The Regulation of Skin Pigmentation*

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Visible pigmentation of the skin, hair, and eyes depends primarily on the functions of melanocytes, a very minor population of cells that specialize in the synthesis and distribution of the pigmented biopolymer melanin. Melanocytes are derived from precursor cells (called melanoblasts) during embryological development, and melanoblasts destined for the skin originate from the neural crest. The accurate migration, distribution, and functioning of melanoblasts/melanocytes determine the visible phenotype of organisms ranging from simple fungi to the most complex animal species. 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 connected to underlying fibroblasts in the dermis and to keratinocytes in the overlying epidermis. Those three types of cells are highly interactive and communicate with each other via secreted factors and their receptors and via cell/cell contacts to regulate the function and phenotype of the skin.

Overview: Architecture of the Skin

Epidermal melanocytes occur at an approximate ratio of 1:10 among basal keratinocytes and distribute the melanin they produce to ∼40 overlying suprabasal keratinocytes via their elongated dendrites and cell/cell contacts (presented schematically in Fig. 1). Although melanocytes and stem cell keratinocytes in the basal layer of the epidermis are very stable populations that proliferate extremely slowly under normal circumstances, keratinocytes in the upper layers of the epidermis proliferate relatively rapidly. That upward pressure carries them toward the surface of the skin along with their ingested melanin to form a critical barrier for the organism against the environment and the many stresses that originate there. Thus it is not the melanin within melanocytes only, but in combination with the pigment in more superficial layers, that gives skin its characteristic color. Although melanocytes in other locations of the body (e.g. hair follicles, eyes, inner ear, etc.) interact with surrounding cells in manners distinct from those in the epidermis, the basic processes involved in producing the melanin and the organelles within which it is synthesized (term melanosomes) are comparable, as are the factors that regulate melanogenesis. This review will restrict itself to epidermal pigmentation, and readers interested in factors influencing pigmentation at other sites should consult recent reviews (1–6) and books (7, 8) on those topics.

Biochemical Considerations

At this time, more than 125 distinct genes are known that regulate pigmentation either directly or indirectly (9). Many of those affect developmental processes critical to melanoblasts, others regulate the differentiation, survival, etc. of melanocytes, and yet others regulate distinct processes that affect pigmentation. Many of those genes (>25 at latest count) affect the biosynthesis or function of melanosomes, the discrete membrane-bound organelles within which melanosomes are synthesized. Melanosomes, which are closely related to lysosomes and are within the family of lysosome-related organelles (LROs), require a number of specific enzymatic and structural proteins to mature and become competent to produce melanin (10, 11). As space does not allow a full consideration of melanosomal biogenesis and the specific functions of melanosomal proteins, readers are referred to several recent reviews on this topic (12, 13). Suffice it to say that the critical enzymes include tyrosinase (TYR), Tyrp1, and Dct, mutations of which dramatically affect the quantity and quality of melanosomes synthesized. Critical structural proteins include Pmel17 (also known as gp100) and MART1, both of which are required for the structural maturation of melanosomes. A large number of proteins are involved in the sorting/trafficking of proteins to melanosomes, and mutations in any of those typically lead to inherited hypopigmentary disorders (8). Melanocytes can produce three distinct kinds of melamins: two types of eumelanin, which are the predominant pigments found in dark skin and black hair, and pheomelanin, which is associated with the red hair/freckled skin phenotype. As melanosomes mature and their constituent proteins are delivered, the organelles themselves become cargos carried by various molecular motors from the perinuclear area to the cell periphery (14, 15), after which they are transferred to neighboring keratinocytes.

The type(s) of melanin produced depends on the function of melanogenic enzymes and the availability of substrates. Biosynthesis of melanin depends on TYR, and mutations disrupting TYR function result in an inherited pigmentary disorder known as albinism. TYR performs the critical rate-limiting activity of hydroxylation tyrosine to l-3,4-dihydroxyphenylalanine (DOPA), which is rapidly converted to DOPAQuinone. If cysteine is available, it will stoichiometrically react with nascent

