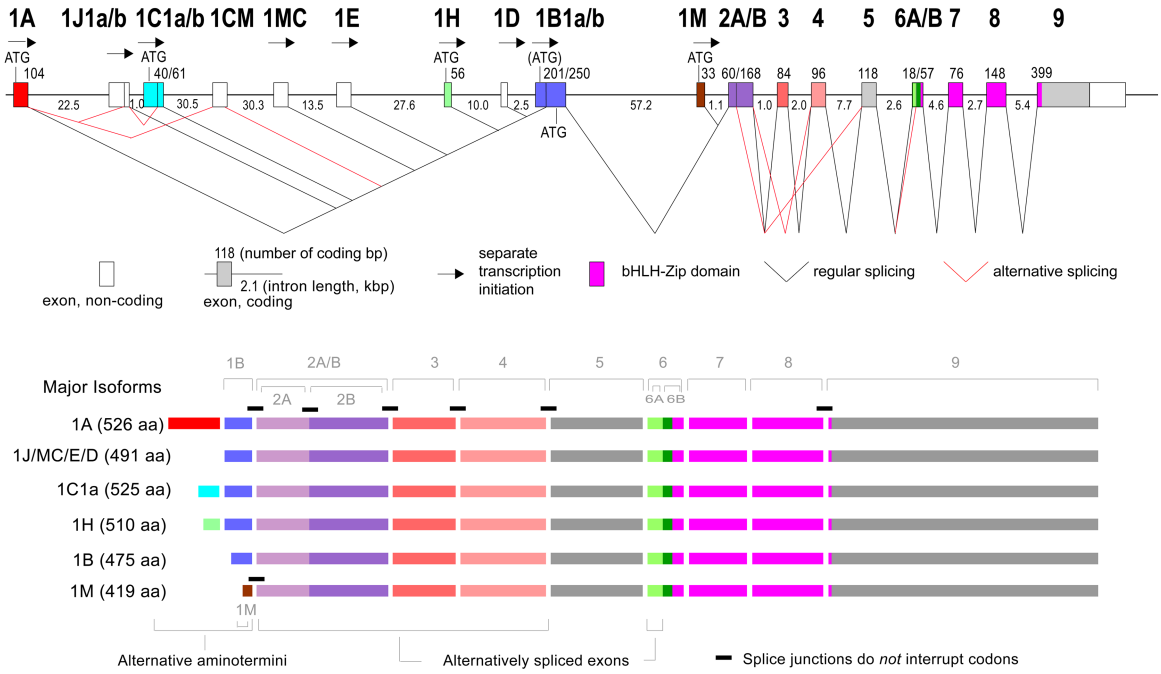

Figure 2. Schematic representation of the human *MITF* gene and protein isoforms. Exon/intron distribution and protein isoforms differing at their N termini are shown. Note that exon 1MC is based on similarity with the mouse sequence. For detailed annotated sequences, see Supplemental Figures S1 (for human MITF) and S2 (for mouse Mitf).

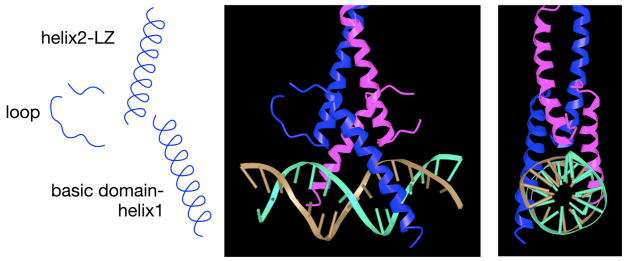

Figure 3. Structure of mouse MITF cocrystalized with dsDNA. Protein: Ribbon view of a dimer of two monomeric bHLH-LZ domains of MITF, comprised of 118 residues each (protein database: 4ATI). DNA: cartoon view of a 16-nt dsDNA comprising an M-box motif with flanking sequences (Pogenberg et al. 2012). The *left* part of the figure schematically represents the different parts of the bHLH-LZ domain of the cocrystal structure of MITF.

syndrome [WS] IIa and the more severe Tietz syndrome) (Leiden Open Variable Database, https://databases.lovd.nl/shared/genes/MITF). There are also compound heterozygotes, as demonstrated recently in individuals with a novel syndrome called COMMAD (coloboma, osteopetrosis, microphthalmia, macrocephaly, albinism, and deafness), depicted in Figure 1B (George et al. 2016). Unlike in many *Mitf* mutant mice, however, human phenotypes are seen in heterozygous individuals, and there is only one report of a homozygous WSIIa individual (Rauschendorf et al. 2019). Most intriguing is the observation that one particular germline mutation has an increased susceptibility to develop melanoma and renal cell carcinoma. This mutation, independently found by two groups using whole-genome sequencing or candidate gene approaches, affects a sumoylation site previously studied in vitro (Murakami and Arnheiter 2005; Bertolotto et al. 2011; Yokoyama et al. 2011). Together with findings of somatic mutations in melanoma cells showing correlations between MITF activity and tumor aggressivity (Garraway et al. 2005; Hoek and Goding 2010), *MITF* has emerged as a key factor important for not only developmental biology and evolution but also oncology.

MITF genomic organization

The human *MITF* gene, from the start of exon 1A to the poly(A) site in exon 9, comprises close to 229,000 bp on the short arm of chromosome 3 (Fig. 2). In other vertebrates, gene size and exon composition may vary, but the principle gene organization is similar to that in humans. Furthermore, multiple blocks of homology are found across vertebrates both in exonic and nonexonic sequences (http://genome.ucsc.edu).

As shown in Figure 2, there are nine upstream exons, each with its own transcriptional start site (exon 1MC is based on similarity to mouse exon 1MC, and a tenth exon, 1CM, may be primate-specific and lack its own start site). Five of them (1A, 1C, 1H, 1B, and 1M) contain ORFs with their own start codons, and four of them (1J, 1MC, 1E, and 1D) do not. In addition, there are eight downstream exons (exon 2–9) usually found in all transcripts. Except for exon 1M, which is spliced directly to exon 2A, all other upstream exons give rise to transcripts that include exon 1B1b as the common link to exon 2A. As indicated in Figure 2, there are multiple alternative splicing events giving rise to different mature transcripts and distinct proteins. Importantly, junctions between some exons do not interrupt codons, while others do, with the consequence that the former can be spliced out without penalty for the downstream ORF, while elimination of the latter usually leads to a change in the downstream ORF and premature protein chain termination or nonsense-mediated decay of the corresponding mRNAs. Intriguingly, it is exon 1B1b and the exons encoding part of the functionally critical basic domain and the HLH-Zip domain that cannot be spliced out without leading to truncated proteins or mRNA decay (see also Supplemental Fig. S3). Based on experiments in mice, the upstream exons all have their preferential expression patterns: For instance, 1A is ubiquitously expressed, 1H accumulates to high levels in the heart (Steingrímsson et al. 1994, 2004), 1D in the RPE (Takeda et al. 2002; Bharti et al. 2008), and 1M in neural crest-derived melanocytes (Hodgkinson et al. 1993). These expression preferences reflect predominantly the regulation of the corresponding enhancers/promoters by tissue-preferred transcriptional regulators.

MITF gene organization thus allows for the generation of several distinct protein isoforms differing at their N termini. Although evidence from cell culture indicates that presence of exon 1B1b confers a distinct mode of regulation on *MITF*, enabling it to be recruited to the lysosome and be phosphorylated by the mTORC1 complex (see below; Martina and Puertollano 2013; Ngeow et al. 2018), there is as yet no direct in vivo evidence in either mice or humans that the different N termini confer tissue-specific activities to the different proteins or that the 5′-untranslated portions of the respective mRNAs play any specific roles. Deletion of exon 1A in mice, for instance, does not visibly alter pigmentation in skin and eye although it leads to the above-mentioned decrease in nephron numbers (Phelep et al. 2017). Alterations in composition and levels of other Mitf transcripts, however, precludes conclusions regarding a specific role of the 1A exon in kidney. A selective deletion of exon 1D, which during development normally contributes ~30% to total Mitf RNA in the RPE, just slightly delays the onset of RPE pigmentation but then is fully compensated by up-regulation of other isoforms, in particular Mitf-H (Bharti et al. 2012). The elimination of exon 1M in humans with 5′ splice site mutations at 1M that are expected to lead to readthrough into intron 1 and termination 18 codons downstream is associated with the typical pigmentary phenotype of WS IIa (Online Mendelian Inheritance in Man [OMIM]: WSIIA.0001) (Tassabehji et al. 1994; Haddad et al. 2011). This means that isoforms in which other upstream exons are spliced to exon 2A do not compensate for the lack of MITF-M. This observation, however, does also not allow one to conclude that the 1M exon has a specific function; exon 1M is just associated with the most

