



The physiology of melanin deposition in health and disease



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Abstract Eumelanin is the major pigment responsible for human skin color. This black/brown pigment is localized in membrane-bound organelles (melanosomes) found in specialized cells (melanocytes) in the basal layer of the epidermis. This review highlights the steps involved in melanogenesis in the epidermis and the disorders in skin pigmentation that occur when specific steps critical for this process are defective. Melanosomes, which contain tyrosinase, a major enzyme involved in melanin synthesis, develop through a series of steps in the melanocyte. They are donated from the melanocyte dendrites to the surrounding keratinocytes in the epidermis. In the keratinocytes, the melanosomes are found singly or packaged into groups, and as the keratinocytes move upward in the epidermis, the melanosomes start to degrade. This sequence of events is critical for melanin pigmentation in the skin and can be influenced by genetic, hormonal, and environmental factors, which all play a role in levels of melanization of the epidermis. The effects these factors have on skin pigmentation can be due to different underlying mechanisms involved in the melanization process leading to either hypo- or hyperpigmentary disorders. These disorders highlight the importance of mechanistic studies on the specific steps involved in the melanization process.

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Introduction

Pigmentation of the skin depends on the synthesis and deposition of the pigment melanin in the epidermis by melanocytes. Melanin, a complex biopolymer, is formed in specialized membrane-bound organelles, melanosomes, present in melanocytes.^{1–6} Melanocytes are derived from precursor cells, melanoblasts, which develop in the neural crest during early embryonic development.^{1,3,6} The melanoblasts

subsequently migrate from the neural crest in the developing organism to the skin, the hair bulb, the eyes, and the leptomeres where they further differentiate into melanocytes (Figure 1).^{1–3,6} In the skin, the melanoblasts are localized to the dermis where they differentiate into melanocytes during weeks 10 to 12 of development.^{1,3,6} The melanocytes then secondarily migrate to the epidermis and localize at the dermal/epidermal junction by weeks 12 to 14 of development (Figure 1).^{1,3,6} The migration of melanoblasts from the neural crest to the dermis of the skin, their differentiation into melanocytes and then their movement to the epidermis are critical factors in the determination of visible pigmentation in the skin.^{1,3,6} Also

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critical for this process is the functional interaction of the melanocytes with the surrounding keratinocytes in the epidermis and the transfer of melanin from the melanocytes to the keratinocytes (Figure 1).^{1-4,6} Deficiencies or irregularities in any one of these critical steps can lead to a number of different pigmentary disorders, which can be the result of decreased or increased melanization of the epidermis. Under certain circumstances, pigmentary change may result from the presence of melanin in the dermis. This review highlights these important physiologic processes.

The physiology of the melanocyte

Melanocytes in the basal layer of the epidermis, though they are solely responsible for production of all the melanin found in the epidermis, comprise less than 1% of the cells present in the epidermis.^{5,7} Among the cells in the epidermis, the melanocytes are highly unique in that they not only produce a membrane-bound organelle containing melanin, the melanosome, but they are also involved in the transfer of this organelle to the surrounding keratinocytes, which results in the deposition of melanin in the epidermis.^{4-6,8-10} The functional interaction between the melanocytes and keratinocytes is of major importance in skin pigmentation. Each melanocyte is surrounded by approximately 36 keratinocytes, which form what has been termed the epidermal melanin unit; this is the fundamental integrated multicellular unit that is responsible for melanin pigmentation in the skin.^{10,11} Within this unit, the tips of the dendrites from the polydendritic melanocytes make contact with the surrounding keratinocytes (Figure 2).^{4,9,10} E-cadherin, which is a member of a family of transmembrane proteins mediating cell-cell adhesion, is expressed on the cell surfaces of both melanocytes and keratinocytes where it functions as a major cell adhesion molecule between these cells.¹² This interaction is unlike that present between individual keratinocytes, which make contact with each other via desmosomes.¹³

Of the two main types of melanin present in the skin and hair of humans, eumelanin (brown/black melanin) and pheomelanin (yellow/red melanin), eumelanin is the major melanin present in melanosomes in melanocytes in the skin.^{1,2,4,6,11,13-15} Depending on the skin phototype (I-VI based on the response to ultraviolet [UV] exposure), pheomelanin may also be present.^{1,2,6,15,16} The biogenesis of melanosomes is a multistep process, which involves unique sorting and trafficking pathways.^{1,2} The initial steps involve the synthesis of tyrosinase in the rough endoplasmic reticulum, where it is packaged into vesicles and transported to the Golgi network.^{1,2,6,17} In the Golgi, further sorting and processing is carried out and vesicles containing tyrosinase and enzymes involved in formation of melanin bud off of the Golgi. These vesicles develop into the first of the four stages involved in the maturation of the melanosome (Figure 1).^{1,2,6,11,17} Stage I melanosomes are round spherical vesicles that contain

tyrosinase, tyrosinase-related proteins, and an amorphous matrix (Figure 1).^{1,2,6,11} The exact pathway for formation of these stage I melanosomes is not completely understood. Stage I melanosomes develop into stage II melanosomes as they elongate and a pigment cell-specific protein, PMEL, a main component of stage II melanosomes, organizes into parallel, proteinaceous fibrils, which are mediated by amyloid-related interactions (Figure 1).^{1,2,6,11} In stage III melanosomes, melanin synthesis is initiated by tyrosinase via the oxidation of L-tyrosine to dopa (3,4-dihydroxyphenylalanine) and dopa to dopaquinone.^{1,2,18,19} These steps are common to synthesis of both eumelanin and pheomelanin after which these two biosynthetic pathways diverge.^{1,2}

Eumelanin is deposited on the fibrils in stage III melanosomes (Figure 1).^{1,2,6,11} In stage IV melanosomes, melanin deposition in the melanosome is completed and occludes the internal structure of the melanosome (Figures 1 and 3).^{1,2,6,11} During these stages in melanosome development, additional proteins involved in this process are imported into the melanosomes.^{1,2,5,6,17,20} These stages of development apply mainly to melanosomes in which eumelanin is synthesized.⁶ There are slight differences in maturation of melanosomes containing pheomelanin, mainly in the spherical nature of the mature melanosome and the lack of an internal fibrillar structure.⁶ Production of melanin in melanocytes in the epidermis can be assessed by examination of tyrosinase activity in the melanocytes using histochemical analyses (Figure 2).^{21,22}

As the melanosomes mature, they are transported by motor proteins (kinesins) along microtubules from the perinuclear area to the tips of the melanocyte dendrites (Figure 1).^{4,17,23} Rab27a, a GTPase on the melanosome surface, recruits the adaptor protein melanophilin, which in turn recruits the actin motor protein, myosin-Va to the melanosome.^{17,24-27} Myosin-Va then associates with the peripheral actin network in the tip of the melanocyte dendrites dispersing the melanosomes (Figure 1).^{17,24-28} This dynamic interaction between myosin-Va and the melanosome via melanophilin, enables the association of melanosomes with actin filaments in the tips of the melanocyte dendrites.²⁴⁻²⁶ Critical to visible pigmentation in the skin is the transfer of these melanosomes from the melanocyte dendrites to the surrounding keratinocytes (Figure 1). If this process is interrupted or not completed, melanin will not be deposited into the keratinocytes, and the skin will have very much diminished color; hence, the uniqueness of the human skin pigmentary system.

