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Update on the regulation of mammalian melanocyte function and skin pigmentation

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Abstract

Melanogenesis is the unique process of producing pigmented biopolymers that are sequestered within melanosomes, which provides color to the skin, hair and eyes of animals and, in the case of human skin, also protects the underlying tissues from UV damage. We review the current understanding of melanogenesis, focusing on factors important to the biochemistry of pigment synthesis, the biogenesis of melanosomes, signaling pathways and factors that regulate melanogenesis, intramelanosomal pH, transport and transfer of melanosomes, and pigmentary disorders related to the dysfunction of melanosome-related proteins. Although it has been known for some time that many of the factors that affect melanogenesis are derived from keratinocytes, fibroblasts, endothelial cells, hormones, inflammatory cells and nerves, a number of new factors that are involved in that regulation have recently been reported, such as factors that regulate melanosome pH and ion transport.

Keywords

eumelanin; pheomelanin; pigmentary disorders; pigmentation; regulation; skin

The color of the skin, hair and eyes of mammals (and other species) is derived from the production and distribution of pigmented biopolymers known as melanins. Melanins are synthesized within specialized membrane-bound organelles termed melanosomes that are exclusively produced in melanocytes and in retinal pigment epithelial cells. The precise migration, distribution, differentiation, proliferation and function of melanoblasts (the melanocyte precursor) and melanocytes determine the visible phenotype of those tissues, and also regulates melanocyte function in a number of minor sites in the body where they also reside, such as the inner ear, brain, heart and so on.

In human skin, melanocytes are localized at the dermal/epidermal border in a characteristic regularly dispersed pattern. Each melanocyte at the basal layer of the epidermis is functionally related to underlying fibroblasts in the dermis and to keratinocytes in the epidermis. Those three types of cells are highly interactive and communicate with each other

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through secreted factors and their receptors and via cell-to-cell contacts to regulate the function and phenotype of the skin.

Biochemical pathway of eumelanin & pheomelanin synthesis

Melanins can be produced in two chemically distinct types, black-to-brown eumelanin and yellow-to-reddish-brown pheomelanin [1]. Eumelanin and pheomelanin are both derived from the common precursor dopaquinone (DQ), which is formed following the oxidation of the common amino acid L-tyrosine by tyrosinase (TYR), which involves a transient intermediate L-3,4-dihydroxyphenylalanine (DOPA) (Figure 1). Eumelanins are dark and highly polymerized while pheomelanins contain sulfur and are lighter and less polymerized.

Eumelanogenesis

The first step in eumelanogenesis after the production of DQ involves the spontaneous cyclization of the quinone to produce cyclodopa, which then rapidly undergoes a redox exchange with another DQ molecule to produce one molecule each of DOPAchrome and DOPA [2]. DOPAchrome is then spontaneously decomposed by decarboxylation at neutral pH to form 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) in a 70:1 ratio [3]. However, in the presence of an enzyme specifically produced in melanocytes termed DOPAchrome tautomerase (DCT), also known as TYR-related protein-2 (TYRP2), DOPAchrome undergoes tautomerization to exclusively produce DHICA [4]. The ratio of DHICA to DHI in natural eumelanins is thus determined by the activity of DCT [5]. Finally, DHI and DHICA are further oxidized and polymerized to form eumelanins (Figure 1).

Recently, the redox exchange reaction between DHI and DQ has been studied and elucidated [6]. It was concluded that during eumelanogenesis, DHI oxidation takes place by a redox exchange with DQ, although such a reaction is likely to be less efficient for DHICA. Therefore, DHICA may require oxidation to the quinone form by the direct action of TYR in humans [7] or by TYR-related protein-1 (TYRP1) in mice [8,9]. Interestingly, human TYRP1 is unable to catalyze the DHICA oxidation [10], and mouse TYR is unable to catalyze the DHI oxidation. The activities of these two TYR-related proteins, TYRP1 and DCT, greatly affect the quantity and quality of eumelanins, their ratio of DHI- to DHICA-derived subunits and the degree of their polymerization [11,12]. Fibrillar melanosomes are characteristic of eumelanin production, and result from the expression of a structural matrix protein known as Pmel17 [13], which is an amyloid protein required for the generation of the internal fibrils (those fibrils are typically lacking in melanosomes containing pheomelanin).