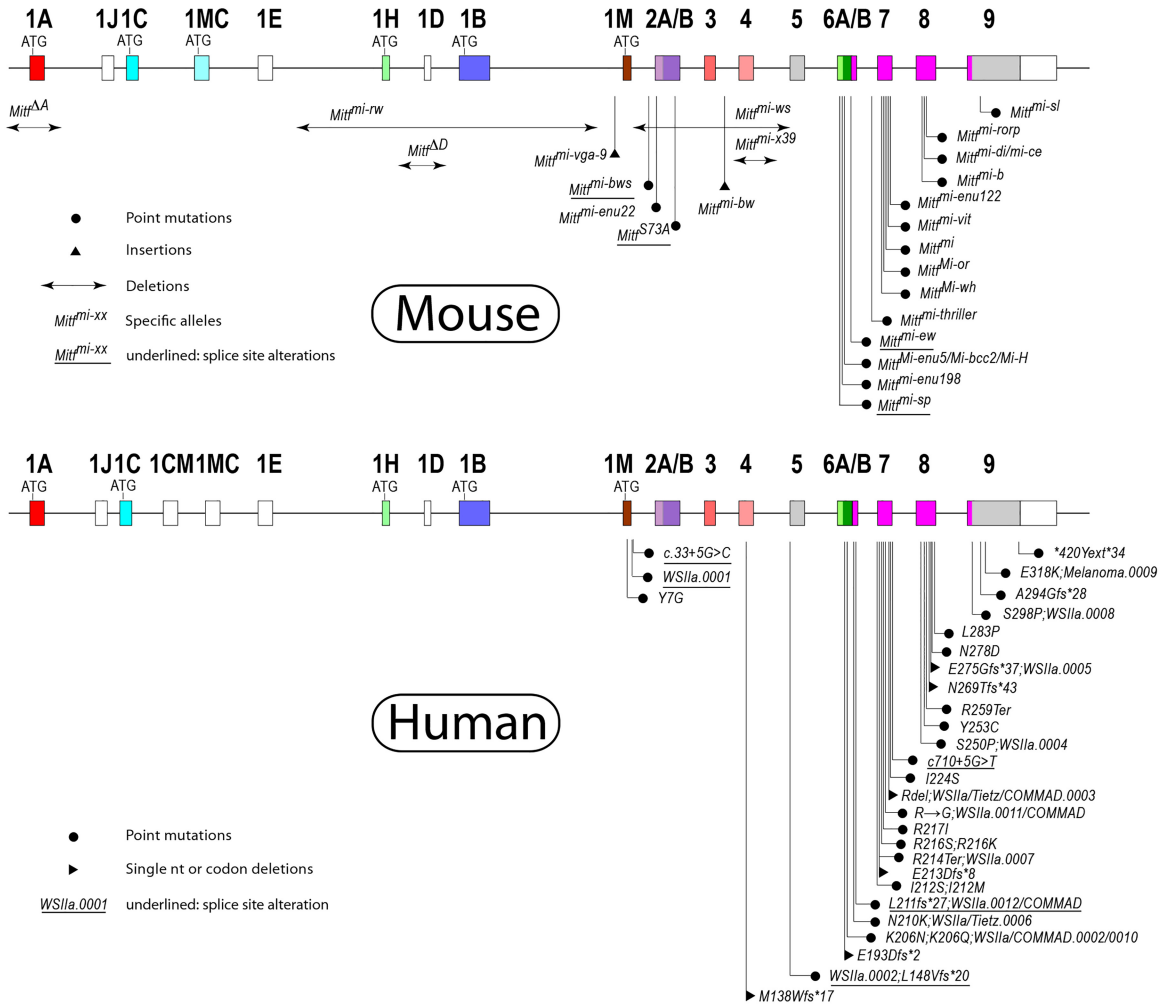

Figure 4. Representative spontaneous, ENU-induced and engineered mouse *Mitf* mutations (*top* panel) and selected symptomatic human *MITF* mutations (*bottom* panel). (For detailed references for mouse mutations, see Mouse Genome Informatics, http://www.informatics.jax.org/phenotypes.shtml; for human mutations, see Leiden Open Variable Database, https://databases.lovd.nl/shared/genes/MITF.)

abundant MITF isoform in melanocytes, and its lack likely reduces the total amount of MITF below a threshold level. The effect of other alternative splicing events, such as inclusion or exclusion of exon 6A encoding six residues upstream of the basic domain, leads to proteins designated as the (+) or (−) 6A isoforms that slightly differ in DNA-binding activities (Pogenberg et al. 2012). Although the inclusion of exon 6a is regulated by MAPK signaling (Primot et al. 2010), it is difficult to attribute a specific function to exon 6A as the overall Mitf RNA or protein levels may be altered in the above mentioned *Mitf*$^{mi\text{-}sp}$ mice, which are unable to incorporate this exon, and no exon 6A-specific interaction partners have yet been reported.

In addition to the various splice isoforms, MITF proteins also show a plethora of posttranslational modifications, including serine and tyrosine phosphorylation, ubiquitination, and sumoylation (Fig. 5). Much of our knowledge on their functional importance comes from in vitro studies with limited supporting evidence in vivo. For instance, the mutation of the phosphorylatable Ser298 (S298) to proline is associated with WS IIA (OMIM: WSIIA.0008) (Takeda et al. 2000a), but this mutation may not be responsible for the phenotype, as the corresponding protein is functional in vitro (Grill et al. 2013). In mice, a change of the conserved codon encoding the phosphorylatable S73 into one encoding an alanine in exon 2B, with the aim to decipher the role of S73 phosphorylation in vivo, had the unexpected molecularly dramatic effect of elimination of the entire exon 2B from mRNA, likely because of disruption of an exonic splice enhancer sequence that includes the S73 codon and interacts with the splicing factor SRSF5 (Arnheiter et al. 2008; Bismuth et al. 2008; Debbache et al. 2012). In vitro, even the change into another one of the six possible serine codons led to the same exon exclusion, indicating as expected that it is the sequence of the RNA—not of the protein—that is responsible for the phenomenon (H Arnheiter, unpubl.). Phenotypically,

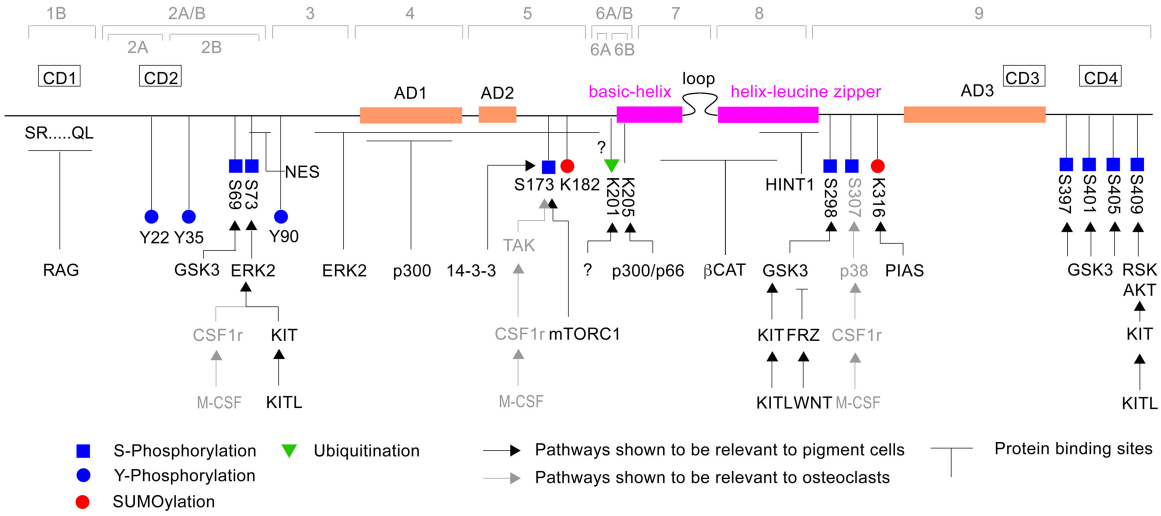

Figure 5. Schematic representation of MITF posttranslational modifications relevant to pigment cells or osteoclasts and resulting from activation of the indicated signaling pathways. (CD) Conserved domain; (AD) activation domain.

however, absence of exon 2B just led to a slight increase in pigmentation on a genetically sensitized background (Debbache et al. 2012). Rescue strategies using mutated genomic (BAC) Mitf transgenes further showed that the entire exon 2 (i.e., exon 2A and 2B) is largely dispensible (Bauer et al. 2009). On the other hand, as mentioned above, the E318K mutation, affecting sumoylation at K316, is associated with increased susceptibility to melanoma (Bertolotto et al. 2011; Yokoyama et al. 2011).

Regulation of MITF expression

Since little is known of how the multiple upstream promoters that drive expression of predominantly nonmelanocyte isoforms are regulated, we focus here on the transcription factors and signaling pathways controlling expression of mRNA encoding the melanocyte-specific isoform MITF-M.

There are three critical regions known to be required for expression of MITF-M that have been defined to date. First, expression of MITF-M is eliminated in *mi-black-eyed white* (*Mitf*$^{mi\text{-}bw}$) mice, in which a LINE1 element is inserted into intron 3 (Yajima et al. 1999). As the name suggests, these mice lack all neural crest-derived melanocytes, but the RPE is intact even though the splicing patterns and expression levels of RPE-expressed isoforms are also altered (Takeda et al. 2014). While there is clear genetic evidence for the effect of the line element insertion, it remains unknown whether it disrupts an enhancer or affects MITF-M expression through other means. Second, an enhancer located ~92 kb upstream of the transcriptional start site of Mitf-M is regulated by the Med23 subunit of the mediator complex (Xia et al. 2017) that links transcription factors to RNA polymerase. Third, the MITF promoter lying immediately upstream of the melanocyte isoform is controlled by several transcription factors and signaling pathways implicated in melanocyte and melanoma biology (Fig. 6; if known, binding sites are indicated in Supplemental Figs. S1, S2). The major transcription factors that are known to regulate MITF are highlighted below.

Activators of MITF mRNA expression

CREB, a bZIP transcription factor, recognizes a TGACG TCA motif within the MITF promoter. CREB enables MITF-M expression to be responsive to elevated cAMP levels downstream from the melanocortin 1 receptor that is implicated in control of hair and skin pigmentation at least in part via its ability to regulate *MITF* expression. The ability of CREB to activate MITF-M in response to elevated cAMP signaling has also been reported to be dependent on SOX10 (SRY-related high-mobility group box 10) (Huber et al. 2003), another key regulator of MITF-M expression (see below). Because CREB regulates MITF-M

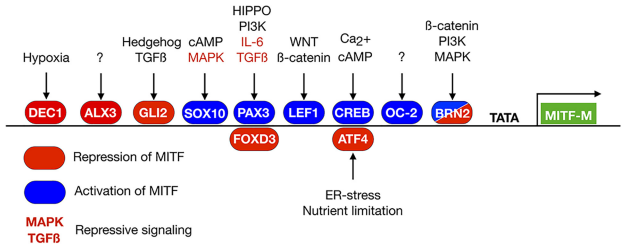

Figure 6. Schematic diagram of transcription factors regulating the MITF-M promoter positively or negatively and their response to signaling pathways. Transcription factor binding sites, as far as identified, are indicated in Supplemental Figures S1 and S2. Note that the precise binding sites for DEC1 and ALX3 (aristaless-like homeobox 3) are not known. Also, ATF4 (activating transcription factor 4) may repress Mitf-M transcription by directly competing with CREB (cyclic AMP regulatory element-binding protein)-binding (Ferguson et al. 2017).

expression, MITF-responsive downstream genes implicated in pigmentation also respond to cAMP signaling (Bertolotto et al. 1996, 1998a,b; Price et al. 1998b).