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The abbreviations used are: LRO, lysosome-related organelle; ACTH, adrenocorticotropic hormone; ASP, Agouti signal protein; DCT, DOPAchrome tautomerase; DKK, Dickkopf; DHI, 5,6-dihydroxyindole; DHICA, DHI-2-carboxylic acid; DOPA, l-3,4-dihydroxyphenylalanine; EMI, epithelial-mesenchymal interactions; EMT, epithelial-mesenchymal transitions; BFGF, basic fibroblast growth factor; HGF, hepatocyte growth factor; HOX, homeobox; MC1R, melanocortin 1 receptor; MITF, microphthalmia transcription factor; αMSH, α-melanocyte-stimulating hormone; NGF, nerve growth factor; POMC, pro-opiomelanocortin; SCF, stem cell factor; TYR, tyrosinase; UV ultraviolet radiation.
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DOPAquinone to yield 3- or 5-cysteinyldopas, which then oxidize and polymerize, giving rise to yellow-red soluble melamins known collectively as phaeomelans (16, 17). As intramelanosomal cysteine is depleted, the excess DOPAquinone spontaneously cyclizes to form an orange intermediate known as DOPAchrome. The carboxylic acid of DOPAchrome will be spontaneously lost generating 5,6-dihydroxyindole (DHI), which rapidly oxidizes and polymerizes to form dark brown/black, high molecular weight insoluble polymers, known as DHI-melanin. However, if DOPAchrome tautomerase (DCT) is present, DOPAchrome will tautomerize without losing its carboxylic acid group to form DHI-2-carboxylic acid (DHICA), which can oxidize and polymerize to form yet a third type of melanin, known as DHICA-melanin, which is a lighter brown color, moderately soluble and of intermediate size (18). Human skin normally contains mixtures of all three types of melamins, and the ratio of those in part determines visible pigmentation (19).

Developmental Considerations

Melanoblasts originating in the neural crest must develop, migrate to appropriate sites, survive, differentiate to melanocytes, and then function to produce normal pigmentation patterns. A large number of genes (>25) are known to be involved in those processes, mutations in which cause developmental pigmentary diseases. In addition to genes expressed by melanocytes, signaling factors originating from adjacent tissues play critical roles in guiding those processes. Epithelial-mesenchymal interactions (EMI) refer to proximate paracrine or juxta-crine cross-talk between stromal fibroblasts and tissue epithelia and differ from epithelial-mesenchymal transitions (EMT), which refer to the transdifferentiation of epithelial cells to a fibroblast-like phenotype. EMI as well as EMT is required for the development of various organs; the key signaling pathways involved in EMI include homeobox (HOX), fibroblast growth factors, sonic hedgehogs, Wnt/β-catenin/Lef1, and bone morphogenesis proteins. EMI also play crucial roles in skin development and in melanocyte development/function as well. HOX genes influence the normal development of skin appendages, the pigimentary system, and stratified epidermis during embryogenesis. Transgenic and knock-out mice studies reveal that not only HOX genes but also the Wnt/β-catenin/Lef1 signaling pathways are involved in melanoblast development. Taken together, EMI are indispensable for site-specific organogenesis including liver, lung, kidney, peripheral nerves, digits, skin, and skin appendages. Readers are referred to recent reviews on this topic for further information (6–8, 20).

Regulation of Constitutive Skin Pigmentation

Skin colors in humans range from extremely fair/light to extremely dark depending on racial/ethnic background, but the density of melanocytes in a given area (e.g. the back or arms) is virtually identical in all types of skin (21). Keratinocytes in fair skin tend to cluster their poorly pigmented melanosomes above the nuclei, whereas in dark skin the heavily pigmented melanosomes are distributed individually in keratinocytes, thus maximizing their absorption of light (shown schematically in Fig. 1). There is a large intra-individual variation in melanocyte density in different areas of the body, e.g. the difference between skin on the palms/soles compared with other areas of the body. Constitutive melanocyte density in the skin can be affected by the environment, e.g. by chronic ultraviolet radiation (UV) (which can increase melanocyte density by 3- or 4-fold) or by toxic compounds such as hydroquinone (which can selectively and permanently destroy melanocytes in the skin). Inherited pigmentary disorders can also result in increased melanocyte density (e.g. freckles) or in decreased melanocyte density (e.g. vitiligo). (An excellent resource for pigmentary genes, their functions, and their involvement in pigmentary diseases can be found at ifpcs.med.umn.edu/micemut.htm.)

Epidermal melanocytes proliferate slowly, if at all, under normal circumstances, and they are quite resistant to apoptosis because of their high expression of Bcl2 (22). Melanocyte density and differentiation is influenced by the environment, including UV and factors secreted by neighboring keratinocytes and fibroblasts (shown schematically in Fig. 2). For example, it was recently shown that fibroblasts in the dermis of the palms/soles secrete high levels of DKK1, which suppresses melanocyte growth and function by inhibiting the Wnt/β-catenin signaling pathway (23, 24). DKK1 inhibition of Wnt signaling in melanocytes dramatically inhibits the melanogenic pathway, ranging from effects on transcriptional regulators (such as MITF) to downstream melanogenic proteins. DKK1 also affects keratinocytes in overlying epidermis, reducing their uptake of melanin and inducing a thicker less pigmented skin phenotype.4 The dermis in adult skin retains the expression patterns of HOX genes (25), which regulate patterning in primary and

Facultative skin pigmentation is the term coined for increased skin color due to some type of physiological regulation. Many factors regulate constitutive skin color, the most obvious of them being UV in what is commonly termed the tanning reaction (28, 29). Recent studies have outlined the complex kinetics of responses of the skin to UV radiation, which result in tanning over the course of several weeks (30).