Pheomelanogenesis

Pheomelanogenesis proceeds through several distinctive steps at the monomer level. The first step is the reductive addition of cysteine to DQ to produce two major types of cysteinylDOPA (CD) isomers, 5SCD and 2SCD; the second step is the redox exchange between CD and DQ to produce CD-quinones and DOPA; the third step is the cyclization of CD-quinones through dehydration to form the ortho-quinonimine (QI) [14–16], after which QI is rearranged with/without decarboxylation to form the 1,4-benzothiazine intermediates that are finally polymerized to pheomelanin [17,18]. We noted above that DQ plays a pivotal role in promoting eumelanogenesis, and it should be noted here that DQ is also involved in the production of CD in the first step of pheomelanogenesis and the oxidation of CD. In other words, DQ plays pivotal roles not only in eumelanogenesis, but also in pheomelanogenesis (Figure 1). Note that the activities of TYR, TYRP1 and DCT are

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involved in the production of eumelanin, but by contrast, only TYR and the precursor amino acid cysteine are necessary for the production of pheomelanin.

A three-step pathway for mixed melanogenesis

Melanocytes produce mixtures of eumelanin and pheomelanin, and therefore, melanogenesis should be considered as 'mixed melanogenesis'. Ito *et al.* reported a three-step pathway for mixed melanogenesis [19], wherein the total amount of melanin produced is proportional to DQ production, which is in turn proportional to TYR activity. In fact, melanogenesis proceeds in three distinct stages. The first stage is the production of CD isomers, which continues as long as the cysteine concentration is above 0.13 μM. The second stage is the oxidation of CD to produce pheomelanin, which continues as long as CD is present at concentrations above 9 μM. The last stage is the production of eumelanin, which begins only after the majority of CD and cysteine is depleted. Therefore, the ratio of eumelanin to pheomelanin is determined by TYR activity and the availability of tyrosine and cysteine in melanosomes [2].

Signaling pathways involved in regulating melanogenesis

Microphthalmia-associated transcription factor (MITF) is a key regulator of mammalian pigmentation that is regulated by environmental factors, including UV, and by factors secreted from keratinocytes, fibroblasts and other cells. MITF controls not only melanogenesis, but also differentiation, dendricity, proliferation and apoptosis through various pathways and mechanisms (Figure 2) [20]. All three of the melanogenic enzymes (TYR, TYRP1 and DCT) that play key roles in melanogenesis have been demonstrated to be transcriptional targets of MITF, as are many other melanocyte-specific proteins. Promoter-reporter studies revealed that promoters of TYR [21,22], TYRP1 [23,24] and DCT [23] are activated by cotransfected MITF. The human TYR promoter contains an M-box (an extended E-box, AGTCATGTGCT), located approximately 100 bp upstream of the transcription start site, and also an E-box (ACATGTGA) at the initiator. Interestingly, the E-box is more important for the promoter function of MITF than is the M-box [21]. The p53 tumor suppressor protein was also demonstrated to participate in the increased melanogenesis that occurs after UV irradiation and acts via two mechanisms:

- It stimulates expression of proopiomelanocortin (POMC) in epidermal keratinocytes, which in turn activates neighboring melanocytes via the melanocortin 1 receptor (MC1R);
- It directly stimulates the expression of the genes encoding TYR and TYRP1 (shown in reporter assays) in melanocytes, and potential binding sites for p53 have been identified in the TYRP1 promoter [25].

Tyrosinase mRNA levels are increased via a p53-dependent mechanism upon UV irradiation of melanoma cells in culture, and p53 is required for the thymidine dinucleotide-induced increase of TYR function in mouse epidermis [26].

Other transcription factors, such as dimerization cofactor of hepatocyte nuclear factor 1 (DcoH)/hepatocyte nuclear factor 1 (HNF1) α, have also been shown to be involved in regulating TYR transcription in skin melanocytes [27]. According to Hou and coworkers, mouse embryonic melanocytes require the coordinated action of MITF and the transcription factor Sox10 for TYR induction, because both pigmentation and TYR expression in Sox10-deficient neural tube explant cultures can only be rescued by exogenous Sox10, which acts upstream of MITF, but not by exogenous MITF alone [28]. The promoter of the human *TYRP1* gene also possesses an M-box (AATCATGTGCT) which is localized approximately 210 bp upstream of the start of transcription and, unlike the mouse promoter, the human

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promoter harbors the TATA sequence [29]. While the M-box is necessary for promoter upregulation by MITF [23], the TYRP1 promoter also binds and is activated by the paired box 3 (Pax3) transcription factor [30].