PAX3, a paired box homeodomain transcription factor, is necessary for melanocyte development and regulation of melanocyte stem cell activation via its capacity to control MITF-M expression and activity (Lang et al. 2005; Medic and Ziman 2010). Mutations in PAX3, like those in MITF, give rise to WS. PAX3 is repressed by TGFβ signaling in the skin (Yang et al. 2008) and is down-regulated by interleukin 6 receptor signaling (Kamaraju et al. 2002). Since PAX3 promotes PI3K-mediated activation of the BRN2 promoter (Bonvin et al. 2012), it is possible that PAX3 also mediates responsiveness of the MITF-M promoter to this signaling pathway. Importantly, PAX3 lies downstream from Hippo signaling that controls organ size and confers responsiveness to mechanical stress (Meng et al. 2016). Since the Hippo pathway effectors YAP and TAZ have been identified as transcription cofactors for PAX3, neural crest-specific deletion of *Yap* and *Taz* lead to neural crest defects and low MITF expression (Manderfield et al. 2014). The Hippo–PAX3–MITF axis is also implicated in melanoma. GNAQ and GNA11, encoding heterotrimeric Gαq family members that bear activating mutations in a high proportion of uveal melanomas, activate YAP via a Trio–Rho/Rac pathway (Feng et al. 2014). Consequently, Gαq signaling leads to YAP-dependent uveal melanoma growth presumably mediated in part via the YAP-PAX3-MITF axis. Similarly, activation of YAP/PAX3 via collagen stiffness has been implicated in gene regulation in cutaneous melanoma cell lines (Miskolczi et al. 2018). In this study, increased collagen abundance in melanomas correlated with nuclear YAP and increased collagen stiffness could promote expression of MITF via YAP/PAX3. However, when fibroblasts were present, this effect was disrupted by TGFβ signaling that redirected YAP away from PAX3 toward a YAP/TEAD/SMAD complex. Inhibition of the YAP/PAX3 complex by TGFβ may also be important in generating quiescent stem cells, where TGFβ signaling is necessary and is accompanied by down-regulation of MITF (Nishimura et al. 2010).

Significantly, the forkhead transcription factor FOXD3 can repress *Mitf* by preventing PAX3 binding to the *Mitf* promoter (Thomas and Erickson 2009). Therefore, in development, FOXD3 controls a neural/glial versus melanoblast fate switch by indirectly controlling *Mitf* (Curran et al. 2009; Thomas and Erickson 2009). *FOXD3* is also widely expressed in melanoma and can confer BRAF inhibitor resistance, presumably in part via its ability to repress *MITF* expression but also through activation of human epidermal receptor 3 (ERBB3/HER3) (Abel et al. 2013).

SOX10 plays a major role in neural crest development as well as in melanocyte biology (Harris et al. 2010). In development, a large part of the role for SOX10 can be explained by its ability to directly bind the MITF promoter in cooperation with PAX3 (Lee et al. 2000; Verastegui et al. 2000; Potterf et al. 2001) and up-regulate *MITF* expression (Elworthy et al. 2003). *SOX10* is highly expressed in melanoma but is not frequently mutated, consistent with it having an important function in melanoma initiation and maintenance (Shakhova et al. 2012; Cronin et al. 2013). Significantly, SOX10 transcriptional activity is inhibited by ERK-mediated phosphorylation that prevents SOX10 SUMOylation normally required for its transcriptional activity (Han et al. 2018). Thus, changes in *MITF* expression following elevated ERK activity in $BRAF^{V600E}$ melanomas may arise in part via loss of SOX10 transcriptional competence.

Significantly, SOX10 can interact directly with PGC1α (PPARGC1A) (Shoag et al. 2013), a transcription factor coactivator whose expression is also activated by MITF (Haq et al. 2013; Vazquez et al. 2013). Since PGC1α protein stability is increased by cAMP levels downstream from MSH signaling and PGC1α can activate *MITF* expression (Shoag et al. 2013), these observations may account for how SOX10, together with CREB, is required for efficient activation of the MITF promoter by cAMP (Huber et al. 2003).

WNT/β-catenin can activate MITF expression via a LEF1/TCF site in the MITF promoter and is crucial for the generation of neural crest-derived melanoblasts and establishment of the melanocyte lineage (Dorsky et al. 2000; Takeda et al. 2000b). WNT/β-catenin is also important for activation of melanocyte stem cells in the adult hair follicle (Rabbani et al. 2011) where it presumably up-regulates MITF expression to promote the transition from a dormant stem cell to a proliferating transit-amplifying cell. In addition, mutations in β-catenin leading to its stabilization have been identified in melanoma (Rubinfeld et al. 1997) and will also potentially lead to activation of MITF.

ONECUT-2 is a cut homeodomain transcription factor (Jacquemin et al. 2001) that has also been identified as a regulator of *MITF*. ONECUT-2 is expressed in melanocytes and binds the *MITF* promoter, and a mutation in its binding site can reduce MITF promoter activity by ~75%. However, nothing is known of how ONECUT-2 expression or activity are regulated in melanocytes or melanoma.

Repressors of MITF mRNA expression

ATF4 (activating transcription factor 4) is a bZIP transcription factor and key mediator of the integrated stress response (Harding et al. 2003) that can repress MITF mRNA expression (Falletta et al. 2017). ATF4 mRNA is translated under conditions that drive phosphorylation of the eIF2α translation initiation factor. Activation of kinases that phosphorylate eIF2α such as PERK that lies downstream from ER-stress, GCN2 that is responsive to amino acid limitation, and PKR that is activated by interferon and inflammatory signaling, all increase ATF4 protein expression while also suppressing global translation, including that of MITF (Fig. 7). Significantly, ATF4 can repress MITF mRNA expression (Falletta et al. 2017), reportedly via competition with CREB for binding to the CRE (Ferguson et al. 2017). As such, any of the upstream activators of ATF4 translation has the potential ability to repress *MITF* transcription, as has been shown to date for glucose

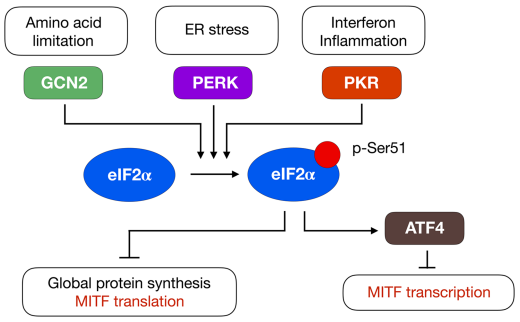

Figure 7. Schematic view of the translational control of MITF. Nutrient limitation, inflammation, and ER stress all lead to eIF2α phosphorylation, in turn leading to global inhibition of translation, including that of MITF, but an increase in translation of ATF4, which, as shown in Figure 6, inhibits Mitf-M transcription.

(Ferguson et al. 2017), glutamine limitation (Falletta et al. 2017), and TNFα (Falletta et al. 2017) that is known to promote melanoma dedifferentiation in vivo (Landsberg et al. 2012).

JUN, a bZIP transcription factor, is regulated by a range of stress-activated signaling pathways. In melanoma, inflammatory signaling downstream from TNFα leads to activation of JUN and consequent down-regulation of MITF, which in turn suppresses JUN expression (Riesenberg et al. 2015). The resulting feed-forward mechanism then leads to down-regulation of MITF as observed in mouse melanoma models where TNFα signaling can lead to dedifferentiation and resistance to adoptive T-cell therapy (Landsberg et al. 2012). However, it is currently not clear whether JUN binds *MITF* regulatory elements directly.

$BHLHB_2$/DEC1/BHLHE40 is a bHLH transcription factor that represses MITF under hypoxic conditions (Cheli et al. 2011; Feige et al. 2011) found frequently within growing tumors as a consequence of the chaotic tumor-associated vasculature (Gilkes et al. 2014). $BHLHB_2$ is up-regulated during hypoxia by the hypoxia responsive transcription factor HIF1α (Cheli et al. 2011; Feige et al. 2011) that together with its partners, HIF1β and HIF2α, are bHLH factors that play a critical role in mediating the transcriptional response to hypoxia. The observation that MITF can also directly activate HIF1 expression (Buscà et al. 2005) suggests that MITF may increase cell survival by enhancing the adaptive response to hypoxia, a role reminiscent of MITF's capacity to increase mRNA expression encoding ATF4 (Falletta et al. 2017), the critical mediator of the integrated stress response (see also below). Collectively, these observations suggest that the HIF1α–$BHLHB_2$–MITF axis creates a feedback loop that can regulate phenotypic plasticity in melanoma.

ALX3 (aristaless-like homeobox 3) is a transcription factor expressed in neural crest-derived mesenchyme and in lateral plate mesoderm (ten Berge et al. 1998). Significantly, ALX3 has been implicated in modulating pigmentation patterning in rodents, at least in part via its ability to suppress MITF expression (Mallarino et al. 2016). Chromatin immunoprecipitation (ChIP) experiments suggest that ALX3 may bind the MITF promoter directly. The precise localization of its binding sites, however, has yet to be confirmed by other means and little is known about how ALX3 activity might be regulated in melanocytes or in melanoma.

GLI2, a transcription factor downstream from Hedgehog signaling, has also been reported to repress MITF (Javelaud et al. 2011). GLI2 mRNA expression can also be induced by TGFß (Dennler et al. 2009) that can repress MITF expression via multiple mechanisms (see above). Although GLI2 binds the MITF promoter, its binding site, however, was not implicated in TGFß-mediated MITF repression (Pierrat et al. 2012). Nevertheless, it is clear that GLI2 plays a key role in melanoma where it promotes invasion and BRAF inhibitor resistance, consistent with a role in down-regulating MITF (Alexaki et al. 2010; Faião-Flores et al. 2017).

BRN2 (POU3F2) is a POU domain transcription factor whose expression is controlled by three signaling pathways relevant to both melanocyte development and melanoma: BRAF/MAPK signaling (Goodall et al. 2004b); PI3K signaling via PAX3 (Bonvin et al. 2012); and WNT/β-catenin signaling (Goodall et al. 2004a). BRN2 does not appear to be expressed in melanocyte development (Goodall et al. 2004a) or in melanocytes but can be expressed in neural crest cell cultures (Cook et al. 2003), probably because of its up-regulation by PI3K or MAPK signaling. In melanoma, BRN2 is widely expressed and has been reported both to up-regulate (Wellbrock et al. 2008) and down-regulate (Goodall et al. 2008) *MITF* expression. Significantly, BRN2 has a key role in driving melanoma invasion (Goodall et al. 2008; Arozarena et al. 2011; Thurber et al. 2011; Zeng et al. 2018; Fane et al. 2019) and is expressed in a mutually exclusive fashion with MITF in tumors (Goodall et al. 2008) and 3D melanomasphere cultures (Thurber et al. 2011). This mutually exclusive expression most likely is due to a positive feedback loop arising as a consequence of MITF promoting expression of the transient receptor potential cation channel subfamily M member 1 (*TRPM1*; also called *melastatin*) (Miller et al. 2004; Margue et al. 2013). Embedded within the *TRPM1* gene is micro-RNA-211 (miR-211) that is a potent suppressor of *BRN2* (Boyle et al. 2011). The feedback loop afforded by repression of *MITF* by BRN2 and MITF-mediated activation of miR-211 provides the potential for the generation of a bi-stable state in which BRN2 and MITF would be expressed in mutually exclusive subpopulations of cells. However, a negative feedback loop arising if BRN2 were an activator of MITF, as reported (Wellbrock et al. 2008), could explain why BRN2 and MITF are generally coexpressed in melanoma cells in culture.