UV radiation is the most significant factor influencing human skin pigmentation. As a direct effect of UV, especially UVA, immediate pigment darkening occurs within minutes and persists for several hours followed by persistent pigment darkening, which occurs within several hours and lasts for several days (31). These rapid increases in pigmentation do not result from acute melanin synthesis but rather from the oxidation and polymerization of existing melanin and the redistribution of existing melanosomes. Delayed tanning also occurs several days after UV exposure but takes longer because it involves the activation of melanocyte function. Exposure to UV leads to increased expression of MITF (the master transcriptional regulator of pigmentation) and its downstream melanogenic proteins, including Pmel17, MART-1, TYR, TRP1, and DCT (30, 32), leading eventually to increases in melanin content. Increased levels of PAR2 in keratinocytes also result from exposure to UV, which increases uptake and distribution of melanosomes by keratinocytes in the epidermis (33).

Epidermal melanocytes and keratinocytes also respond to UV exposure by increasing their expression of αMSH and ACTH, which up-regulates the expression and function of MC1R and consequently enhances melanocyte responses to those melanocortins. Variants of MC1R that function weakly are found in individuals with red hair and fair skin who contain predominantly pheomelanin and have a relative inability to tan. Endothelin 1 expression by keratinocytes is also increased by UV and enhances the expression of MC1R, although it works through its own receptor (EDNRB) on melanocytes. Interleukin-1 secretion by keratinocytes is also elicited by UV and it stimulates the secretion of ACTH, αMSH, endothelin 1, and bFGF by keratinocytes. Other melanogenic factors produced by keratinocytes in response to UV include SCF and NGF. The tanning response also relies on stimulation of secretion of NGF by keratinocytes, which prevents melanocyte apoptotic cell death following UV exposure (34). Stimulation of p53 in keratinocytes by UV increases expression of the POMC gene, leading to increased secretion of αMSH and stimulation of MC1R function in neighboring melanocytes (35). UV can also affect fibroblasts in the dermis; growth factors secreted from those cells in response to UV include HGF, bFGF, and SCF, all factors that stimulate pigmentation via their receptors on melanocytes (36). Retinoic acid up-regulates the differentiation (i.e. melanogenesis) and proliferation of mammalian melanocytes (37), an effect that seems to be mediated through increased expression of melanocortin receptors.

**Role of Melanin in Photoprotection of the Skin**

Lightly pigmented skin has a dramatically increased risk of skin cancers, including melanomas, much higher (15–70-fold) than in darker skin (38, 39). Because skin pigmentation is primarily regulated by the MC1R, its gene is considered a susceptibility gene for melanoma (40).

UV is harmful to human skin because of its production of various types of cellular damage, most notably oxidative dam-
age and two major types of DNA damage: cyclobutane pyrimidine dimers and 6,4-photoproducts (41). Such molecular lesions have significant long-term effects on tissue if not repaired quickly and correctly. There is increasing evidence that DNA damage/repair itself can induce skin pigmentation. Small DNA fragments, such as thymine dinucleotides, enhance pigmentation of melanocytic cells and can stimulate TYR mRNA levels and responses to MSH (42). p53, which regulates the cell cycle and the repair of DNA damage, as well as the induction of apoptosis (32), can also up-regulate POMC/MSH expression by keratinocytes in response to UV, thereby inducing pigmentation (35).

The involvement of MC1R with UV induction of skin pigmentation is complex and is regulated at many levels (43). MC1R regulates melanocyte function primarily via MITF, which in turn regulates melanogenesis and dendricity. MITF expression is stimulated relatively quickly, and significant increases are seen within 1 day of UV exposure. The downstream targets of MITF, e.g. TYR, Pmel17, and DCT, respond more slowly and reach maxima from 1–3 weeks after UV exposure. It takes several weeks after UV exposure before significant increases in melanin synthesis or melanocyte density occur. In addition to its role in pigmentation, MC1R regulates many other properties of melanocytes, such as the activation of DNA repair and other anti-photocarcinogenic activities that are important for protection against the deleterious effects of UV (44). Although UV increases expression of melanogenic genes similarly in skin of different racial/ethnic groups (29), there are some significant differences including melanin redistribution, protection against DNA damage, and induction of apoptosis in melanin-containing keratinocytes (21, 45). UV stimulates the transfer of melanin from the lower epidermis upward and prevents DNA damage in the lower epidermis more significantly in dark skin than in fair skin (29, 45). UV induces significantly more apoptosis in dark skin than in fair skin, which suggests a more efficient removal of UV-damaged cells; this may play a role in the decreased photocarcinogenesis of darker skin.