DOPAchrome tautomerase is expressed very early during melanoblast differentiation in the developing embryo, approximately when MITF begins to be expressed. The 5′-regulatory region of the DCT gene contains an M-box (GGTCATGTGCT) positioned approximately 135 nucleotides upstream of the transcription start site [31] and the promoter responds to MITF coexpression [23]. Another important transcription factor specifically involved in regulating the expression of DCT is SOX10, which also regulates the expression of MITF. A human DCT promoter–reporter construct was demonstrated to be activated by SOX10 [32,33] and SOX10 and MITF act in a synergistic manner to activate that promoter [34,35]. These data are in accordance with the observation that mouse heterozygous embryos carrying the $Sox10^{dom}$ mutation transiently lack DCT expression (around days 11–12) in the melanoblast lineage, and MITF alone is incapable of triggering DCT transcription in these early MITF-positive cells [33]. In a recent report, another member of the Sox family, Sox5, inhibited the Sox10-stimulated activity of the DCT promoter in melanocytes [36]. A conserved cAMP-response element (CRE)-like element in the DCT promoter might also contribute to gene expression through direct regulation by CRE-binding (CREB) protein [21]. Distal enhancers have been described for the *TYR* and *TYRP1* genes in mice [37]. An upstream regulatory region for human TYR is reportedly located approximately −9 kb upstream from the start of transcription [38,39]. This sequence, which shows a homology with a similar distal locus found in the mouse and functions as an enhancer in transfection assays, may prove to be important for the pigment cell-specific expression of human TYR.

Hoek *et al.* reported a DNA microarray study using SK-Mel28 human melanoma cells that overexpress MITF, which confirmed the regulation of the above genes and identified other possible MITF target genes involved in melanosome biogenesis and function [40]. Among them, the lysosomal trafficking regulator (*LYST*) gene is known as a pathogenic gene involved with Chediak–Higashi syndrome (CHS), an autosomal recessive disease with immunodeficiency [41], and Hermansky–Pudlak syndrome 4 (*HPS4*) mutations cause Hermansky–Pudlak syndrome (HPS) [42]. Recently, Levy *et al.* demonstrated that expression of DICER, a central regulator of miRNA maturation, is strongly induced during melanocyte differentiation via direct transcriptional regulation by MITF. DICER plays a crucial role in melanocyte survival involving, in part, the post-transcriptional processing of miRNA-17, which leads to downregulation of the proapoptotic protein Bcl-2-interacting mediator of cell death (BIM) [43].

Melanogenesis is regulated hormonally by α-melanocyte stimulating hormone (α-MSH) and its receptor MC1R through the cAMP/PKA signaling cascade (Figure 2). The *POMC* gene encodes a precursor that is processed to form α-MSH, adrenocorticotropic hormone (ACTH) and β-endorphin. The *POMC* gene is active mainly in the pituitary, but POMC-derived peptides are also produced in keratinocytes and in melanocytes [44,45]. Although α-MSH is a potent inductor of pigmentation in mammalian skin, β-MSH and ACTH also possess melanogenic activities [44,46,47]. The hormonal stimulation by α-MSH mediated through MC1R results in increased intracellular cAMP levels and leads to the activation of PKA. This hormonal signaling pathway has specific consequences in melanocytes as the MITF promoter possesses a CRE element that is bound by CREB proteins that are phosphorylated by PKA and that then activate the transcription of MITF. Interestingly, α-MSH is able to control melanogenesis independently of MC1R, possibly by acting directly in melanosomes [48].

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Another receptor that is upregulated by MITF is the endothelin receptor B (EDNRB), which serves as a receptor for endothelins, and is also regulated by MITF. Moreover, signaling by endothelins 1 and 3 activates MAPK, which then subsequently phosphorylates MITF and also stimulates MITF expression [49].

Hormonal signaling is also involved in skin responses to UV irradiation. In keratinocytes, UVB exposure induces the expression of *POMC* via p53, which leads to the secretion of α-MSH and the upregulation of melanogenesis via the MC1R [46,50]. In addition, the expression of corticotropin-releasing hormone (CRH) is also stimulated by UVB in melanocytes, which is mediated by the CREB–PKA signaling with consequent stimulation of POMC expression through the CRH-R1 receptor [50]. As noted above, the *POMC* gene has been reported to be p53-responsive following UV irradiation, and the *POMC* promoter contains a p53 binding site that is necessary for its highest activity [51]. The *in vivo* tanning response and induction of POMC mRNA are dependent on p53, further suggesting that the p53 protein is an important mediator of UV-induced melanogenesis via increased transcription of POMC [51]. However, POMC-knockout mice display normal melanin pigmentation, and some *POMC* mutations occur in humans who do not show any pigmentary change, although patients with several severe *POMC* mutations show pigmentary changes ranging from brown hair to red hair, which implies a more complex regulation of UV-induced pigmentation via induction of *POMC* transcription [52,53]. Hormonal regulation also underlies the switch of pigment type formation in the skin. α-MSH signaling of the MC1R receptor is inhibited by the agouti signaling protein (ASP, ASIP in humans) that can compete with α-MSH in binding to MC1R. High expression of ASP is associated with yellow pigmented bands in mouse hair. Thus, MC1R and its ligands, α-MSH and ASIP, regulate the switch between eumelanin and pheomelanin synthesis in melanocytes [44,54]. ASP was demonstrated to downregulate *MITF* gene expression, and hence its targets, by antagonizing the effect of α-MSH, thus favoring pheomelanogenesis by reduced production of eumelanin [55]. Interestingly, a similar profile of genes was inversely regulated by ASP and α-MSH in a microarray analysis [56]. Recently, it has been demonstrated that the receptor-binding domain of ASIP efficiently antagonizes the MSH–MC1R signaling by reducing cAMP levels, while it induces no changes in pigmentation, demonstrating that the negative regulation of differentiation by ASP signaling is independent of the cAMP–CREB pathway [54]. Other hormones, such as steroids, can also influence pigmentation [44,46], and a recent study reported that even cholesterol is capable of increasing the expression of MITF and its target genes in melanocytes, possibly through the upregulation of the CREB protein [57].