Although BRN2 can bind elements within the MITF promoter, and can clearly suppress a proapoptotic gene expression program (Herbert et al. 2019), it is also possible that it exerts its transcriptional function by cooperating with other sequence-specific transcription factors rather than regulating transcription by itself. Notably, mass spectrometry (MS) analysis of BRN2-associated

proteins failed to identify a significant association with non-DNA-binding transcription cofactors. Instead, BRN2 is associated with the DNA-damage-response factors Ku70/Ku80 and PARP1 and plays a role in DNA damage repair by enhancing nonhomologous end-joining at the expense of homologous recombination (Herbert et al. 2019). Thus, an ability of BRN2 to exchange cooperating DNA-binding cofactors under different conditions—for example, in vitro versus in vivo—might explain how it could switch from a repressor to an activator of MITF. Alternatively, it has also been suggested that BRN2's ability to regulate MITF may reflect heterogeneity in MAPK signaling (Wellbrock and Arozerena 2015). Notably, BRN2's ability to bind DNA appears to be controlled by an intramolecular conformation switch regulated in part by two phosphorylation sites within its N-terminal region that can be modified by p38, a stress-activated kinase downstream from UV and ROS (Herbert et al. 2019). Thus, it seems likely that the interplay between stress and MAPK signaling will be important in determining BRN2 function in the regulation of MITF. Evidently, deciphering precisely how BRN2 regulates MITF in response to specific microenvironmental cues is important given that MITF and BRN2 mark distinct subpopulations of melanoma cells in vivo (Goodall et al. 2008; Thurber et al. 2011) and that the interplay between BRN2 and PAX3 has recently been reported to control the dynamics of MITF expression in response to BRAF inhibition (Smith et al. 2018).

In addition to transcriptional regulation, as might be expected for a key transcription factor, MITF is also subject to posttranscriptional regulation via both control of its mRNA polyadenylation by CPEB4 (Pérez-Guijarro et al. 2016) and the action of microRNAs that play a key role in melanoma biology (Bell and Levy 2011; Kunz 2013). To date, several microRNAs have been described as inhibiting MITF expression, including miR-26a in melanoma (Qian et al. 2017), miR-340 in osteoclasts (Zhao et al. 2017), and miR-137 (Bemis et al. 2008), miR-148 (Haflidadóttir et al. 2010), miR-155 (Arts et al. 2015), and miR-182 (Yan et al. 2012). No doubt future research will identify additional miRs that can affect MITF expression and for now we refer the reader to the microRNA target prediction Web page for the 3′ untranslated region (UTR) (TargetScanHuman, http://www.targetscan.org/cgi-bin/targetscan/vert_72/view_gene.cgi?rs=ENST00000328528.6&taxid=9606&showcnc=0&shownc=0&shownc_nc=&showncf1=&showncf2=&subset=1).

MITF DNA-binding specificity

MITF binds DNA as a homodimer or heterodimer with the related family members TFEB and TFE3 (Hemesath et al. 1994), which are widely expressed. The expression of TFEC, the fourth member of the MiT family capable of forming heterodimers with MITF, is more restricted and so may contribute to gene expression only in specific tissues (Rehli et al. 1999; Kuiper et al. 2004; Bharti et al. 2012). Although in zebrafish one report suggests that TFEC is a key regulator of the hematopoietic vascular niche during development, it is not known whether TFEC performs a similar function in mammals (Mahony et al. 2016).

The basic region of bHLH and bHLH-LZ factor dimers binds so-called E-box sequences usually comprising a 6-bp CANNTG motif. Specificity is dictated both by a combination of the central bases of the motif and its flanking sequences together with the amino acid sequence of the basic region that directly recognizes DNA. Initial studies examining the first known targets of MITF that were present in promoters of genes implicated in pigmentation indicated that MITF bound a specific E-box variant termed the M-box (Lowings et al. 1992; Bentley et al. 1994; Yavuzer and Goding 1994) exemplified by a core CATGTG E-box element with additional flanking residues (Fig. 8). Later studies examining other genes revealed that MITF could also recognize the 6-bp palindromic CACGTG motif (Fig. 8). Notably, the arrangement of amino acids in the MITF basic region, highlighted in the MITF bHLH-LZ domain–DNA cocrystal structure (Fig. 3; Pogenberg et al. 2012), means it is unlikely to bind with high affinity to CA**GC**TG E-boxes recognized by other bHLH/bHLH-LZ transcription factors such as AP4. This is reflected in the ChIP-seq (ChIP followed by high-throughput DNA sequencing) data where CAGCTG motifs are associated with a small minority of MITF-associated peaks. The MITF binding detected in the ChIP-seq data sets might arise either through stabilization of its association with a CAGCTG element via a cooperative interaction with other transcription factors or possibly through a chromatin loop between MITF bound to a canonical site and a factor such as AP4 bound to a CAGCTG recognition motif.

A further level of specificity was revealed when it was shown that MITF prefers a 5′T and/or 3′A residue flanking the core 6-bp CACGTG or CATGTG elements to bind well (Aksan and Goding 1998). These observations were subsequently confirmed in genome-wide ChIP-seq studies of MITF where the vast majority of sites are represented by CACGTG motifs with a minor population of CATGTG motifs present in differentiation-associated genes (Strub et al. 2011). The requirement for the flanking 5′T–3′A may be to restrict binding to many MITF recognition

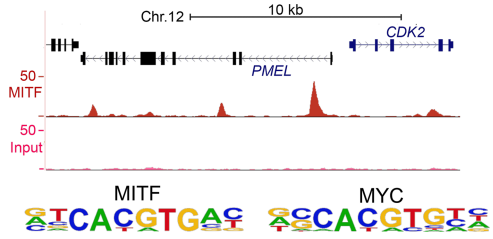

Figure 8. Schematic view of MITF ChIP-binding peaks over a portion of human chromosome 12 comprising the *CDK2* and *PMEL* genes. The majority of MITF-bound sites are CACGTG E-box motifs flanked by A and/or T, with a minority being equally flanked CATGTG "M-box" motifs present mostly in differentiation-associated genes such as pMEL. The flanking sequences enable discrimination between MITF- and MYC-binding sites.

motifs by MYC, which has a preference for sites lacking the T–A flanking sequences, and other bHLH-LZ factors able to recognize similar 6-bp elements as heterodimers with MAX (Fisher et al. 1993; Solomon et al. 1993). Indeed, recent genome-wide analysis has confirmed that the flanking sequences represent major discriminators between MYC–MAX binding and MITF, although a restricted subset of E-box motifs is able to bind both MYC and MITF (Hejna et al. 2018). Collectively, these studies indicate that sequence specificity of MITF means that it has a largely complementary repertoire of targets to MYC (Fig. 8).

Since the nomenclature of the MITF-binding sites has been a point of confusion to some, we propose that the term M-box be reserved for MITF-binding sites containing a core CATGTG motif (which is frequently associated with differentiation genes); other E-box-containing MITF-binding sites, including the major palindromic CACGTG motif (which is associated with a large range of target genes and is also part of the 10-bp CLEAR [coordinated lysosomal expression and regulation]-box described for TFEB and TFE3) (Settembre and Medina 2015), should simply be called E-box motifs.

MITF posttranslational regulation

MITF is subject to a variety of posttranslational modifications. Surprisingly, however, only a few of the potential modifications have been well-characterized, and it seems likely that many more levels of regulation remain to be discovered. The signaling pathways and downstream posttranslational modifications that have been mapped and associated with some function are depicted in Figure 5 and are outlined below.

MAPK kinase pathway activation downstream from receptor tyrosine kinases, as well as NRAS and BRAF, leads to MITF phosphorylation by ERK on S73 and by RSK on S409 (Hemesath et al. 1998). S73 phosphorylation by ERK was initially proposed to be required for MITF degradation (Wu et al. 2000; Xu et al. 2000) and also for recruitment of the p300/CBP transcription cofactor by MITF (Price et al. 1998a). However, other studies did not find increased association between CBP and S73 phosphorylated MITF (Sato et al. 1997). Moreover, although UBC9 was proposed to be the ubiquitin ligase responsible for degradation of MITF in response to S73 phosphorylation (Xu et al. 2000), UBC9 is more usually described as an E2 SUMO conjugating enzyme (Knipscheer et al. 2008). Furthermore, both Hemesath et al. (1998) and Wellbrock and Marais (2005) have shown that an S73A mutation does not affect MITF protein stability, at least in the assays undertaken. However, the RSK phosphorylation site at S409 does appear to affect MITF protein stability via priming for GSK3 phosphorylation (see below; Ploper et al. 2015).

More recently, phosphorylation of S73 by ERK was revealed to act as a priming event for phosphorylation of S69 by GSK3, with dual phosphorylation activating an adjacent CRM1-dependent nuclear export signal (Ngeow et al. 2018). Thus, acute activation of BRAF was able to promote efficient nuclear export of MITF-M ,while inhibition of GSK3 prevented export. Since GSK3 is inhibited by both WNT and PI3K signaling, the MITF export signal is responsive to two key signaling pathways downstream from receptor tyrosine kinases, which are also deregulated in melanoma. Under normal conditions, however, MITF-M is predominantly nuclear owing to a constitutive nuclear localization signal within its basic region (Takebayashi et al. 1996; Fock et al. 2018) and the absence of a domain that mediates cytoplasmic retention of exon 1B-containing isoforms (Martina and Puertollano 2013; Ngeow et al. 2018). Flux through the nuclear import–export cycle regulated by both GSK3 and ERK and their associated signaling pathways is likely to play a key role in MITF function. Indeed, as mentioned, an S73A mutation or elimination of the corresponding exon 2B in mice led to slightly darker pigmentation visible on a genetically sensitized background (Debbache et al. 2012). Similarly, exon 2B-deleted human MITF used in zebrafish rescue experiments increased melanocyte numbers (Taylor et al. 2011), and deletion of MITF exon 2B, because of a splice alteration, is associated with human melanomas (Cronin et al. 2013). While these observations are compatible with the original proposal of an increase in MITF protein stability, they may as well result from an increase in nuclear steady state levels without changing protein stability.