**Disrupted Regulation of Skin Pigmentation**

The regulation of skin pigmentation sometimes goes awry, leading to pigmentary disorders of many types. Intracellular pH is an important consideration to the regulation of TYR function (46), not only because intramelanosomal pH dramatically affects catalytic functions but also because a correct pH gradient is critical to the sorting pathway responsible for delivery of melanosomal proteins (47). There are many instances where normal levels of functional TYR are produced by melanocytic cells and yet little or no melanin is formed; it is even thought that intracellular pH plays an important role in regulating pigment production in various types of skin according to racial/ethnic origin (48).

TYR function is also regulated by proteasome activity. This occurs during normal pigmentation and is possibly regulated by intracellular levels of fatty acids, but it becomes especially important in the degradation of mutant TYR that occurs in some forms of albinism. The endoplasmic reticulum-associated degradation system is exquisitely sensitive to almost any small perturbation in TYR structure or its chaperone-like protein Tyrp1, leading to extensive hypopigmentation of tissues. Those interested in the mechanisms of hypopigmentation are referred to a recent review of the topic (49).

All forms of albinism result from the dysfunction of TYR and/or other melanogenic proteins, which leads to impaired pigmentation of the skin, hair and eyes (50). By its nature, only pigmented tissues are affected; to date, five types of albinism have been defined that map to five distinct pigment-related loci. Mutations in any of those genes impact TYR activity either directly or indirectly: oculocutaneous albinism (OCA) type 1 (TYR) and OCA3 (TYRPI) by leading to proteasomal degradation of TYR, OCA2 (P) and OCA4 (MATP) by disrupting the sorting of functional TYR to melanosomes. OA1 (OA1) impairs melanosome biogenesis and pigmentation by an as yet unknown mechanism and thereby disrupts the production of melanin (51).

The biogenesis of melanosomes is closely related to the biogenesis of LROs. Mutations that affect LRO formation and/or function usually also affect pigmentation of melanocyte-containing tissues. The most obvious of these conditions is Hermansky-Pudlak syndrome (52), which has pleiotropic clinical effects (8). So far, eight distinct types of Hermansky-Pudlak syndrome have been identified, and all map to genes encoding proteins critical to protein trafficking (53). However, more than 15 such genes have been identified in mice, so ultimately it is expected that several more forms of Hermansky-Pudlak syndrome in humans will be identified. The functional analysis of those genes is providing tremendous insights into trafficking mechanisms of proteins in general (54).

Acquired melanin pigmentation disorders can involve a lightening or darkening of the skin. Diminished skin color most commonly results from decreases in epidermal melanin content, e.g. leukoderma and hypopigmentation are caused by defects in melanin formation (reviewed in Ref. 8). The absence or loss of melanocytes is another mechanism of skin lightening, e.g. as found in vitiligo. In contrast, darkening of the skin may result from an increased number of melanocytes that produce excessive amounts of melanin (epidermal melanocytosis, lentigines) or increased amounts of melanin produced by a normal population of melanocytes (epidermal melanosis, freckles). Alternatively, skin darkening can result from abnormal distribution of melanin (e.g. dermal melanosis, pigmentary incontinence). Up-regulation of the melanogenic paracrine cytokine network is intrinsically involved in several types of acquired hypermelanoses (e.g. lentigo senilis and UVB melanosis).

**Approaches to Regulating Skin Pigmentation**

The regulation of human skin pigmentation has been a long-standing goal for cosmetic and pharmaceutical applications. It has implications regarding social standing, cosmetic appearance, and of course photoprotection of the skin against cancer and photoaging. A number of approaches to stimulate pigmentation have been tried, including activation of MC1R by agonists and bioactive derivatives, topical application of factors that bypass the MC1R, factors to stimulate TYR function, fac-
tors to increase melanosome transfer, etc. Most of those experiments have met with limited or no success, in part because of the challenge in penetrating the skin barrier and in part because of the quest for specificity, i.e. to stimulate melanocyte function without affecting other types of cells in the skin. Interested readers are referred to a recent review examining approaches to up-regulating skin pigmentation (55).

Conversely, inhibition of skin pigmentation is also a goal in many cultures, approaches to which include inhibition of MC1R function and disruption of TYR activity, for example. Again, effective agents have had limited success, at least in providing reversible inhibition of skin pigmentation. Readers interested in approaches to down-regulating skin pigmentation are referred to a recent review of this topic (49).

In summary, pigmentation of human skin has dramatic consequences at a variety of distinct levels, such as social attraction and protection from the environment. The skin is responsive to many factors that regulate its structure and appearance in an extremely complex manner.

REFERENCES