We recently reported two fibroblast-derived paracrine factors, dickkopf-related protein 1 (DKK1) and neuregulin-1 (NRG1), that regulate melanogenesis. DKK1, which is secreted at high levels by fibroblasts in the dermis of the palms/soles, suppresses melanocyte growth and function by inhibiting the Wnt/β-catenin signaling pathway [58,59]. NRG1, which is highly expressed by fibroblasts derived from darker skin, significantly increases pigmentation in a reconstructed skin model and in cultured human melanocytes. It is considered that NRG1, acting through the ErbB3 or ErbB4 receptor, leads to the activation of intracellular signaling that include the PI3K and the MAPK pathways to regulate melanogenesis [60].

Finally, the post-transcriptional regulation of melanogenesis independent of MITF should be noted, since MITF is required, but is not sufficient, to induce the expression of melanogenic enzymes [61]. In fact, Newton *et al.* demonstrated that the inhibition of TYR activity by resveratrol is not due to changes in MITF, but instead is explained both by direct TYR inhibition and by a post-transcriptional effect that reduces the amount of fully processed TYR. They also reported that the intracellular elevation of cAMP increases protein levels for

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MITF, TYR and DCT, but there is no concomitant increase in TYR or DCT mRNAs [62]. Recently, Bellei *et al.* demonstrated that downregulation of p38 expression leads to an increase in the levels of differentiation-associated markers such as melanin synthesis and the expression of TYR and TYR-related proteins. They also demonstrated that the mechanism involved in the p38-mediated regulation of melanogenesis is the ubiquitin–proteasome pathway, where melanogenic enzymes are degraded [63].

Regulation of melanogenesis by pH

Fuller *et al.* reported that melanosomes of melanocytes derived from light human skin have low levels of TYR activity and are more acidic than the melanosomes of melanocytes derived from dark human skin, which have high TYR activity [64]. Since then, the importance of melanosomal pH to skin pigmentation has been widely accepted, although the mechanisms and molecular players involved in the regulation of melanosome pH remain to be elucidated. One of the main factors involved in this pH regulation is vacuolar (V)-ATPase; its presence in melanosomes has been demonstrated by proteomic analysis [65]. Recently, Cheli *et al.* reported that activation of the cAMP pathway by α-MSH or forskolin increases the pH of melanosomes and regulates the expression of several V-ATPases and ion transporters [66]. Notably, cAMP upregulates the expression of V-ATPase subunits, which should result in the acidification of melanosomes. V-ATPases are a key component in the regulation of organellar pH and, more specifically, of melanosome acidification.

However, the pH of organelles is also greatly influenced by their internal ionic equilibrium, which is mainly regulated by ion pumps, such as Na^+/K^+-ATPase, and by ion-specific channels, such as chloride, potassium and sodium channels [67]. The role of these ion pumps and channels in melanosomes still needs further investigation. Furthermore, ion exchange through the vast family of solute carriers (SLCs) is also predicted to play a pivotal role in the regulation of melanosomal pH. In fact, a potassium- dependent sodium/calcium exchanger, the NCKX5 protein encoded by *SLC24A5*, has been detected in melanosomes [68], and has been demonstrated to control melanogenesis in both human and mouse melanocytes [69]. In addition, SLC45A2, formerly termed MATP or AIM1, is also localized in melanosomes and is the pathogenic gene for oculocutaneous albinism (OCA) type 4 [70]. The P protein, encoded by the *OCA2* gene, is mutated in OCA type 2; this protein is related to the sodium/sulfate transporter of the SLC13 family. The P protein has also been implicated in regulating melanosomal pH. Indeed, melanosomes in melanocytes derived from pink-eyed dilution mice were reported to have a less acidic pH that did not favor melanin synthesis [71,72]. However, these observations are not in agreement with a number of other studies demonstrating that melanogenesis is stimulated by agents that increase melanosomal pH [64,73–75], or that cAMP increases melanogenesis and melanosomal pH. The exact role of these pumps, channels and exchangers in the regulation of melanosome pH and in modulating skin pigmentation needs to be further investigated.