Deposition of melanin in the epidermis

Exactly how melanin is deposited into the keratinocytes in human skin is still not completely understood. Both the melanocytes and the keratinocytes actively participate in this process. There are a number of theories and models regarding how this occurs (Figure 4).^{4,29,30} Insights into this process have been gained from both light and electron microscopic

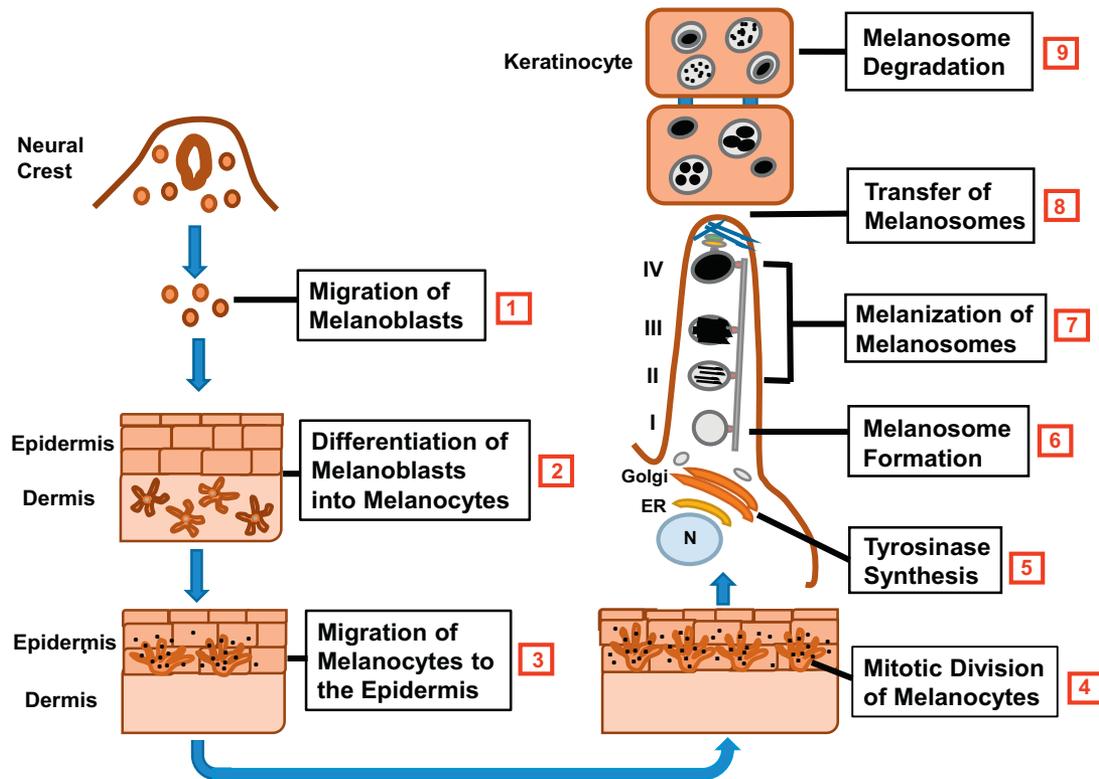


Fig. 1 Stages in melanocyte development, melanosome formation and melanization, and melanin transfer to keratinocytes. Melanoblasts develop in the neural crest and migrate to the dermis of the skin (1). In the dermis, the melanoblasts differentiate into melanocytes (2). These melanocytes then migrate to the epidermis (3) where they can undergo mitotic division (4). Within the melanocyte, tyrosinase is synthesized by the rough endoplasmic reticulum and transported to the Golgi complex where it is modified (5). Tyrosinase and tyrosinase-related proteins bud off as vesicles from the Golgi and then further develop into stage I melanosomes (6). Melanization of the melanosomes takes place and stage II, III, and IV melanosomes form (7). The melanosomes are transported along microtubules via the motor protein kinesin to the tip of the melanocyte dendrite, where the melanosome, via Rab27a and melanophilin, interacts with myosin-Va, which associates with the peripheral actin network in the melanocyte dendrite. Transfer of melanosomes from the melanocyte to the surrounding keratinocytes takes place (8). As the keratinocytes differentiate and move upward in the epidermis to the stratum corneum, the melanosomes start to degrade (9). In hypopigmentary disorders of the skin, there are deficiencies in different aspects of the processes described above as follows: piebaldism, improper migration of melanoblasts from the neural crest (1) and/or failure to differentiate into melanocytes (2); albinism, defects in tyrosinase synthesis (5) and failure of melanization of melanosomes (7); Hermansky-Pudlak syndrome, defects in melanosome biogenesis (6); Griscelli syndrome, failure of transfer of melanosomes to keratinocytes (8); and pityriasis alba, deficiency in transfer of melanosomes to keratinocytes (8). In vitiligo, the areas of hypopigmentation are due to destruction or loss of melanocytes (not shown). In hyperpigmentary disorders of the skin, there are also deficiencies in several aspects of these processes as follows: postinflammatory hyperpigmentation of the skin, hyperplasia of epidermal melanocytes (4), increased melanogenesis (7), and increased transfer of melanosomes to the keratinocytes (8); melasma, increased tyrosinase activity (5), increased formation of melanosomes (6), increased melanization of melanosomes (7), increased transfer of melanosomes (8); and Addison disease, increased tyrosinase synthesis (5), increased melanin synthesis (7), increased transfer of melanosomes (8). In addition, several of the hyperpigmentary disorders can lead to melanin deposition in the dermis, such as postinflammatory hyperpigmentation, melasma, and pigmentary incontinence (not shown).

studies of human skin as well as studies in which melanocytes and keratinocytes have been cocultured and this process examined *in vitro*.^{8,31–40} In one model, known as the cytophagocytosis or dendritic phagocytosis model, the tip of the melanocyte dendrite makes contact with the keratinocyte and is pinched off and taken up by cytophagocytosis by the keratinocyte (Figure 4A).^{9,29,30} This model is supported by studies on coculture of melanocytes and keratinocytes in which this process was examined by both time-lapse video or digital microscopy^{8,31–35} and electron microscopy.^{32–35} In the coculture studies, the melanocyte dendrites have been shown to

make contact with the keratinocytes (Figure 5).^{32–35} According to this model, melanosome transfer occurs when the tip of the melanocyte dendrite is pinched off and taken up by phagocytosis by the keratinocyte (Figures 6 and 7). This process is aided by a band of actin filaments in the cytoplasm of the melanocyte just beneath the cell membrane (Figure 8).^{32,34}

Phagocytosis of the tip of the melanocyte dendrite by the keratinocyte is aided by a cortical band of actin filaments beneath the keratinocyte cell membrane (Figure 9).^{32,34} Breakdown of these bands of actin filaments in both the melanocytes and keratinocytes, by an agent, such as

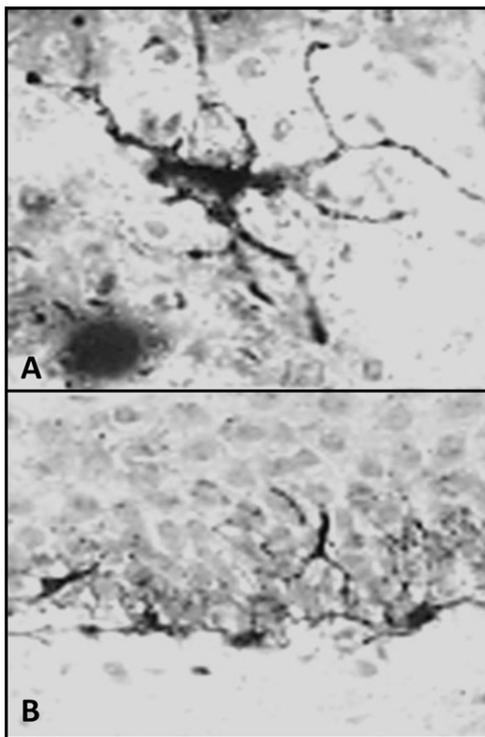


Fig. 2 Basal epidermal cells and melanocytes in the skin stained with dihydroxyphenylalanine (DOPA). (A) An epidermal sheet of skin from a guinea pig ear was obtained by separation of the epidermis from the dermis. After incubation with DOPA, tyrosinase in the basal melanocytes converts DOPA to black DOPA-melanin. (B) A vertical cross section through the skin showing the melanocytes in the basal layer of the epidermis making contact with the surrounding keratinocytes.

cytochalasin, leads to loss of melanosome donation from the melanocytes to the keratinocytes.^{32,34} This indicates that these cellular bands of actin filaments are needed for this autophagocytotic process to occur in these cells in culture.^{32,34} In an extension of this model, known as the filopodia-phagocytosis model, filopodia, which are narrow extensions of the cellular membrane, extend from the melanocyte and make contact with a keratinocyte.^{10,29,30} Melanosomes are transported along the filopodia, which is engulfed by the keratinocyte, and phagocytosis takes place with the melanosomes transferred to the keratinocytes.³⁶