GSK3, a kinase that usually uses a priming phosphorylation site at the +4 position to modify its targets (Frame and Cohen 2001), has been reported to target S298 (Takeda et al. 2000a) and three sites at the MITF C terminus (Ploper et al. 2015) in addition to phosphorylating MITF on S69 to regulate nuclear export. The C-terminal sites at S405, S401, and S397 are evolutionarily conserved and present in all MiT members (Supplemental Fig. S3). Their phosphorylation by GSK3 in MITF can be primed by the previously described RSK-mediated phosphorylation at S409 (Hemesath et al. 1998). Significantly, phosphorylation at the C-terminal GSK3 sites is suppressed by WNT signaling (Ploper et al. 2015), a critical developmental pathway required for expression of MITF in the neural crest (Dorsky et al. 2000; Takeda et al. 2000b) that is also required for activation of melanocyte stem cells (Rabbani et al. 2011) and is frequently deregulated in melanoma (Rubinfeld et al. 1997; Delmas et al. 2007). Phosphorylation of the C-terminal GSK3 sites appears to destabilize MITF protein (Ploper et al. 2015), perhaps accounting for how RSK phosphorylation at S409 (Hemesath et al. 1998) could regulate MITF. Note, however, that an S409A mutated MITF is fully functional in BAC rescue transgenic mice (Bauer et al. 2009), suggesting that at least during development, C-terminal phosphorylations may be irrelevant. In contrast, a mutation at S298 to proline was reported to affect DNA binding and be associated with WS IIa (Takeda et al. 2000a). S298, however, is not in the vicinity of any potential priming site for GSK3 phosphorylation and lies at the C-terminal end of the MITF leucine zipper; that is, at a considerable distance from the DNA. Since, as mentioned, Grill et al. (2013) showed that mutation of S298 to proline is fully capable to bind DNA and activate a Tyrosinase reporter in vitro, the evidence that S298 is a bona fide GSK3

phosphorylation site is not strong; its mutation in a WS IIa patient (Takeda et al. 2000a) therefore may be coincidental rather than causal.

AKT is a serine threonine kinase that lies downstream from both mTORC2 and PI3K signaling. Recent evidence suggests that AKT can, like RSK, phosphorylate MITF on S409 (reported as S510) (Wang et al. 2016) a site conserved and phosphorylated in the MITF-related factor TFEB (see Supplemental Fig. S3, where S409 in MITF-M corresponds to S516 in MITF-A) (Palmieri et al. 2017). Using a phosphorylation mimetic "S510D" mutant, Wang et al. (2016) reported that MITF was degraded more rapidly, suggesting that AKT promotes MITF degradation. This would be consistent with results from the Ploper et al. (2015) study that reported that phosphorylation on S409 primes for phosphorylation by GSK3 at S405, S401, and S397, leading to destabilization of MITF

AKT-mediated phosphorylation of MITF was also reported to stimulate MITF interaction with p53 and promote *CDKN1A* expression, while nonphosphorylated MITF was able to activate better tyrosinase expression (Wang et al. 2016).

SUMO is a small ubiquitin-like peptide that is coupled to target proteins to modify their function or subcellular localization or alter their interaction with partner proteins (Zhao 2018). MITF is SUMOylated on two lysine residues: K182 and K316 (Miller et al. 2005; Murakami and Arnheiter 2005). Significantly, a germline E318K mutation prevents SUMOylation at K316 and predisposes to melanoma in humans (Bertolotto et al. 2011; Yokoyama et al. 2011). In mice, the same mutation leads to mild hypopigmentation but increases nevus counts after conditional induction of a $BRAF^{V600E}$ mutation in melanocytes and accelerates tumor formation after conditional induction of a double $BRAF^{V600E}/PTEN^{-/-}$ mutation (Bonet et al. 2017). Although analysis of SUMO site mutations appears to suggest that SUMOylation of MITF may regulate its target specificity (Murakami and Arnheiter 2005; Bertolotto et al. 2011), this has not been explored in detail, and recent evidence indicates that the E3I8K mutation can impair $BRAF^{V600E}$-induced senescence (Bonet et al. 2017). However, precisely how SUMO modification affects MITF function and what regulates SUMOylation remain to be determined. In addition to UBC9, discussed above, one candidate is PIAS3, a SUMO E3 ligase identified as binding the MITF leucine zipper domain that can suppress MITF's transcriptional activity (Levy et al. 2002, 2003). Interestingly, phosphorylation on the RSK and AKT target at S409 substantially reduced PIAS3 interaction with MITF (Levy et al. 2003). However, while PIAS3 interaction with MITF is clear, it has not formally been shown to modify MITF.

SRC family members are key nonreceptor tyrosine kinases that lie downstream from receptor tyrosine kinases, including KIT (Lennartsson and Rönnstrand 2012). Interestingly, when cells express a constitutively active KIT^{D816V} receptor tyrosine kinase, formation of a triple complex between KIT, SRC, and MITF-M leads to SRC-mediated phosphorylation of three MITF-M N-terminal tyrosines: Y22, Y35, and Y90 (Phung et al. 2017). Since both SRC and KIT are cytoplasmic and MITF-M predominantly nuclear, the three proteins should not be able to form a triple complex. However, expression of the KIT^{D816V} mutant promoted increased cytoplasmic accumulation of MITF-M. SRC-mediated tyrosine phosphorylation of MITF-M was also reported to increase the ability of MITF-M to regulate transcription of a range of target genes, although how exactly this is achieved remains to be understood.

p38 is a stress-activated protein kinase activated by stressors such as UV and reactive oxygen species. p38-mediated phosphorylation of MITF has been reported to occur on S307 in osteoclasts (Mansky et al. 2002) and facilitates MITF's capacity to activate transcription in these cells. Despite the obvious link between p38 and UV irradiation in melanoma, the role of p38 phosphorylation of MITF in the melanocyte lineage has yet to be examined.

Caspase activation after apoptosis induction leads to cleavage of MITF after D345. Interestingly, expression of a noncleavable mutant (D345A) rendered cells more resistant against TRAIL-induced apoptosis, suggesting that the cleavage products may sensitize cells to TRAIL. Caspase cleavage generates a transcriptionally active amino terminal fragment, that is, however, rapidly degraded and hence unable to maintain sufficient BCL2 levels. On the other hand, siRNA-mediated reduction of MITF, equally unable to maintain BCL2, did not sensitize cells to TRAIL-induced apoptosis, suggesting that the carboxyl terminal fragment may have a proapoptotic function, as was indeed found (Larribere et al. 2005).

Ubiquitination is likely to control MITF protein stability. While identity of the ubiquitin ligase and attachment sites are not clear, an shRNA library screen led to the identification of USP13 as an MITF deubiquitination enzyme (Zhao et al. 2011). Consequently, ectopic expression of USP13 stabilized MITF and up-regulated MITF target genes, whereas USP13 knockdown decreased MITF protein, but not mRNA, and blocked melanoma cell proliferation.

A range of additional phosphorylation events have been detected in various high-throughput proteomic screens (PhosphSitePlus, http://www.phosphosite.org/proteinAction?id=1001&showAllSites=true); however, the significance of these modifications is yet to be determined.

MITF cofactors

Much as other transcription factors, MITF interacts with cofactors to regulate gene expression. Broadly speaking, these fall into two classes: (1) DNA-binding cofactors that interact with MITF to increase MITF's DNA-binding affinity or facilitate MITF targeting to specific repertoires of genes and (2) non-DNA-binding MITF-interacting factors that contribute to MITF's ability to regulate transcription by modifying or remodeling chromatin or which may themselves recruit additional transcription factors or cofactors. Since little is known of the DNA-binding

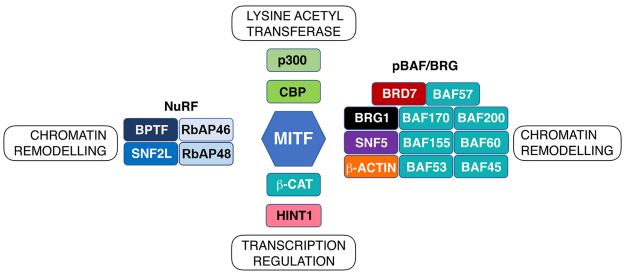

Figure 9. MITF interaction partners. Schematic showing some of the well-characterized MITF interaction partners. The NURF and pBAF/BRG complexes facilitate chromatin remodeling by MITF and may include alternative subunits. p300 and CBP are highly related lysine acetyl transferases. β-Catenin facilitates transcription activation of some differentiation-associated genes by MITF, while HINT (histidine triad nucleotide-binding protein) is a negative regulator of MITF function. Other interacting partners (not shown) have been identified, but their function in association with MITF is poorly understood.

cofactors that facilitate MITF targeting, we focus below on non-DNA-binding cofactors (Fig. 9).

p300/CBP was one of the earliest non-DNA-binding cofactors for MITF to be identified (Sato et al. 1997; Price et al. 1998a). p300 and CBP are highly related proteins with lysine acetyltransferase activity that bind to the N-terminal region of MITF (Sato et al. 1997; Price et al. 1998a), with recruitment being reported to be facilitated by phosphorylation of S73 (Price et al. 1998a), though this has yet to be substantiated. p300 and CBP act as transcription cofactors for many transcription factors and can modify histone lysines, thereby facilitating the chromatin remodeling that accompanies transcription regulation (Dancy and Cole 2015). In addition to targeting nucleosomes, they can also acetylate their associated transcription factors, though acetylation of MITF has not been published.

β-Catenin is a key transcription factor downstream from WNT signaling that plays a critical role in development and disease, most notably in promoting proliferation and activation of stem cells (Clevers 2006). As discussed above, in the melanocyte lineage, β-catenin activates expression of MITF (Dorsky et al. 2000; Takeda et al. 2000b), a function related to its ability to activate melanocyte stem cells (Rabbani et al. 2011) and promote proliferation in melanoma. However, β-catenin also acts as a key cofactor for MITF, binding the bHLH domain and enhancing the ability of MITF to drive expression of differentiation-associated genes (Schepsky et al. 2006). Whether the interaction between MITF and β-catenin is regulated is not known at present.

The SWI/SNF chromatin remodeling complex is frequently mutated in cancer (Masliah-Planchon et al. 2015). Notably, the SWI/SNF complex plays a key role as an MITF cofactor in controlling expression of MITF target genes (de la Serna et al. 2006). MS analysis and downstream validation suggests that MITF can interact with a PBAF complex containing both BRG1, the catalytic subunit of the SWI/SNF complex, together with the helicase CHD7 (Laurette et al. 2015). In melanoma, BRG1 cooperates with MITF to suppress apoptosis by regulating expression of the antiapoptotic melanoma inhibitor of apoptosis (ML-IAP/BIRC7) (Saladi et al. 2013) that is preferentially expressed in melanomas (Vucic et al. 2000). BRG1 is also required for proliferation in vitro and for development of the melanocyte lineage in vivo (Laurette et al. 2015). However, because the SWI/SNF complex acts as a cofactor for many transcription factors, the relative contribution of the SWI/SNF–MITF interaction to these processes is difficult to decipher. Nevertheless, ChIP-seq assays revealed that both SOX10 and MITF target BRG1 to a wide range of MITF regulatory elements in cells (Laurette et al. 2015).