Genome-wide association studies have revealed several new candidate genes that regulate melanogenesis, such as *SLC24A4*, interferon regulatory factor 4 *(IRF4)* and two-pore segment channel 2 (*TPCN2)* [76]. Among them, it has recently been revealed that human two-pore channel 2 protein (TPC2), encoded by *TPCN2*, localized on lysosomal membranes, induces nicotinic acid adenine dinucleotide phosphate (NAADP)-dependent calcium ion transport from acidic organelles [77]. This protein is possibly also involved in the pH regulation of melanogenesis.

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Transport of melanosomes from melanocytes to keratinocytes & between keratinocytes

Melanosomes mature in a well-characterized series of steps, divided into stages I, II, III and IV, according to their structures and content of pigment. 'Early' melanosomes refers to stages I and II (little or no pigment), while 'late' melanosome refers to stages III and IV (some to complete pigment). In melanocytes, the 'late' melanosomes bind to microtubules and undergo actin-dependent transport toward the cell periphery, following which they are transferred to keratinocytes [78]. The sum of these transport and transfer processes are critical to the eventual distribution of melanin pigments in the skin. Thus, the intracellular transport of melanosomes is directed by microtubules (composed of α:β-tubulin dimers) and actin filaments (composed of actin monomers). Microtubules act as tracks for the transport of several intra-cellular organelles, including lysosomes and sorting vesicles [79,80]. The trafficking of sorting vesicles to their target organelles is controlled by two classes of microtubule-associated motor proteins – kinesins and cytoplasmic dyneins. Kinesins power the plus-end-directed microtubule-based motility, while cytoplasmic dyneins drive the minus end motility [81,82]. Dyneins and kinesins also have well-established roles in retrograde and in anterograde transport of melanosomes [83–86]. We have demonstrated that the movement of early melanosomes is dominated by dyneins and spectrin [87].

It has also been accepted that the Rab27a–melanophilin (Mlph)–myosin Va (Myo5a) tripartite complex is related to the capture of melanosomes within the distal, actin-rich regions of the dendrites [88–92]. Moreover, Rab27a links synaptotagmin-like protein2-a (Slp2-a) with phosphatidylserine, thereby docking melanosomes at the plasma membrane, which suggests the role of Slp2-a as a regulator of melanosome exocytosis [93].

Despite the fact that it has been known for decades that the transfer of melanosomes from melanocytes to their neighboring keratinocytes and their subsequent distribution throughout the epidermis of the skin and hair is critical to pigmentation of those tissues, the factors involved in those processes have remained stubbornly enigmatic [94,95]. Several hypotheses have been proposed to explain that transfer, including the cytophagocytosis of melanocyte dendrite tips [96], the exocytosis of melanosomes into the extracellular space and their subsequent uptake by phagocytosis into keratinocytes [97,98], and filopodia-mediated melanosome transfer [99–101]. Recently, yet another hypothesis to explain this was proposed by Singh *et al.*, the filopodial-phagocytosis model, which would explain melanosome transfer from melanocytes to keratinocytes and even between keratinocytes under both constitutive and facultative (i.e., post-UVR) conditions [102].

Pigmentary disorders related to dysfunction of melanosome proteins

Most forms of congenital hypopigmentation derived from defects in melanin synthesis are forms of oculocutaneous albinism (OCA), which can be divided into nonsyndromic OCA and syndromic OCA. Nonsyndromic OCA arises from the absence or severe dysfunction of TYR and other key pigment factors (including P, TYRP1 and SLC45A2), which result in OCA types 1–4. Syndromic OCA, HPS, CHS and Griscelli syndrome (GS) have a number of additional features, including a bleeding diathesis, immunodeficiency and neurological dysfunction [103].

Oculocutaneous albinism

Oculocutaneous albinism is an inherited autosomal recessive disorder, which is characterized by hypomelanosis in most normally pigmented tissues, including the skin, hair and eyes, accompanied by reduced visual acuity with nystagmus and photophobia. The defects in melanin biosynthesis or transport result in a deficiency or complete absence of

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melanin in affected patients. OCA can be categorized into six types – OCA1A, OCA1B, OCA1-temperature-sensitive (OCA1-TS), OCA2, OCA3 and OCA4 [103].