In a second model, the membrane fusion or tunneling nanotube model, the tip of the melanocyte membrane fuses with the keratinocyte membrane and a tunneling nanotube or channel forms and serves as a conduit for transfer of melanosomes from the melanocyte to the keratinocyte (Figure 4B).^{9,29,30,37} These nanotubes contain actin, which is thought to be important in the transport process.^{30,37}

In a third model, the shedding-phagocytosis model, melanosomes are shed in membrane-bound vesicles from melanocytes.^{9,29,30} These vesicles then either fuse with the keratinocyte cell membrane, and melanosomes are transferred to the keratinocytes by phagocytosis or these vesicles are

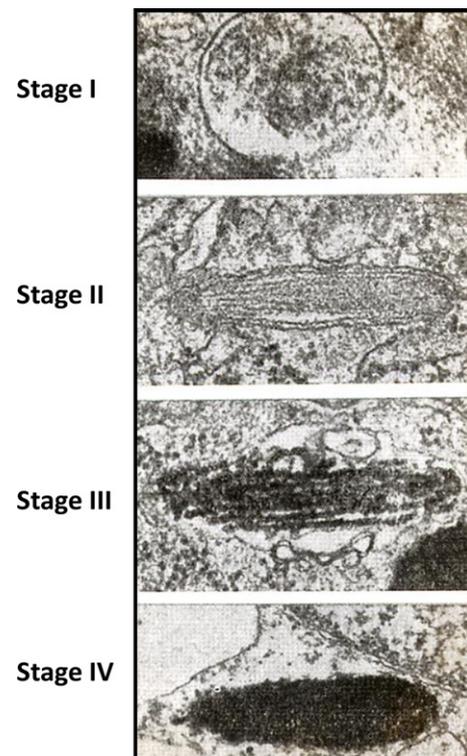


Fig. 3 Stages in the development of human melanosomes containing eumelanin. Stage I melanosomes are spherical vesicles with tyrosinase, tyrosinase-related proteins, and an amorphous matrix. Stage II melanosomes are elongate and develop proteinaceous fibrils. In stage III melanosomes, melanin is deposited on the fibrils. In stage IV of human melanosomes, melanin deposition is complete. (Courtesy of Dr. George Szabo).

present extracellularly and then secondarily internalized by the keratinocyte via phagocytosis (Figure 4C).^{9,29,30,38,39}

A fourth model for melanosome transfer, the exocytosis/endocytosis model, proposes a mechanism in which individual melanosomes are exocytosed by the melanocyte dendrite and endocytosed by the keratinocytes (Figure 4D).^{9,29,30} This model is supported by electron microscopic studies of human skin samples and studies from cocultures of melanocytes and keratinocytes.⁴⁰

As a result, a number of different mechanisms for transfer of melanosomes from melanocytes to keratinocytes have been proposed, which provide explanations on how melanin present in melanocytes is deposited in keratinocytes in the epidermis. There are studies that support each of these models. Deposition of melanin in the epidermis is necessary and critical for the coloration seen in the skin. Whether one or more of the mechanisms proposed is used in human skin pigmentation and whether a particular set of circumstances could dictate the specific mechanism taking place is not known. This is an exceedingly interesting area that needs to be investigated further.

Once the melanosomes have been transferred to the keratinocytes, they localize in the keratinocytes either singly or in

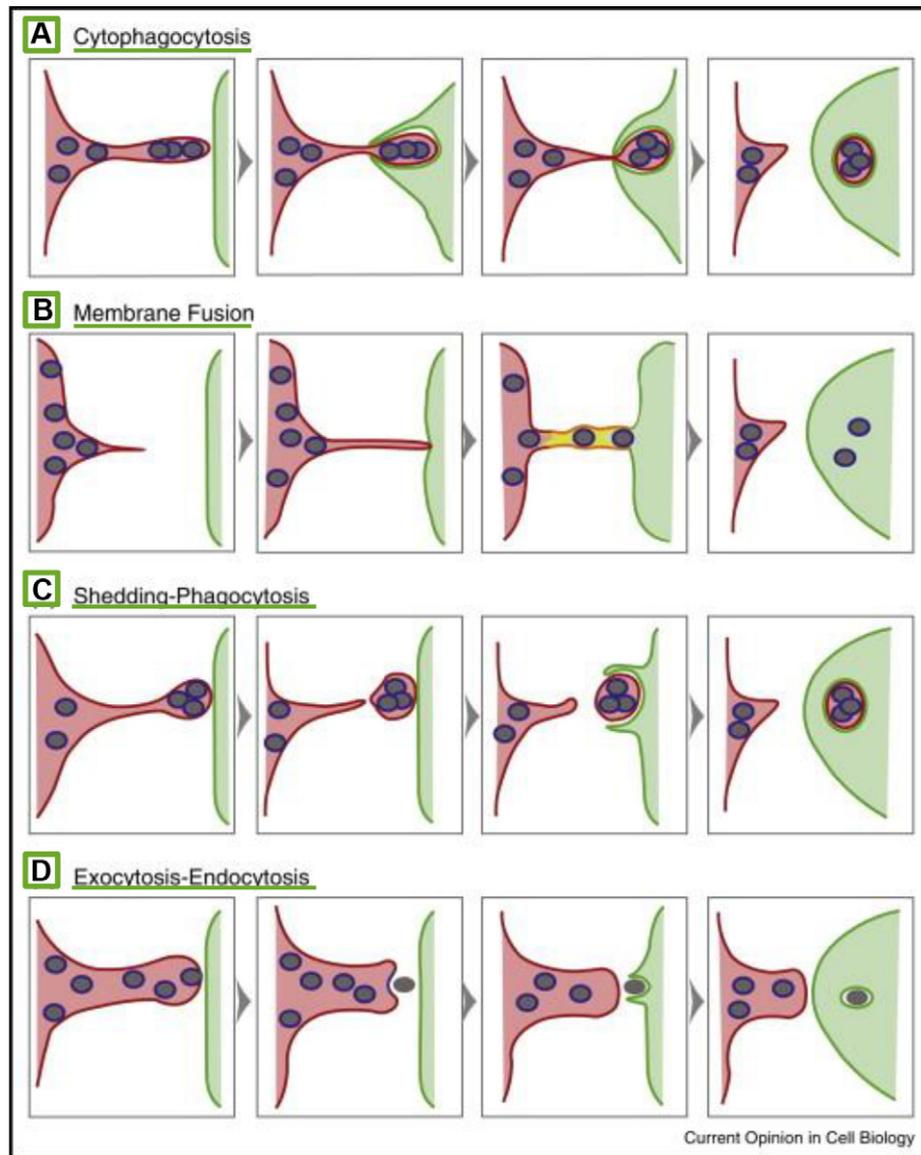


Fig. 4 Models for deposition of melanin in the epidermis. **(A)** In the cytophagocytosis or dendritic phagocytosis model, the tip of the melanocyte dendrite is pinched off and taken up by cytophagocytosis by the keratinocyte. **(B)** In the membrane fusion or tunneling nanotube model, the tip of the melanocyte membrane fuses with the keratinocyte membrane and a tunneling nanotube serves for transfer of melanosomes from the melanocyte to the keratinocyte. **(C)** The shedding-phagocytosis model proposes that melanosomes are shed in vesicles from the melanocyte and taken up by phagocytosis into the keratinocytes. **(D)** The exocytosis/endocytosis model proposes that individual melanosomes are exocytosed by the melanocyte dendrite and endocytosed by the keratinocytes. (Reproduced with permission from Wu X, Hammer JA. Melanosome transfer: it is best to give and receive. *Curr Opin Cell Biol* 2014;29:1-7. Copyright, Elsevier).

groups surrounded by a membrane (Figure 10).^{11,41-44} This is under genetic control (as will be discussed below). As the keratinocytes differentiate and move upward in the epidermis through the stratum spinosum and stratum granulosum to the stratum corneum they transport the melanosomes with them, which results in melanin being present in keratinocytes throughout the epidermis. During this process, the melanosomes start to degrade.^{11,43-45} The degree to which this degradation occurs affects the level of melanin pigmentation in the skin.