In addition to many subunits of the SWI/SNF and PBAF complexes, the MS analysis by Laurette et al. (2015) confirmed the interaction between MITF and a range of known binding partners, including TFE3, TFEB, and β-catenin. Other interactors reported in this study include factors implicated in DNA replication, including MCM3, MCM5, and MCM7 as well as RFC1, RFC2, RFC4, and RFC5; proteins implicated in the ubiquitin cycle (HERC2, NEURL4, UBR5, USP7, and USP11); and components of the TRRAP chromatin remodeling complex. Consistent with MITF shuttling in and out of the nucleus (see below), several nuclear pore components were identified, including IPO5, together with RNA polymerase III cofactors and cohesin subunits (SMCA1, SMC3, STAG2, and PDS5). Although the biological consequences of many of the interactions identified remain to be determined, interaction with several components of the NURF chromatin remodeling complex (BPTF, SMARCA1 [SNF2L], SMARCA5 [SNF2H], and RBBP4 [RbAP48]) led Koludrovic et al. (2015) to examine the role of NURF in melanoma/melanocyte biology. The results indicate that NURF is implicated in a gene expression program that overlaps with MITF, and is required for proliferation, migration, and morphology in development. Significantly, mice with a melanocyte lineage-selective inactivation of BPTF exhibit defects in melanocyte stem cell proliferation and differentiation (Koludrovic et al. 2015). How many of the defects linked to inactivation of BPTF can be ascribed to its interaction with MITF versus interaction with other transcription regulators is not entirely clear, though the defects associated with BPTF loss would be consistent with a partial failure of MITF function.

In addition to the cofactors outlined above, MITF also interacts with histidine triad nucleotide-binding protein 1 (HINT1/PKCI) (Razin et al. 1999). Although the interaction has been best-characterized in mast cells, it also occurs in melanoma (Motzik et al. 2017). Transcription activation by MITF is inhibited by its direct interaction with HINT1 that can be diminished by activation of the KIT RTK in melanoma cells or FC receptor epsilon on mast cells. Significantly, HINT1 is released from MITF by diadenosine tetraphosphate (Ap_4A) that is produced by the action of Lysyl-tRNA synthetase (LysRS) (Carmi-Levy et al. 2008). Consequently, knockdown of the Ap_4A hydrolase leads to up-regulation of MITF targets (Yannay-Cohen et al. 2009). Since LysRS plays a key role

in protein translation, these observations suggest that the LysRS–HINT1–MITF axis may function as a means to couple transcription of MITF targets to translational control.

MITF target genes and biological role

Over the years following the isolation of the MITF gene, the number of potential target genes has increased dramatically. ChIP-seq analysis of genome-wide MITF binding (Strub et al. 2011; Webster et al. 2014) has indicated that MITF binds between 12,000 and 100,000 genomic sites depending on the background threshold set and the ChIP efficiency. Of these, ~9400 lie within 20 kb of an annotated RefSeq gene (Strub et al. 2011). However, like with most transcription factors, binding to a specific DNA element does not necessarily translate to regulation of the nearby gene. Indeed, of the genes bound by MITF in the Strub et al. (2011) study, only 465 genes could be clearly designated as directly regulated MITF target genes (240 down-regulated and 225 up-regulated). Even genes with high levels of MITF occupancy at a specific site may not exhibit regulation. Why not all MITF-bound genes are regulated by MITF is not known, but this may happen for several reasons. For example, the culture conditions under which gene expression is measured may not allow MITF to regulate a bound gene, the absence of functional cofactors or an inability to interact with them may not permit MITF to regulate a gene, or, as for any transcription factor, a certain time of association with DNA (the dwell time) is required to regulate gene expression, and dwell time does not necessarily correlate with occupancy as measured by ChIP-seq (Lickwar et al. 2012).

Nevertheless, there are many MITF target genes that are bound by MITF and whose expression is changed upon depletion or overexpression of MITF. Note, however, some studies suggest binding of MITF to a gene's promoter, but unbiased ChIP-seq analysis shows binding is located elsewhere, within an exon or 3′ to the gene, for example. Here, we focus on a small selection of identified target genes related to specific biological processes regulated by MITF, and refer the reader to other reviews or articles for additional discussions of the field (Hoek et al. 2008; Cheli et al. 2010; Strub et al. 2011). The selected target genes and the biological processes in which MITF has been reported to play a major role are indicated in Figures 10 and 11. It needs to be kept in mind, however, that the regulation of specific target genes may be bi- or multiphasic and not simply proportional to MITF activity levels as schematically shown in Figure 10. The selected processes include the following:

Differentiation: MITF can promote differentiation-associated functions, including regulation of genes implicated in pigmentation such as *TYR*, *TYRP1*, *DCT*, *MLANA*, *SILV*, and *SLC24A5* (Cheli et al. 2010) or cell adhesion such as carcinoembryonic antigen-related cell adhesion molecule 1 (*CAECAM*) (Ullrich et al. 1995), all of which

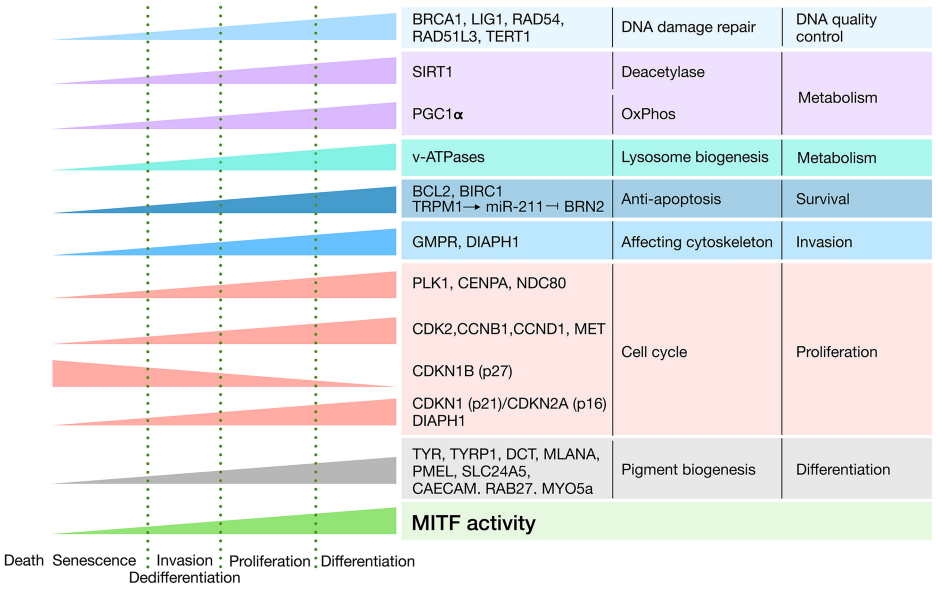

Figure 10. Schematic diagram of target gene regulation by different activity levels of MITF. The selected target genes are associated with the major biological functions of MITF as indicated at the *right* and in Figure 11. The model, known as the "rheostat model," shows that high MITF activity levels are associated with cell differentiation and reduced proliferation and that progressively decreasing MITF activity levels are associated with proliferation, dedifferentiation/invasion (as shown for melanoma cells), senescence, and eventually cell death. Note, however, that this schematic integrated view does not reflect the relative induction levels of each target gene. In fact, it is likely that the different "activity levels" of MITF, brought about by absolute protein levels in conjunction with posttranslational modifications and the availability of interacting proteins, are associated with differential regulation, for instance, of proliferation- and differentiation-linked target genes. Furthermore, target gene regulation need not necessarily be directly or indirectly proportional to MITF activity levels and may well be biphasic or multiphasic.

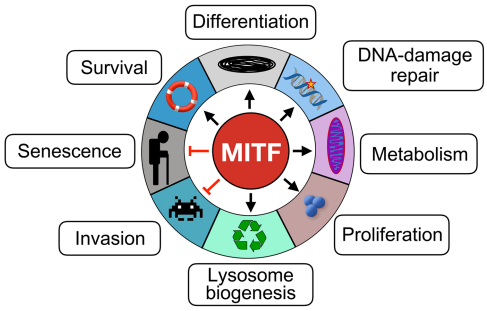

Figure 11. Summary view of the hallmarks of the biological functions of MITF.

contain at least one M-box comprising the CATGTG core-binding site with appropriate 5′T and/or 3′A flanking sequences. MITF also positively regulates genes such as *RAB27a* (Chiaverini et al. 2008) and *MYOSIN5a* (*MYO5a*) (Alves et al. 2017) that contribute to melanosome transport. Note that differentiation-associated genes may not uniquely have CATGTG MITF-binding motifs but may also contain other elements recognized by MITF.

Not surprisingly, given the role of MITF in controlling genes implicated in pigmentation, increasing evidence implicates MITF in the response to UV irradiation. Most notably, recent evidence from Malcov-Brog et al. (2018) suggests that in response to UV irradiation, MITF exhibits damped oscillations in its expression, enabling it to act as a UV protection timer. Oscillatory MITF expression was reported to arise both as a consequence of an MITF-HIF1α transcriptional feedback loop and also through a posttranscriptional loop involving microRNA 148a.

Survival: In development, MITF is required for melanoblast survival (Hodgkinson et al. 1993). As seen in mutant mouse embryos, in the absence of functional MITF protein, MITF RNA-positive melanoblasts emerge from the neural crest but die within 2 d, most likely by apoptosis (Opdecamp et al. 1997; Nakayama et al. 1998). Since MITF is a positive regulator of *BCL2* (McGill et al. 2002), an antiapoptotic BCL2 family member (Kalkavan and Green 2018), it is possible that a reduction of MITF below a threshold level renders melanoblasts hypersensitive to proapoptotic triggers. This would be consistent with the fact that *Bcl2*-null mice turn gray, with the levels of the proapoptotic BH3-only protein Bim apparently playing a key role in setting the apoptotic threshold in a range of tissues (Bouillet et al. 2001). However, it is clear that since *Mitf*-null mice are white, whereas *Bcl2*-null mice are gray, other factors must play a role. The nature of the apoptotic triggers in *Mitf*-null mice is not known but may be related to the activity of ATF4, the key mediator of the integrated stress response (Harding et al. 2003). As discussed above, ATF4 is expressed as a consequence of translation reprogramming mediated by phosphorylation of the eIF2α translation initiator factor by stress-activated kinases such as PERK and GCN2 (Fig. 7; Pakos-Zebrucka et al. 2016). Since ATF4 can activate expression of *CHOP*/*DDIT3*, which in turn represses *BCL2*, it is plausible that loss of MITF, combined with ATF4 expression in the neural crest (Suzuki et al. 2010) could reduce the levels of *BCL2* expression below a threshold required for cell survival. MITF also appears to regulate apoptosis by directly up-regulating the expression of the antiapoptotic factor BIRC7 (ML-IAP) (Dynek et al. 2008; confirmed by ChIP-seq in Strub et al. 2011).