In patients with OCA1A (TYR-negative OCA; Mendelian Inheritance in Man [MIM] 203100), TYR activity is completely lacking owing to a mutation of its encoding gene (*TYR*) [104]. Melanin formation does not occur throughout the patient's life, because the first step of melanin synthesis is blocked. Therefore, the OCA1A phenotype is characterized by completely white hair, pinkish skin and red pupils [105].

Oculocutaneous albinism type 1B (yellow-mutant OCA, MIM 606952) patients completely lack detectable pigment at birth and are initially indistinguishable from patients with TYR-negative OCA (OCA1A). However, such patients rapidly develop a yellowish hair pigment in the first few years of life and then continue to slowly accumulate pigment in the hair, eyes and skin over their lifetime. In these patients, TYR activity is greatly decreased, but is not completely absent. A point mutation in the *TYR* gene causes a small change in TYR conformation, or causes the formation of a new splicing site associated with decreased enzyme activity [106,107].

Oculocutaneous albinism type 1-temperature-sensitive patients have white hair and skin and blue eyes at birth. At puberty, they develop progressively darker hair at cooler areas of the body (extremities) but retain white hair in the warmer areas (scalp, axilla) [108]. A missense mutation in the *TYR* gene of those patients causes an amino acid substitution that makes the enzyme temperature-sensitive, with very low activity at 35°C and a complete loss of activity above 37°C. The lack of correct enzyme folding results in the retention of TYR in the endoplasmic reticulum and its eventual degradation by proteasomes [109,110].

Oculocutaneous albinism type 2 (MIM 203200) patients have mutations of the *P* gene [104]. The *P* gene encodes a melanosomal membrane protein that may regulate the processing and/or transport of TYR [111]. To date, the P protein has an undefined function. The phenotypes of OCA2 have much variety. Patients with complete loss of melanin are indistinguishable from patients with OCA1A, while those with brown hair resemble OCA1B patients [105]. In addition, albinos with a *P* gene mutation, who would be expected to have yellow hair, may have red hair if *MC1R* mutations coexist [112]. It should be noted that OCA2 function may be independent of its activity on TYR folding or transport [113].

Oculocutaneous albinism type 3 (MIM 203290), also known as *TYRP1* gene-related OCA or Rufous OCA, was first described in South African black individuals and is caused by mutations in the *TYRP1* gene [104,111]. OCA3 patients have now also been reported in Caucasian and in Asian Indian populations [114,115]. As noted above, human TYRP1 is unable to catalyze the oxidation of DHICA in contrast to murine TYRP1 which is able to oxidize DHICA [10]. TYRP1 also serves as a type of chaperone to TYR, enhancing its stability so that decreased function of TYRP1 impacts melanin synthesis in several ways [116].

Oculocutaneous albinism type 4 (MIM 606574) results from mutations of the *SLC45A2* gene and is considered one of the most common and severe types of OCA in Japan. The clinical phenotype of OCA4 reported in Japanese patients is variable and is similar to OCA2. OCA4 albinism is rare in Caucasian patients [117].

Hermansky–Pudlak syndrome

Hermansky–Pudlak syndrome (MIM 203300) is a genetically heterogeneous group of related autosomal recessive conditions described in humans and in mice. There are eight known human *HPS* genes that cause different subtypes of HPS (HPS 1–8) and at least 14

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murine *HPS* genes, eight of which are orthologous to the human genes [118]. Defects in proteins encoded by these genes can affect the biogenesis and/or function of intracellular organelles found in specialized secretory cells, such as pigment cells (melanocytes and retinal pigment epithelial cells), platelets, T cells, neutrophils and lung type II epithelial cells. The organelles affected by *HPS* genes belong to the family of organelles known as lysosome-related organelles [118].

Hermansky–Pudlak syndrome is similar to albinism, but with additional clinical features. The pigment phenotype of HPS patients is extremely variable and can range from a minimal to a severe reduction in skin, hair and ocular pigmentation. In general, there is no tanning response after sunlight exposure in these patients [104]. Mutations in two of the *HPS* genes (*HPS1* and *HPS4*) cause the most common and most severe clinical subtypes, in which affected individuals have OCA, prolonged bleeding (due to platelet storage pool deficiency) and can suffer morbidity from granulomatous colitis and premature mortality from pulmonary fibrosis [118].

The most common type of HPS (HPS1) is found in Puerto Rican patients and is caused by a 16-bp frameshift duplication in exon 15 [118]. The HPS1 protein is ubiquitous and is primarily localized to the cytosol, with a small proportion being membrane-associated.