The level of melanization of the skin (ie, visible pigmentation in the skin) is related to a number of critical biological processes (Figure 1), including:

- Migration of melanoblasts from the neural crest to the skin
- Differentiation of melanoblasts into melanocytes in the dermis
- Movement of melanocytes from the dermis to the basal layer of the epidermis
- Production of melanosomes in the melanocytes

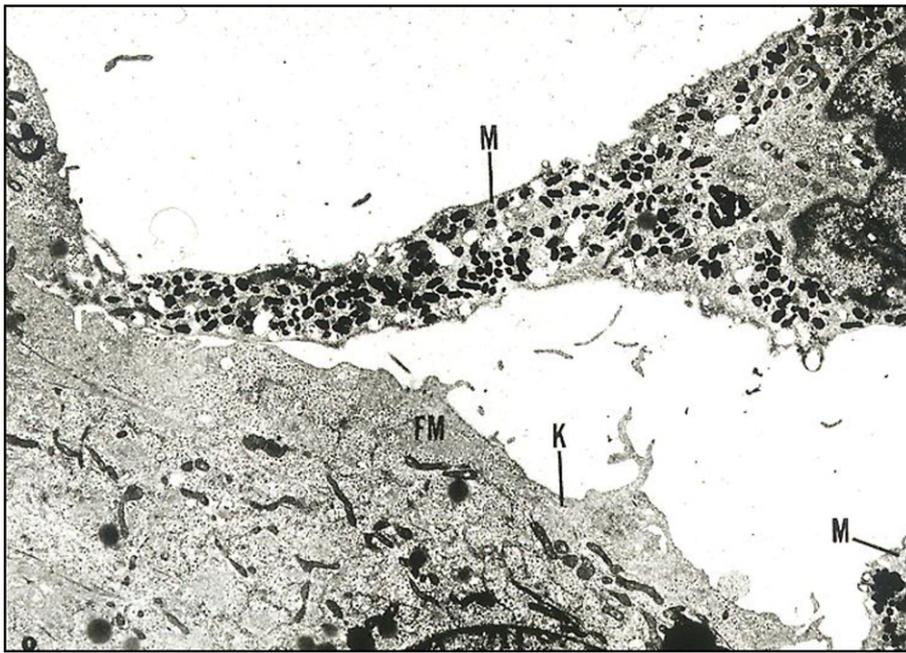


Fig. 5 Association of a melanocyte with a keratinocyte. Electron micrograph of a section of a melanocyte and keratinocyte from guinea pig ear skin in coculture. The tip of the melanocyte (M) dendrite is in contact with a keratinocyte (K). Microfilaments (FM) are present beneath the keratinocyte cell membrane. (Reproduced with permission from Wikswo MA, Szabo G. Effects of cytochalasin B on mammalian melanocytes and keratinocytes. *J Invest Dermatol* 1972;59:163-169. Copyright, The Williams & Wilkins Co.)

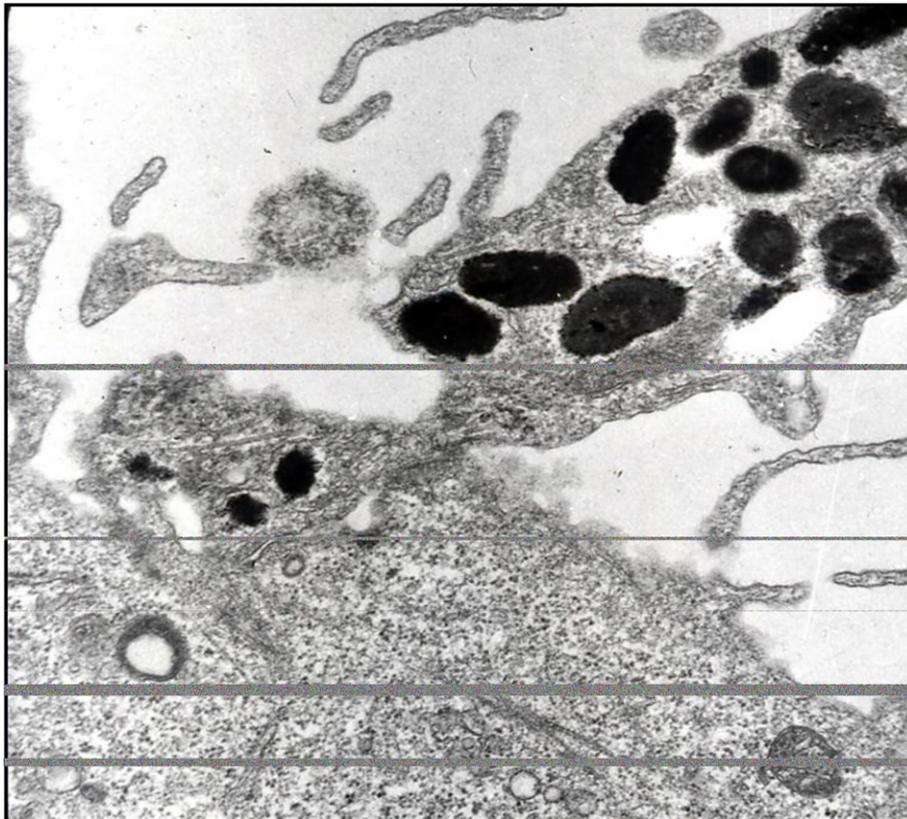


Fig. 6 Pinching off of the tip of a melanocyte dendrite in contact with a keratinocyte. Electron micrograph of a section of a melanocyte and keratinocyte in coculture obtained from guinea pig ear skin in coculture. The tip of the melanocyte dendrite is in contact with a keratinocyte and is being pinched off during phagocytosis by the keratinocyte.

- Melanization of melanosomes in the melanocytes
- Transport of the melanosomes to the tips of the melanocyte dendrites
- Transfer of melanosomes from the melanocytes to the keratinocytes
- Degradation of melanosomes within the keratinocytes

Disturbances in any one of these processes can lead to increased or decreased melanin pigmentation in the skin, as will be discussed below.

Variations in melanization of the skin

The levels and uniformity of melanization of normal human skin can vary over a wide range and these variations can be due to genetic, hormonal, or environmental factors. The intrinsic or constitutive coloration of the skin is mainly under genetic and hormonal control; more transient changes in levels of skin pigmentation (facultative skin pigmentation) are largely influenced by environmental factors, such as UV radiation (UVR) or by some type of physiologic regulation.^{5,6,46–48} The amount of melanin present in the keratinocytes is a significant factor in determination of skin pigmentation and is one of the main determinants of differences

in skin color between individuals¹¹; however, the differences in intrinsic skin pigmentation from dark to light that are observed among the various racial/ethnic populations are not due to differences in the number of melanocytes present in the epidermis.

The number of melanocytes in the basal layer of the epidermis, in any given area of the body, is roughly similar in Caucasian skin, compared with African/American skin or Asian skin.^{49–52} These differences in melanin pigmentation and the functional activities of the epidermal melanin units are under genetic control. Three of the major determinants in skin pigmentation include the following: (1) the nature of the deposition of melanosomes within the keratinocytes, (2) the degree of melanization of the melanosomes, and (3) the distribution and degradation of melanosomes in the keratinocytes.^{11,41–45}

In Caucasian skin, the melanosomes in the keratinocytes are predominantly arranged in groups of two to eight and surrounded by a membrane (Figure 10A).^{11,41–44} In African/American skin, single melanosomes are predominantly present in the keratinocytes rather than in groups surrounded by a membrane, as they are in Caucasian skin (Figure 10C).^{11,41–44} The melanosomes are also larger in size, and there are more Stage IV melanosomes; thus there is more melanin in these melanosomes.^{11,41–45} In Asian skin, the distribution of melanosomes in the keratinocytes is intermediate between African/American and Caucasian skin. The



Fig. 7 Tip of a melanocyte dendrite phagocytosed by a keratinocyte. Electron micrograph of a cross-section of a keratinocyte in coculture with melanocytes from guinea pig ear skin. The keratinocyte is phagocytosing the tip of the melanocyte dendrite which contains numerous melanosomes.