Importantly, MITF has also been reported to regulate expression of DICER (Levy et al. 2010), a critical regulator of microRNA processing. DICER was not picked up as an MITF target gene in genome-wide binding studies (Strub et al. 2011), perhaps because MITF binding is found a great distance upstream of the DICER transcriptional startsite (up to 150 kb), beyond the usual bioinformatic cutoff applied to link binding with gene regulation. Since DICER is necessary for melanocyte development and survival of melanocytes in culture (Levy et al. 2010), the regulation of DICER by MITF, and the downstream consequences for microRNA expression, may be a significant contributor both to the melanocyte-development defects observed in *Mitf*-null mice as well as MITF-dependent melanocyte and melanoma biology.

Lysosome biogenesis and autophagy: More recently, in *Drosophila* and in mammalian melanocytes, MITF has been implicated in transcriptional activation of all 15 subunits of the V-ATPase complex that regulates acidification of organelles, including lysosomes and endosomes (Zhang et al. 2015), with MITF also promoting expression of genes associated with driving lysosome biogenesis (Ploper et al. 2015). By controlling lysosome function, the v-ATPase complex also increases activity of the lysosome-bound mTORC1 (Zoncu et al. 2011) that promotes global protein synthesis. This makes sense, as elevated protein synthesis is necessary for cell proliferation, which is also promoted by MITF, but can potentially also lead to feedback regulation in that MITF, particularly the isoforms containing the 1B1b exon, can be retained in the cytoplasm in response to phosphorylation by activated mTORC1 (Martina and Puertollano 2013; Ngeow et al. 2018) (for more details see below). Recent evidence also indicates that MITF can control the expression of the lysosomal acid ceramidase ASAH1 that controls sphingolipid metabolism (Leclerc et al. 2019). Significantly ectopic expression of ASAH1 could rescue the cell cycle defects associated with MITF depletion, consistent with a role for ASAH1 in promoting melanoma proliferation (Realini et al. 2016). Moreover, MITF in melanoma has recently been identified as a regulator of a subset of genes implicated in autophagy (Moller et al. 2019), a lysosome-dependent process that degrades and recycles unwanted organelles and may represent a key survival strategy of cells under nutrient restriction. Notably, experimental depletion of MITF reduced the autophagy response to starvation, whereas overexpression of MITF increased the number of autophagosomes.

Proliferation: MITF's role in cell proliferation is complex. The reported role of MITF in cell cycle progression was initially confusing as some groups reported a proproliferative function for MITF (Widlund et al. 2002), including designating MITF as a lineage survival oncogene

(Garraway et al. 2005), while other studies suggested that MITF was antiproliferative (Carreira et al. 2005). This apparent paradox was resolved by the so-called rheostat model for MITF function (Carreira et al. 2006). In this model (Fig. 10), low levels of MITF are associated with dedifferentiation, increased invasion, and elevated protein levels of the p27 (CDKN1B) cyclin-dependent kinase inhibitor (Carreira et al. 2006) and hence reduced proliferation, whereas cells with high MITF activity are also cell cycle-arrested owing to increased expression of p21 (CDKN1A) (Carreira et al. 2005) and $p16^{INK4a}$ (CDKN2A) (Loercher et al. 2005) provided that *CDKN2A* is not inactivated, as is frequently the case in melanoma (Bennett 2015). Thus, increasing MITF activity from a low level can promote proliferation by suppressing p27 expression, but increasing MITF activity further can induce a p21/p16-dependent cell cycle arrest.

The role of MITF as a proproliferative factor is underlined by its ability to up-regulate CDK2 expression (Du et al. 2004), and also positively regulate directly the cyclin genes *CCNB1* and *CCND1* (Strub et al. 2011) as well as the oncogenic hepatocyte growth factor receptor, MET (McGill et al. 2006; Beuret et al. 2007; Webster et al. 2014). Genes implicated in mitosis are also direct MITF targets, including *PLK1*, encoding a key regulator of M-phase progression, and components of the CENPA and NDC80 complexes that connect mitotic spindle microtubules to kinetochores (Strub et al. 2011). However, the role of MITF as a proproliferative factor has been challenged by the observation that inducible depletion of MITF using shRNA did not block proliferation in all melanoma cell lines tested but did lead to dedifferentiation (Vlčková et al. 2018). This suggests that other factors may compensate for the absence of MITF or that technical details such as using siRNA, as done in most studies, versus using shRNA, as done by Vlčková et al. (2018), makes a difference. Evidently, further work is needed to clarify this issue.

DNA damage repair: An important set of MITF target genes are those implicated in DNA replication, damage repair, and chromosome integrity. Depletion of MITF leads to an increase in γH2AX foci that are associated with DNA damage (Giuliano et al. 2010). How MITF suppresses DNA damage is not clear but may be related to MITF's capacity to transcriptionally up-regulate a set of key repair factors, including *BRCA1* (Giuliano et al. 2010), *LIG1*, *RAD54*, and *RAD51L3* as well as telomerase encoded by *TERT1* (Strub et al. 2011). MITF also transcriptionally controls *GTF2H1*, encoding a core component of the TFIIH complex that is implicated in both UV-induced nucleotide excision repair as well as in global transcription, and *CDK7* encoding the TFIIH kinase implicated in the restart of transcription that occurs after completion of DNA damage repair (Seoane et al. 2019).

Metabolism: In addition to controlling key components of the cell cycle machinery, MITF also reprograms the metabolic landscape of cells. Notably, MITF is a positive regulator of PGC1α (*PPRGC1A*) that controls mitochondrial biogenesis (Haq et al. 2013; Vazquez et al. 2013). MITF appears not to control glucose uptake but does decrease production of lactate and increases oxidative phosphorylation downstream from PGC1α. Significantly, inhibition of BRAF in melanoma increases MITF expression and consequently drives a shift to oxidative phosphorylation, while inhibition of the MAPK pathway does not appear to affect PGC1α expression in MITF-negative melanomas (Haq et al. 2013).

The ability of MITF to affect melanocyte/melanoma biology by modulating metabolism is also exemplified by its ability to promote expression of the *SIRT1* gene encoding a key NAD-dependent deacetylase that is important in promoting proliferation and suppression of senescence (Ohanna et al. 2014). SIRT1 is a major metabolic sensor in cells and can deacetylate and repress the key lysine acetyl transferase p300 (Bouras et al. 2005). Since p300 is also a transcription cofactor for MITF (Sato et al. 1997; Price et al. 1998a), this mechanism provides a feedback loop that may enable MITF activity to be maintained within a tight window compatible with the metabolic status of the cell reflected in the levels of NAD.

Invasion: Recently MITF was shown to control expression of guanosine monophosphate reductase (GMPR) and, consequently, depletion of MITF leads to increased intracellular GTP levels (Bianchi-Smiraglia et al. 2017). This is important since increased GTP levels lead to elevated levels of active (GTP-bound) RAC1, RHO-A, and RHO-C, key regulators of the actin cytoskeleton. Together with the ability of MITF to regulate *DIAPH1* (Carreira et al. 2006), a gene implicated in actin polymerization, these observations implicate low MITF in promoting invasion. However, although siRNA-mediated depletion of MITF can lead to increased invasion (Carreira et al. 2006; Giuliano et al. 2010), suppression of MITF expression by inducing ATF4 expression did not give the same outcome (Falletta et al. 2017). This might indicate that low MITF levels may be necessary for cell invasion but are not sufficient under conditions where such low levels are achieved by using physiological regulators. However, recent genetic ablation of the TGFβ-pathway antagonist SMAD7 led to melanoma cells adopting a dual invasive/proliferative phenotype in which MITF expression was not suppressed (Tuncer et al. 2019). In other words, in this model, moderate MITF levels are not incompatible with invasion. Therefore, it is possible that two modes of melanoma invasion operate: one with low MITF levels, where invasion is not associated with proliferation, and another with higher MITF levels, where cells are simultaneously proliferative and invasive. Understanding the triggers of such distinct modes of invasion is clearly a key unresolved issue.

Senescence: Depletion of MITF using siRNA leads to change in cell morphology and increased invasiveness (Carreira et al. 2006). However, while invasion is a short-term response to MITF depletion, prolonged siRNA-mediated suppression of MITF leads to senescence associated with an irreversible growth arrest (Giuliano et al. 2010). Although senescence can be triggered by telomere attrition, senescence occurring as a consequence of depletion of MITF appears to arise from increased DNA damage, possibly following reduced levels of DNA damage repair

factors that are regulated by MITF (Strub et al. 2011). In addition, depletion of MITF is also associated with a PARP and NFκB-driven senescence-associated secretome that is able to trigger dedifferentiation of melanoma cells (Ohanna et al. 2011, 2013). While these observations are consistent with MITF playing an antisenescence role, if MITF is instead silenced via up-regulation of ATF4, cells are not senescent (Falletta et al. 2017). It is possible that the senescence triggered by siRNA-mediated MITF depletion may be blocked by ATF4-induced cell cycle arrest.

Finally, other MITF target genes and *HPGDS* and *TPSB2* reported to be regulated by MITF in mast cells (Morii and Oboki 2004) do not appear to be bound by MITF in the genome-wide analyses (Strub et al. 2011) performed to date in melanoma. It is likely that these genes are primarily expressed in mast cells and so may not be bound by MITF in the melanocyte lineage.

TFEB, TFE3, and nonmelanocyte isoforms of MITF

Unlike the melanocyte-specific MITF-M isoform, other isoforms include exon 1B1b that contains residues that facilitate MITF interaction with RAG GTPases at the surface of the lysosome (Martina and Puertollano 2013; Ngeow et al. 2018). As a consequence, exon 1B1b-containing isoforms can be phosphorylated by the lysosome-associated mTORC1 complex on the MITF-M equivalent of S173. Phosphorylation of this residue by mTORC1, or by TAK1 in osteoclasts (Bronisz et al. 2006), leads to MITF cytoplasmic sequestration via interaction with 14-3-3 proteins. This mode of regulation via nuclear cytoplasmic shuttling has been extensively studied for the MITF-related factor TFEB, and to a lesser extent TFE3 (Puertollano et al. 2018). Consequently, understanding their role and regulation is likely to be directly relevant for our understanding the function of the nonmelanocyte isoforms of MITF.