As in other subtypes of HPS, HPS4 patients have great variability in the degree of hypopigmentation, and may present with a severe phenotype similar to that of HPS1 patients [118]. The similarity in phenotypes between HPS1 and HPS4 subtypes is explained by the finding that intracellular HPS1 and HPS4 proteins associate together in a protein complex termed biogenesis of lysosome-related organelle complex 3 (BLOC-3) [119]. The BLOC-3 complex regulates the biogenesis and/or function of lung lamellar bodies, as well as the platelet-dense bodies and melanosomes [118]. Recently, the specific interaction of BLOC-3 with Rab9a and Rab9b isoforms was reported, which suggests that BLOC-3 functions as a Rab9 effector [120].

Hermansky–Pudlak syndrome subtype 2 is caused by mutations in the *AP3B1* gene that encodes the β3A subunit of the heterotetrameric adaptor protein complex AP-3. AP-3 plays a role in mediating cargo protein selection into transport vesicles and in trafficking those membrane proteins to lysosomes [118,121,122]. The HPS2 subtype may be clinically distinguished from the other forms of HPS as it is unique in causing immunodeficiency and manifests with neutropenia and susceptibility to recurrent respiratory illnesses.

The HPS3, HPS5 and HPS6 subtypes are clinically similar. They may present with ocular albinism and bruising, but without pulmonary fibrosis or colitis. The HPS3 protein associates with HPS5 and HPS6 proteins in yet another multimeric protein complex, termed BLOC-2. Immunofluorescent imaging of melanocytes derived from HPS3 patients demonstrated that molecules normally targeted to late melanosomes (e.g., the melanogenic enzymes TYR and TYRP1) are mislocalized. By contrast, the steady-state distribution of molecules targeted to early melanosomes (e.g., silver/Pmel17/gp100 and melan-a/MART1) were found to be normal. DOPA staining detected melanogenic enzymatic activity in melanosomes, suggesting that melanogenic enzymes can access melanosomes via an HPS3-independent pathway. In addition, it has been suggested that the lack of melanin detected in late melanosomes in HPS3 cells is due to the limiting quantity of another (presumably mis-trafficked) molecule [118,123]. HPS5 is a rare type of HPS [118] that encodes a cytoplasmic protein of unknown function that interacts with the HPS-6 protein [111]. All HPS5 patients have been reported to have elevated cholesterol levels, with several also having mildly elevated triglyceride levels. The significance of these elevated lipid levels, and whether they are a result of an underlying membrane trafficking defect, is not yet known [118].

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The *DTNBP1* gene, which is defective in HPS7, encodes the dysbindin protein. A single patient has been reported with HPS7, who presented with OCA, easy bruisability, bleeding tendency In patients with HPS8, the and a decreased lung compliance defective gene is *BLOC1S3*. Patients present with OCA and mild platelet dysfunction with easy bruising, epistaxis and a bleeding tendency [118]. HPS7 and HPS8 proteins are both subunits of BLOC-1. In mice, BLOC-1 functions in a transport pathway from early endosomes to maturing melanosomes, and that transport pathway is obligatory, at least for TYRP1. BLOC-2 seems to function downstream of BLOC-1 in this pathway, but AP-3 seems to regulate different cargo [124]. In mice, BLOC-1 also regulates copper-dependent TYR activity in melanosomes via ATP7A transport [125]. These new insights should be helpful to further understand the pathology of HPS7 and HPS8.

Chediak–Higashi syndrome

Chediak–Higashi syndrome (MIM 214500) is a rare autosomal recessive disorder characterized by OCA and a silvery sheen to the hair. CHS is also characterized by a bleeding tendency, progressive primary neurological impairment and severe immune deficiency due to a lack of natural killer cell function, which results in recurrent pyogenic infection. In addition, it causes a severe hemophagocytic lymphoproliferative syndrome caused by uncontrolled T-cell and macrophage activation. CHS is characterized by massive cytoplasmic lysosomal and nonlysosomal inclusions in granule-containing cells, which are probably responsible for most of the impaired functions in CHS cells. Melanocytes containing giant melanosomes seem to account for the hypopigmentation. Most cases are fatal unless treated by bone marrow transplantation. CHS has been linked to the human gene *CHS1*/*LYST*. The CHS1 protein is predicted to be a cytosolic protein with a role in vesicular transport, although no specific function for this protein has been elucidated despite intense efforts of many groups [126].

Griscelli syndrome

Griscelli syndrome is a rare autosomal recessive disorder characterized by pigmentary dilution of the skin, a silver-gray sheen to the hair, large clumps of pigment within hair shafts and the accumulation of large and abnormal end-stage melanosomes in the center of melanocytes [111]. GS has been linked with defects of the Rab27a–Mlph–MyoVa protein complex formation in melanocytes, which is important for melanosomes to connect to the actin network [111]. Patients with GS can be categorized into three types. Type 1 (GS1; MIM 214450) is manifested with albinism and severe primary neurological impairment, with developmental delay and mental retardation. It has been attributed to mutations of the myosin 5a gene (*MYO5a*) that encodes an organelle motor protein, Myo5a [127].