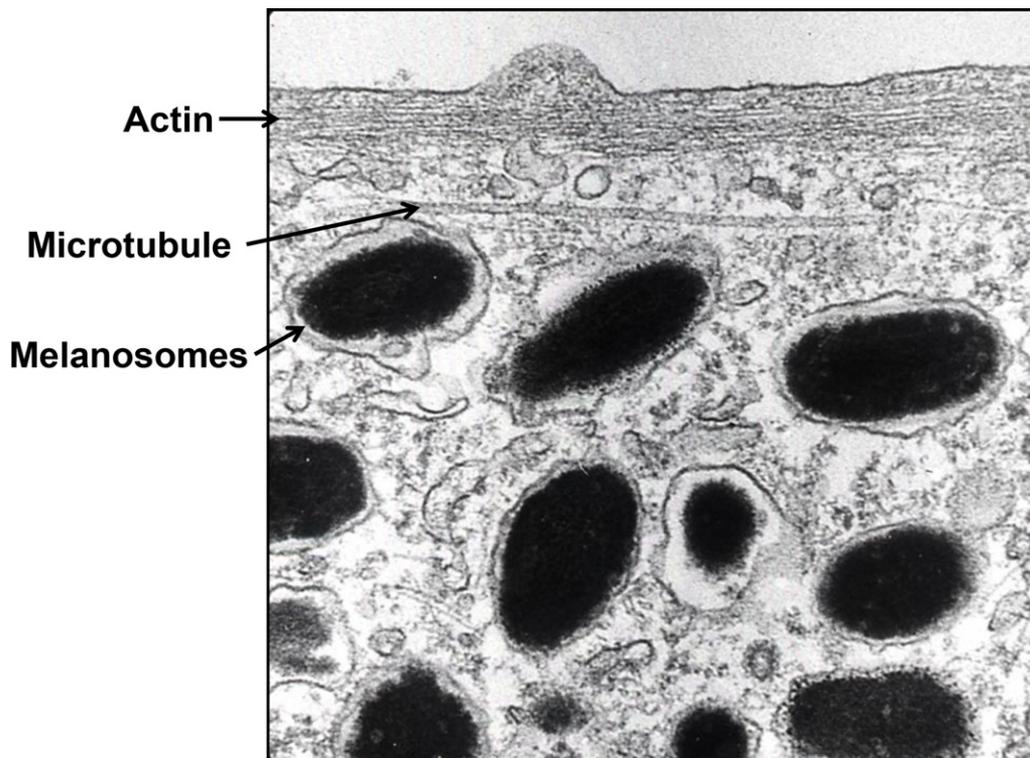


Fig. 8 Electron micrograph of a melanocyte dendrite. Section of a melanocyte dendrite in a coculture of melanocytes with keratinocytes obtained from guinea pig ear skin. Actin microfilaments are present beneath the melanocyte membrane surface. Melanosomes are aligned along a microtubule in the dendrite.

melanosomes are arranged in groups in the keratinocytes (Figure 10B) and are also individually distributed within the keratinocytes.^{11,41–45} Within each group of melanosomes, there may be fewer melanosomes than in Caucasian skin, but the melanosomes are larger in size.^{11,41–45}

Another factor important in skin pigmentation is the degree to which the melanosomes are degraded in the keratinocytes as they undergo terminal differentiation and migrate upward in the epidermis to the stratum corneum. In the epidermis of Caucasian skin, the melanosomes are generally completely degraded and are absent from the corneocytes in the stratum corneum.^{11,43–45} In African/American skin, there is less degradation of the melanosomes in the keratinocytes as they migrate upward through the epidermis to the stratum corneum.^{11,43–45} In Asian skin, the degree of degradation of melanosomes in the stratum corneum is intermediate between that found in African/American or Caucasian skin and individual melanosomes rather than clusters of melanosomes are more prominent in these terminally differentiated cells.^{11,43–45}

The major factors contributing to degree of melanization of the skin of individuals of different racial/ethnic backgrounds are:

- The nature of the deposition of melanosomes in the keratinocytes (eg, singly or in groups surrounded by a membrane)
- The size of the melanosomes in the keratinocytes

- The level of melanization of the melanosomes
- The degree of degradation of melanosomes in the upper layers of the epidermis

In individuals with dark constitutive skin pigmentation, the melanosomes in the epidermal keratinocytes are larger and contain more melanin, they are individually distributed in the keratinocytes and show less degradation in the upper layers of the epidermis and the stratum corneum than in individuals with light constitutive skin pigmentation.

Levels of pigmentation (melanization) in the skin can also be under hormonal control. One of the most important positive regulators of melanogenesis is the melanocortin receptor, in particular melanocortin type 1 receptor (MC1R), which regulates the quantity and quality of melanins produced.^{15,53–55} This receptor on the melanocyte cell surface is stimulated by the melanocortins, melanocyte stimulating hormone (MSH), and adrenocorticotropic hormone (ACTH), which upregulate the expression and function of MC1R.^{15,53–55} In skin, α -MSH is the predominant biologically active form of MSH, which can stimulate melanocyte activity; it has a heptapeptide core that is responsible for its melanogenic activity.^{1,2,5,15} It is produced by both keratinocytes and melanocytes and acts to exclusively enhance the synthesis of eumelanin.^{6,56–59}

When MSH binds to melanocortin receptors present on the melanocyte, it activates adenylyl cyclase, which leads to increased synthesis of cyclic adenosine monophosphate (cyclic

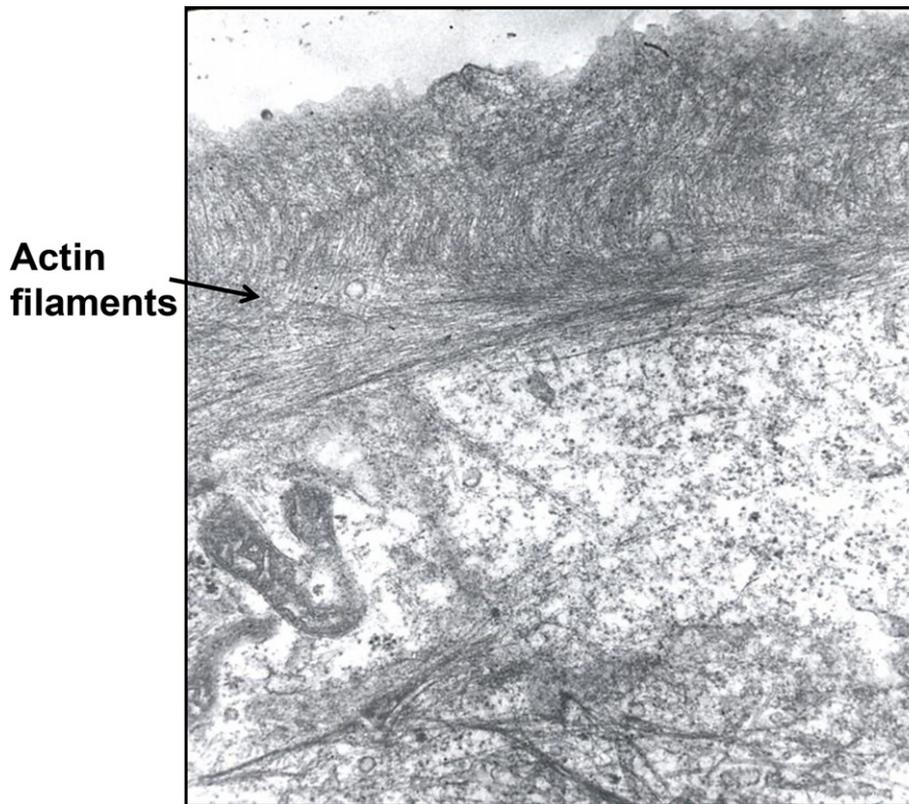


Fig. 9 Electron micrograph of a keratinocyte. Section of a keratinocyte in coculture with melanocytes from guinea pig ear skin. Actin microfilaments are present beneath the keratinocyte membrane surface. (Reproduced with permission from Wikswa MA, Szabo G. Effects of cytochalasin B on mammalian melanocytes and keratinocytes. *J Invest Dermatol* 1972;59:163-169. Copyright, The Williams & Wilkins Co.)