TFEB is phosphorylated on several residues that together regulate its stability or subcellular localization. These include phosphorylation of S3 by MAP4K3 (Hsu et al. 2018), an mTORC1-independent amino acid sensing kinase. Phosphorylation of S3 is necessary for TFEB to interact with the mTORC1–RAG–Ragulator complex at the lysosome surface and consequently is required for TFEB phosphorylation by mTORC1 on two sites: S211, where phosphorylation drives cytoplasmic sequestration via 14-3-3 binding (Roczniak-Ferguson et al. 2012), and S142, which, like the corresponding S73 in MITF-M, is also reported to be an ERK target (Settembre et al. 2011, 2012) and acts as a priming site for GSK3-mediated phosphorylation of S138 and CRM1-dependent nuclear export (Li et al. 2018). TFEB is also phosphorylated by mTORC1 on S122 (Vega-Rubin-de-Celis et al. 2017), for which there is no corresponding serine at this position in MITF, but, mechanistically, how this modification modulates TFEB subcellular localization is not well understood. A comparison of the sequences of human MITF, TFEB, TFE3, and TFEC proteins and their domain structures and posttranslational modification sites is shown in Supplemental Figure S3.

This complex network of phosphorylation events is primarily devoted to regulating TFEB subcellular localization in response to nutrient availability: under nutrient-rich conditions, phosphorylation drives TFEB nuclear export and cytoplasmic retention; under glucose limitation, activation of mTORC2 leads to AKT-mediated inhibition of GSK3 and reduced nuclear export (Li et al. 2018), while amino acid limitation inactivates mTORC1 and releases TFEB from its cytoplasmic anchor (Roczniak-Ferguson et al. 2012). Notably it has recently been shown that the critical mTORC2 subunit RICTOR is targeted by miR-211 (Ozturk et al. 2018), a microRNA whose expression is activated by MITF (Miller et al. 2004; Boyle et al. 2011; Margue et al. 2013), and likely also by TFEB and TFE3. Since inactivation of mTORC2 signaling by miR-211 leads to inactivation of mTORC1, nutrient limitation that triggers nuclear accumulation of MiT family members and increased miR-211 expression activates a feed-forward loop that amplifies MiT family nuclear accumulation and their downstream transcription program (Ozturk et al. 2018). In addition, AKT-mediated phosphorylation of S467 decreases TFEB protein stability such that AKT inhibition can lead to increased nuclear accumulation of TFEB (Palmieri et al. 2017). Importantly, inactivation of the kinases responsible for TFEB cytoplasmic localization or nuclear export is unlikely to be sufficient to promote TFEB nuclear accumulation since increased activity of phosphatases is at least as important. Those shown to play a role to date include the calcium-sensing phosphatase calcineurin (Medina et al. 2015) and PP2 that can dephosphorylate TFEB in response to oxidative stress (Martina and Puertollano 2018).

Once nuclear, TFEB, like MITF, regulates gene expression, and, in cells where multiple members of this family are expressed, they may bind DNA as either homodimers or heterodimers (Hemesath et al. 1994). Like MITF-M (Falletta et al. 2017), TFEB can activate expression of the integrated stress response factor ATF4 (Martina et al. 2016). Also like MITF-M (Ploper et al. 2015; Zhang et al. 2015), TFEB is widely regarded as a key regulator of lysosome biogenesis and autophagy (Settembre et al. 2012, 2013; Martina et al. 2014). TFEB is important in controlling metabolic flexibility in exercising muscle (Mansueto et al. 2016) and can suppress both atherosclerosis (Lu et al. 2017) and neurodegeneration (La Spada 2012; Decressac and Björklund 2013; Li et al. 2016; Palmieri et al. 2017). Importantly, deregulation of the MITF/TFEB/TFE3 family is also implicated in cancer; increased nuclear localization of these key transcription factors is required for pancreatic cancer progression (Perera et al. 2015), whereas Ewing's sarcoma gene (EWS) translocations leading to fusion with the CREB-related transcription factor ATF1 can lead to aberrant expression of MITF and clear cell sarcoma (Li et al. 2003; Davis et al. 2006). Moreover, translocations between different MiT family members are driver mutations for renal cell carcinoma (Inamura 2017) leading to deregulation of MiT-family target genes.

Although less is known of how TFE3 or exon 1B1b-containing isoforms of MITF are regulated, the limited evidence to date (Martina et al. 2014, 2016; Taniguchi et al.

2015; Li et al. 2018; Martina and Puertollano 2018) suggests they will be regulated to a large extent in a similar fashion to TFEB. Given that MITF, TFEB, and TFE3 are highly related, it seems likely that they will share a large set of target genes, although whether each also has a restricted set of unique targets has yet to be determined. Interestingly, transgenic expression of the related TFEC in the RPE can rescue eye defects in *Mitf*$^{mi\text{-}rw}$ mutant mice (Bharti et al. 2008).

Conclusions and future directions

Over the past 25 yr since the MITF gene was isolated, our understanding of its role has undergone a series of transformations. MITF was first recognized as being required for melanocyte development, leading the gene nomenclature committee to rename MITF as melanocyte-inducing transcription factor (for a commentary on this recent name change, see Arnheiter 2017). However, subsequently MITF was implicated in melanocyte differentiation and identified as a key regulator of cell proliferation, acting both as a proproliferative factor but also suppressing the cell cycle and invasion. Later, it was recognized as a critical regulator of metabolism and the DNA damage response as well as lysosome biogenesis and autophagy. Thus, while MITF is equal to other transcription factors in that it binds DNA to up-regulate or down-regulate transcription of its target genes, it is perhaps more than just equal in the sense that it is not dedicated to a specific response and rather coordinates a wide variety of cellular processes, unlike, for instance, the nuclear hormone receptors or the sterol response element-binding factor SREBP. Although additional transcription factors clearly play a critical role in melanocyte biology (Seberg et al. 2017), given MITF's role in nonmelanocyte cell types and in regulating genes implicated in a wide range of cellular functions, this "most important transcription factor" has taken center stage in our understanding of melanocyte and melanoma biology. Even though a great deal of our knowledge of MITF regulation and function has come from work on melanoma, it should not be forgotten that MITF's physiological function is to control the development and differentiation of the cells in which it is expressed, including in melanocytes the response to UV irradiation. It is likely, therefore, that with the exception of cell lineage-specific target genes, the range of other MITF-regulated target genes and biological functions may be similar in all MITF-expressing cell types, including melanoma. Indeed, it would seem to be a highly efficient strategy to place in the hands of a single transcription factor the coordination of many different cellular functions, although this strategy comes at the cost that a deregulated input can then affect many aspects of cell biology. The genetic lesions that drive melanoma initiation and progression and the altered microenvironment within tumors all lead to deregulation of MITF expression, posttranslational modification and function, with the consequence that MITF's ability to coordinate a wide range of biological processes is subverted. For example, ChIP-seq analysis indicates that different target sites have different affinities for MITF (Strub et al. 2011). Since elevated MAPK signaling resulting from BRAF mutations leads to lower MITF expression levels (Garraway et al. 2005) and increased cytoplasmic localization (Ngeow et al. 2018), the effective concentration of MITF in the nucleus will be reduced. Consequently, MITF's capacity to occupy lower affinity target sites will be diminished and regulation of its repertoire of target genes affected.

However, despite our increasing knowledge of the role and regulation/deregulation of MITF, our understanding remains severely limited. In terms of MITF posttranslational modification, the role of just a few phosphorylation sites has been determined, and likely many more modification sites will be uncovered: The role of SUMOylation is ill-defined, the repertoire of ubiquitin ligases required for MITF turnover and their lysine targets remain largely unreported, and the likely regulation of MITF by a plethora of other posttranslational modifications, including acetylation, lysine or arginine methylation, ADP-ribosylation, and so on, remains unknown.

Although the genome-wide occupancy of MITF-M has been determined by ChIP-seq, and many direct MITF target genes have been identified, it seems likely that many more bound genes will be regulated than is currently recognized, since most studies are performed under nutrient-rich conditions in cells in culture that do not necessarily reflect the in vivo microenvironment. In particular, MITF can control both proliferation and differentiation-associated gene sets. However, proliferation and differentiation are two largely mutually exclusive states in vivo. So, how does MITF distinguish between those genes associated with differentiation versus those linked to proliferation and how is this distinction regulated by the levels of MITF and its posttranslational modifications? Several possibilities exist, including cooperation for binding with cofactors that allow MITF to distinguish between different classes of targets in response to prodifferentiation or proproliferation signals. Indeed, a large repertoire of MITF-interacting cofactors has been identified. The role of some, including p300/CBP, the SWI/SNF complex, and, to a certain extent, β-catenin is defined. However, the role of the remaining interacting factors in controlling MITF activity remains to be determined. Equally important, how signaling to MITF might enable MITF to exchange specific cofactors remains unknown. Nevertheless, despite our advanced knowledge, how MITF regulates both proliferation and differentiation remains a key challenge to our understanding of MITF biology, as does how MITF activates some genes and represses others.

Also unknown is whether TFEB, TFE3, and nonmelanocyte isoforms of MITF directly regulate the same sets of genes as MITF-M since their genome-wide occupancy has yet to be explored by robust ChIP-seq analysis. Whether they use the same or different cofactors as MITF is also largely unknown.

Last, determining the extent to which the many in vitro findings are indeed relevant for the in vivo situation in both health and disease will require considerable additional efforts. As stated in this review, there are instances

where clear in vitro findings do not necessarily translate into predicted phenotypes in vivo and phenotypes may arise in vivo that one might not have predicted on the basis of in vitro results. For instance, although there is compelling genetic evidence for the importance of MITF in the biology of melanocytes and other cell types, the importance of specific posttranslational modifications seen in vitro is not always matched in genetic tests in vivo. However, we do not hold that such discrepancies are a principal problem of the applied tests. Rather, for the discrepancies to be minimized, it may become necessary to better adapt the conditions under which the different types of experiments are run, in particular concerning metabolic state or stress. Nevertheless, we would argue that it is important not to rush into clinical trials based on in vitro findings, however clean these may be, without first challenging these in vitro findings under many different conditions and in appropriate preclinical models. In this sense then, MITF research will not run out of exciting questions to address for many years to come.

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MITF––the first 25 years

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