GS type 2 (GS2; MIM 607624) presents with albinism and is associated with potentially lethal immune defects and a hemophagocytic syndrome. Bone marrow transplantation is currently the only curative treatment. GS2 is caused by mutations in *RAB27A*, which encodes a small GTPase protein (Rab27a) that is involved in the function of the intracellular-regulated secretory pathway [127].

Griscelli syndrome type 3 (GS3; MIM 609227) results from mutations in the gene that encodes melanophilin (MLPH). Unlike GS1 and GS2, GS3 has only dermatological manifestations [127,128].

Expert commentary

Many research groups are still attempting to elucidate the mechanisms of melanogenesis, with the goal to develop cosmetics and/or treatments for hypopigmentary and/or

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hyperpigmentary disorders, with the ultimate goal of therapies leading to melanoma treatment. We have briefly reviewed updates in our understanding of melanogenesis and pigmentary disorders related to human melanogenesis dysfunction. There is a vast literature on these topics, and interested readers should refer to the original articles and/or recent reviews for full bibliographic citations. The transcription factor MITF plays a key role in regulating signaling pathways in melanogenesis. Identification of factors that regulate MITF, MITF target genes and the MITF-dependent transcriptome is significant to better understand the mechanisms involved. With regard to regulating MITF, factors derived not only from keratinocytes, but also from fibroblasts, play important roles. Melanosome regulation by pH is important and, in fact, pH regulation via ion transporters has been known to be related to the functions of various organelles for some time. In melanosomes, V-ATPase plays a major role in regulating pH, but other important ion transporters have also been identified. The potassium-dependent sodium/calcium exchanger NCKX5 (encoded by *SLC24A5*) has been implicated in regulating pigmentation, as has an NAADP-dependent calcium ion transporter TPC2 (encoded by *TPCN2*). Finally, P and SLC45A2 presumably play some roles as ion transporter proteins, although their specific functions are still being actively characterized.

Five-year view

In the next 5 years, our understanding of factors involved with regulating melanogenesis will be further improved since there are still many factors, proteins and genes with unknown functions that dramatically affect melanogenesis, melanosome transport and visible pigmentation of tissues. Although biochemically the mechanism of switching between eumelanogenesis and pheomelanogenesis depends on TYR activity, as well as the availability of tyrosine and cysteine *in vitro*, the mechanism that regulates that switch *in vivo* is still unsolved. Additional signaling pathways that regulate MITF will provide further understanding, not only of melanogenesis, but also of pigmentary disorders and melanoma. With regard to ion transporters that regulate melanosome pH, characterizing the functions of the P and SLA45A2 proteins will provide us with new insights about their mechanism of action.

We still understand little about melanosome transfer from melanocytes to keratinocytes and between keratinocytes compared with what we know about the critical signaling pathways involved in regulating melanocyte function. Although it remains unproven at this time, the filopodial–phagocytosis model might be an important advance in this area. There are still many cases of OCA and other pigmentary disorders that have not been associated with any genes, although at this time, only approximately 50% of the over 200 known pigment genes have been associated with human pigmentary functions, and thus many remain to be characterized. Many pathogenic genes remain to be characterized, and those should eventually provide more insights into the complex mechanisms that regulate melanogenesis in mammals.

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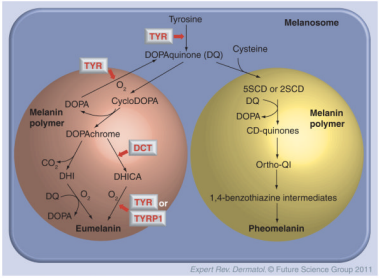

Figure 1. Biosynthetic pathways of eumelanin and pheomelanin

The activities of TYR, TYRP1 and DCT are involved in the production of eumelanin, but only TYR and cysteine are required for the production of pheomelanin from DQ.

CD: CysteinylDOPA; DCT: DOPAchrome tautomerase; DHI: 5,6-dihydroxyindole; DHICA: 5,6-dihydroxyindole-2-carboxylic acid; DOPA: L-3,4-dihydroxyphenylalanine; DQ: Dopaquinone; QI: Ortho-quinonimine; TYR: Tyrosinase; TYRP1: Tyrosinase-related protein-1.

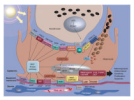

Figure 2. Selected factors and signaling pathway regulating melanocyte function

Various factors that regulate melanocyte function in the skin are shown. Antagonists of receptor binding are shown in red.

Adapted from [20].

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