AMP).^{1,2,4,6,60} The increased levels of cyclic AMP modulate the expression of several melanocyte specific genes, including the gene coding for tyrosinase, leading to increased tyrosinase activity and stimulation of melanogenesis.^{1,2,4,6,60} This leads to increased production of melanosomes, increased deposition of melanin in the melanosomes, and increased transfer of melanosomes from the melanocytes to the keratinocytes.^{2,6,60} In support of this, increased transfer of melanosomes from melanocytes to keratinocytes has been observed, using time-lapse video microscopy, light microscopy, and electron microscopy, in cocultures of mammalian melanocytes and keratinocytes, which have been treated with cyclic AMP or MSH.^{33,61} MSH can additionally increase the number of melanocytes in the epidermis.^{1,2,4,6,60} ACTH, which has the same heptapeptide core present in MSH and is involved in melanogenic activity, has a similar effect on melanogenesis.^{6,60} Thus, stimulation of the melanocortin type 1 receptor on melanocytes in the epidermis by MSH as well as ACTH can upregulate melanogenesis and lead to enhanced darkening of the skin.

Environmental factors can have a significant effect on melanization of the skin. UVR, by both UVA and UVB, is one of the most important external factors influencing human skin pigmentation. It can lead to both immediate darkening of the skin, mainly by UVA, and to longer-term, persistent

pigmentation of the skin, mainly by UVB.^{5,6,14,46,47} The immediate darkening effects by UVA are due mainly to oxidation of existing melanin or melanin precursors and redistribution of the melanosomes in the keratinocytes from a perinuclear localization to a more general disbursement throughout the cell leading to more visible pigmentation.^{6,14,46}

Persistent pigment darkening of the skin and the longer-term effects involve activation of melanocyte function.^{5,6,14,46,47} UVR exposure leads to increased expression of the transcription factor MITF (microphthalmia-associated transcription factor), a transcriptional regulator of melanocyte function that has a number of protein targets, an important one of which is tyrosinase.^{5,6,46} Activation of tyrosinase and some of these other melanogenic proteins (ie, tyrosinase-related protein 1; DOPACHrome tautomerase) leads eventually to increases in melanin synthesis and content in the melanosomes and over an extended of time leads to increases in melanocyte density in the epidermis.^{1,2,5,6,46,62} Over a period of 4 to 5 weeks after exposure to UV, there is increased melanization of the melanosomes and increased cell division of melanocytes, which in turn leads to an increased number of melanosomes transferred to the keratinocytes.^{5,6,46} Under chronic UVR, the melanocyte density can increase by three- or fourfold leading to a significant

increase in melanin deposition in the epidermis and in darkening of the skin.^{5,6,46}

UV can also stimulate the production of α -MSH and ACTH in the skin.^{4-6,63} In response to UVB, keratinocytes and melanocytes are induced to increase expression of α -MSH and ACTH, which stimulate production and activity of the MC1R on melanocytes leading to increased production of eumelanin, enhanced melanogenesis, and darkening of the skin.^{5,6,64} This response to UV is a protective one, because melanin functions to absorb UV, which has harmful effects on skin and subcutaneous tissues.^{1,5,6,46,47} Melanin in the skin can scavenge free radicals and minimize the toxic effects of reactive oxygen species generated by UV and help protect cellular DNA, proteins and lipids from damage; thus, deposition of melanin in the epidermis has a photoprotective effect against sunlight.^{1,5,6,46,47}

Disorders in skin pigmentation

There are a number of important biological processes that are critical for normal pigmentation of the skin. Disturbances in any of these processes can result in pigmentary disorders, which include both increased and decreased melanization of the skin. These disorders are ultimately due to changes in the number or activity of melanocytes present in the basal layer of the epidermis, to altered synthesis of proteins/enzymes needed in the formation or melanization of melanosomes in the melanocytes, or to changes in the transfer of melanosomes from the melanocytes to the keratinocytes. Just a few of the disorders in which these processes are defective and result in either hypopigmentation or hyperpigmentation will be discussed.

Hypopigmentary disorders

Vitiligo is one of the most commonly acquired hypopigmentary disorders.^{28,65-71} It is characterized by loss of epidermal melanin in the skin, which is due to selective destruction of melanocytes in the basal layer of the epidermis.^{28,65-71} These areas are usually very well demarcated and depigmentation is complete. Loss or reduction in the number of melanocytes can result from defects in pigment cell differentiation, proliferation, migration, and/or survival.⁶⁵⁻⁷¹ This disorder is almost always acquired.^{12,67,70-73} The prevalence of vitiligo varies markedly; it has a prevalence of approximately 1% to 2% of the world population.^{65,66,71} The pigmented skin of vitiligo patients shows microdepigmentation and vacuolization of melanocytes and/or keratinocytes.⁷³ Ultrastructural studies show the absence of melanocytes in the vitiliginous lesions.⁷⁰ The etiology and underlying pathogenesis of vitiligo remain poorly understood and its progression is unpredictable.^{28,65,66,70,71}

Vitiligo is thought to be a multifactorial disorder involving the interplay of several factors. Several hypotheses have been

proposed as to the underlying mechanisms involved.^{12,65,67,68,70-73} Two of the main ones are the autoimmune response hypothesis and the autotoxic/metabolic hypothesis.^{12,65,67,70,72,73} The autoimmune hypothesis proposes that there is an autoimmune response against melanocytes leading to a loss of melanocytes.^{65,67,68,70-72,74-76} Autoantibodies to proteins associated with melanocytes, such as tyrosinase and tyrosinase-related proteins, may be present thus

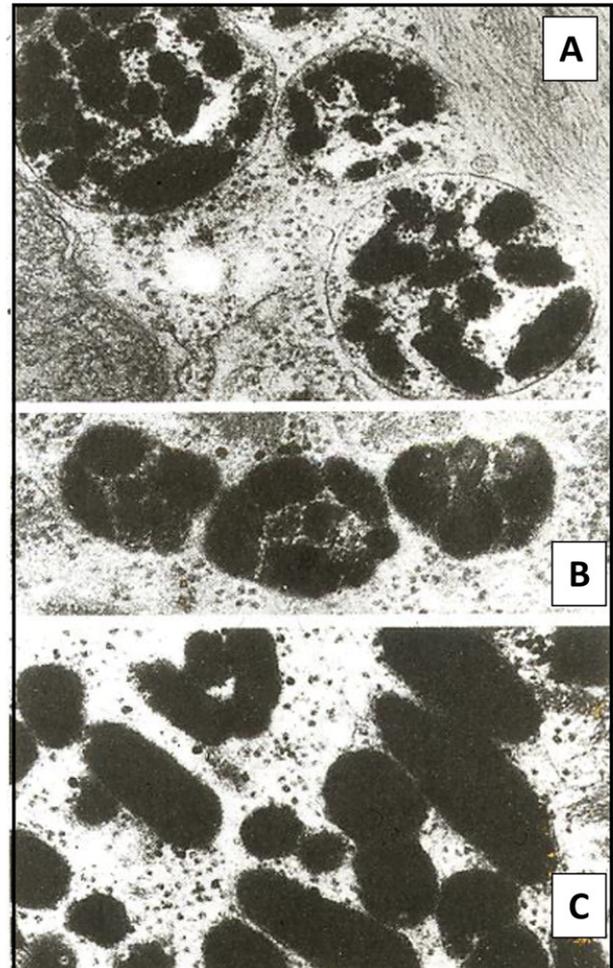


Fig. 10 Fate of melanosomes transferred from melanocytes to keratinocytes in skin from different racial groups. Electron micrograph of sections of the epidermis from human skin from different racial groups showing the differences in distribution of melanosomes transferred from the melanocytes into keratinocytes. (A) In Caucasian skin, melanosomes are arranged in groups of two or more and are surrounded by a membrane; (B) in Asian skin, melanosomes are arranged in groups in the keratinocytes. There are fewer melanosomes per group but they are larger in size than those in Caucasian skin; (C) in African/American skin, the melanosomes in the keratinocytes are predominately single rather than in groups. They are larger than those found in Caucasian or Asian skin and contain more melanin. Variations in these patterns are also observed. (Reproduced with permission from Szabo G, Gerald AB, Pathak MA, Fitzpatrick TB. Racial differences in the fate of melanosomes in human epidermis. *Nature* 1969;222:1081-1082. Copyright, Nature Publishing Group.)

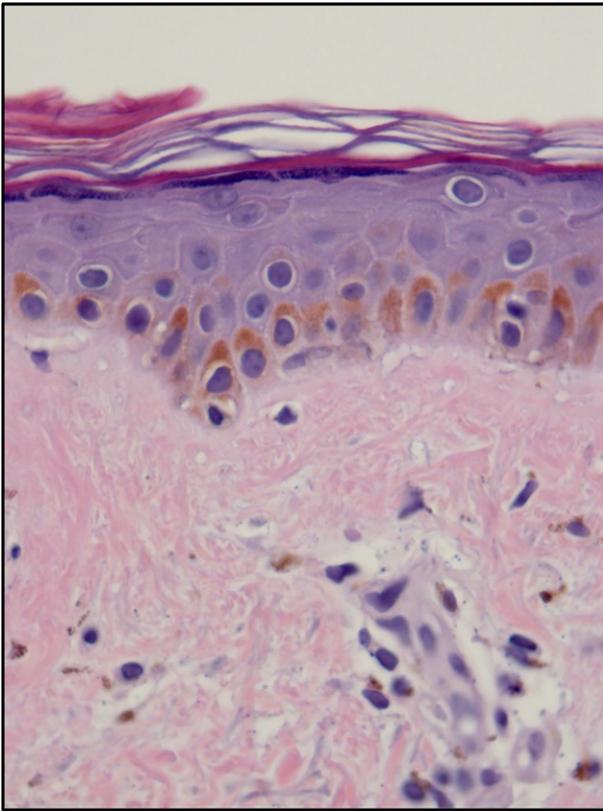


Fig. 11 Photomicrograph of melasma. Increased pigmentation in the skin is primarily in the keratinocytes in the basal layer (unstained; their natural color is brown) due to increased activity of the melanocytes, increased production of melanin and increased transfer of melanin to the keratinocytes. There is also a lesser amount of melanin pigment that began in the epidermal basal layer, but which is now located in the dermis (pigment incontinence) secondary to increased permeability of the dermal–epidermal interface due to inflammation (dermatitis) in the epidermis. Hematoxylin and eosin, $\times 380$.

leading to loss of function of the major enzyme involved in melanin synthesis and to death of melanocytes.^{69,77} A role for cellular immunity playing a role in vitiligo is further supported by evidence that specific cytotoxic T cells (CD8+ T cells) against tyrosinase and other melanocyte proteins are found in some vitiligo patients and are associated with the destruction of melanocytes.^{70,71,74–77}

The autotoxic/metabolic hypothesis proposes that intrinsic metabolic defects in nonlesional melanocytes lead to intracellular oxidative stress, which is an intracellular signal for melanocyte degeneration.^{67,70,72} Oxidative stress could also be generated by various extrinsic triggers, such as sunburn and exposure to toxic chemicals, which lead to melanocyte death.^{67,72} In support of this, individuals with vitiligo have been reported to have compromised antioxidant responses.^{67,72} In addition, recent studies suggest that E-cadherin, which mediates the adhesion between melanocytes and keratinocytes, is absent or deficient at melanocyte membranes and this abnormality is associated with detachment of

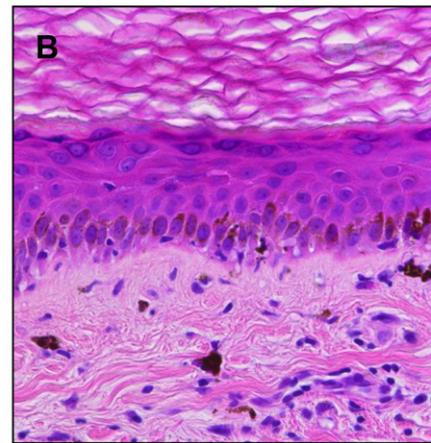
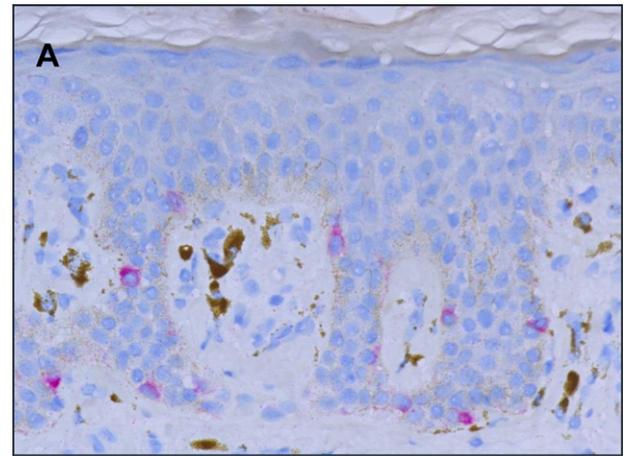


Fig. 12 Photomicrograph of pigment incontinence. (A) Pigment is observed in the dermis due to transfer of melanin (brown) from the epidermis into the papillary (superficial) dermis secondary to inflammation in the epidermis “dermatitis.” This inflammation makes the dermal–epidermal interface more permeable to melanin deposits, so they end up in macrophages (melanophages) in the papillary dermis (Melanin A, Mart 1, immunohistochemical stain, $\times 980$). (B) Progressive movement of melanin (seen as brown granules) into the superficial dermis is observed on the *right side* of the figure. Movement into the central and deeper parts of the dermis is seen on the *left and central parts* of this figure where it is found in melanophages. Hematoxylin and eosin, $\times 560$.

the melanocytes from the keratinocytes in the epidermis; this dissociation is accelerated after oxidative and mechanical stress.¹² This could thus be a factor in loss of melanocytes from the basal layers of the epidermis.¹² As a result, there are a number of factors that could be important in the pathogenesis of vitiligo and the loss of melanocytes in the epidermis. The various pathways proposed may not be mutually exclusive and may converge, ultimately leading to destruction or loss of melanocytes.

Albinism is a congenital disorder characterized by hypomelanosis in most normally pigmented tissues resulting from defects in melanin biosynthesis.^{4,70,78,79} There are several clinical types of albinism. In the most common type,

oculocutaneous albinism (OCA), there is reduced synthesis of melanin in melanocytes in the skin, hair, and eye.^{4,70,78,79} In OCA1, which is one of the most prevalent clinical phenotypes of OCA, deficiencies in melanogenesis are the result of loss of functional activity of tyrosinase due to mutations in the *TYR* gene that encodes tyrosinase.^{70,78,79} A large number of different mutations have been reported in the *TYR* gene in this disorder.^{70,78,83} Only Stage I and II melanosomes are present; internal matrix formation in the melanosomes is normal; however, loss of tyrosinase activity leads to failure of synthesis of melanin (Figure 1).⁷⁹ In different clinical phenotypes of OCA, however, tyrosinase is present indicating that there is a different cause for lack of melanin synthesis.⁷⁹ Cutaneous lack of pigmentation in the skin in OCA is mainly due to lack of melanization of the melanosomes. This loss of melanin in individuals with albinism can lead to massive solar damage and numerous UV light-related skin cancers.^{70,78,79}

Piebaldism is a congenital, autosomal-dominant disorder, characterized by a white forelock and leukoderma on the frontal scalp, forehead, ventral trunk, and/or extremities.^{78–80} The cutaneous lack of pigmentation in the ventral white patches and white forelock is due to a significant or complete loss of melanocytes in the epidermis and hair bulbs.^{78–80} This is caused by improper migration of melanoblasts from the neuronal crest in the embryo and/or their failure to differentiate into melanocytes (Figure 1).^{78–80} Loss of melanocyte formation in the skin is due to mutations in the *c-KIT* gene, which encodes a tyrosine kinase transmembrane receptor on the melanoblast surface.^{79,81–84} These mutations decrease the ability of the *c-KIT* receptor to be activated by the *c-KIT* ligand (stem cell factor, steel factor, or mast cell growth factor) expressed by epidermal keratinocytes and initiate a cascade of signaling events to upregulate melanoblast proliferation, migration, and/or differentiation into melanocytes during embryogenesis.^{78–80} In piebaldism, loss of melanocytes is caused by a loss-of-function in *c-KIT* receptors on melanoblasts, which results in failure of the melanoblasts to migrate to the skin and differentiate into melanocytes leading to loss of melanization of the skin.

Hermansky-Pudlak Syndrome is a rare, autosomal, genetically heterogeneous disorder in which individuals present with the clinical findings of OCA and are characterized by hypopigmentation of the skin, hair, and eyes accompanied by additional nonpigmentary findings (eg, bleeding diathesis, immune-deficiency, lung fibrosis).^{1,70,79,85,86} This is a disorder of melanosome biogenesis, which results from mutations in genes that encode proteins involved in membrane trafficking of melanosome related proteins to maturing melanosomes (Figure 1).^{1,70,79,85,86} Electron microscopy has shown that macromelanosomes as well as stage I to III melanosomes may be present, but not stage IV melanosomes.⁷⁰ These deficiencies in melanosome biogenesis contribute to the loss or reduction in pigmentation of the skin in individuals with Hermansky-Pudlak Syndrome.^{1,70}

Griscelli syndrome (GS) is a rare, autosomal-recessive disorder, which is characterized by diffuse pigmentary dilution in

the skin and silvery-gray hair, in addition to some nonpigmentary abnormalities (eg, neurologic impairment, immune abnormalities, and a hemophagocytic syndrome).^{2,70} This is due to melanosomes congregating in the center of the epidermal melanocytes as a result of failure of melanosomes to be transported within the melanocyte dendrites and transferred to the surrounding keratinocytes (Figure 1).^{2,70}

There are three types of GS (GS1, GS2, GS3) each one having a defect in a different gene involved in melanosome transport in the melanocyte.^{2,70,87–89} These genes code for one of the following three proteins: Rab27a, a GTPase on the melanosome surface; melanophilin, an adaptor protein that binds to Rab27a and in turn recruits a third protein, myosin-Va to the melanosome.^{2,17,24–27,70} Myosin-Va then binds to actin in the tip of the melanocyte dendrite linking the actin network to the melanosome.^{2,17,24–27,70} This linkage provides a dynamic interaction between the actin network and the melanosome and plays a key role in localization of melanosomes to the tip of the melanocyte dendrite and their transfer from the melanocyte to the keratinocyte (Figure 1).^{2,17,24–27,70} In the absence of any one of these proteins, melanosome transfer to the keratinocytes does not take place. The cellular deficiencies present in GS, thus show the importance of transport of melanosomes within melanocyte dendrites for transfer of melanin to keratinocytes and for normal pigmentation of the skin.

Pityriasis alba is an acquired hypopigmentation disorder, which is characterized by hypopigmented macules and patches, typically located on the face but may appear on the shoulders and arms.^{70,84,90} It is thought to result from edema between epidermal cells due to an inflammatory process occurring from low-grade eczematous dermatitis.⁸⁴ The edema between cells prevents melanosome transfer from melanocytes to the surrounding keratinocytes, resulting in patches of skin that show a striking reduction in pigmentation (Figure 1).⁸⁴ This demonstrates the importance of contact between the melanocytes and keratinocytes in order for melanosomes to be transferred to the keratinocytes and for melanization of the epidermis to occur. Separation of epidermal cells (keratinocytes) by edematous fluid, as can occur in pityriasis alba, interferes with this process and results in patches of hypopigmentation.

Hyperpigmentation disorders

Skin hyperpigmentation may result from numerous causes. Perhaps, the most common and/or significant hyperpigmentation disorders occurring in the epidermis are postinflammatory hyperpigmentation, melasma, and Addison disease. Dermal hyperpigmentation can also occur, particularly after inflammation, and will be briefly discussed. Dyspigmentations due to aberrant melanocytic proliferations are numerous and are beyond the scope of this paper.

Postinflammatory hyperpigmentation is an extremely commonly acquired disorder in which there is an excess of melanin

pigment production after cutaneous inflammation or injury.^{91–93} It may occur due to hyperplasia of epidermal melanocytes, to hyperfunction of epidermal melanocytes, or to both together (Figure 1).^{91–93} This leads to increased melanogenesis and to increased melanin deposition in the epidermis (eg, after atopic dermatitis or acne) or in the dermis (eg, after lichen planus).^{93–95} In the latter, melanin enters the dermis via a damaged basement membrane where it is phagocytosed by dermal macrophages, forming melanophages.^{91,92} These macrophages may also migrate into the epidermis where they phagocytose melanosomes and then return to the dermis.^{91,92} This increased melanin deposition leads to formation of hyperpigmented macules and patches in the skin.^{91–93}

A number of factors produced during inflammation, such as prostaglandins, leukotrienes, cytokines, and inflammatory mediators, may play a role in this response and the increased melanogenesis that occurs.^{92–95} The hyperpigmentation that is produced secondary to inflammation may persist for weeks to months in the epidermis and the melanin within dermal melanophages may persist for longer periods of time, such as years.⁹¹ Due to the Tyndale light scattering effect (also known as the Rayleigh phenomenon), such pigment located deeper in the dermis may also impart a blue coloration to the affected skin seen clinically.

Melasma is a commonly acquired disorder characterized by symmetrical patches of hyperpigmentation on the skin, most commonly occurring on the face.^{6,92,96–98} Areas of hyperpigmentation are due to increased deposition of melanin in the epidermis and dermis, where it is found in dermal macrophages (Figure 11).^{6,96–98} No increase in number of melanocytes is observed in these areas but epidermal melanocytes show greater activity with increased production of melanosomes, increased tyrosinase activity, increased synthesis of melanin, especially eumelanin, in the melanosomes, and increased transfer of melanosomes to keratinocytes (Figure 1).^{6,97} Genetic predisposition is considered as one of the main causes involved in the development of melasma along with UV irradiation and endocrine factors.^{6,92,96–98} It is most prevalent in women.^{6,96–98} During pregnancy, elevated levels of estrogen, progesterone and MSH have been associated with melasma.^{6,92} A number of signaling pathways are thought to be involved in the upregulation of tyrosinase and microphthalmia-associated transcription factor, resulting in the stimulation of melanogenesis and the development of melasma.^{6,98} These pathways and the factors triggering them are still being investigated.

Addison disease is an endocrinopathy in which there is acquired hypermelanosis.^{92,99,100} It results from damage to the adrenal glands resulting in a deficiency in glucocorticoids and mineralocorticoids.^{93,99,100} In greater than 50% of the patients it is an autoimmune disorder in which autoantibodies to the adrenal cortex lead to its destruction.^{93,99,100} Hyperpigmentation of the skin is the most striking cutaneous sign of the disorder.^{93,99,100} The adrenal cortical insufficiency and the resulting decreased production of corticosteroids leads to a compensatory overproduction of adrenocorticotropic

hormone (ACTH) by the pituitary.^{93,99,100} ACTH has the same active heptapeptide core as MSH and as a result can bind to the melanocortin-1 receptor on melanocytes and stimulate melanogenesis leading to increased synthesis of tyrosinase and melanin and increased transfer of melanosomes to keratinocytes in the epidermis (Figure 1).^{93,99,100,101} Hyperpigmentation occurs and is typically striking and accentuated in sun-exposed areas, flexural folds, and skin creases, including the creases on the palms.^{92,101,102}

Pigmentary incontinence is a term used to describe a condition in which melanin is deposited in the dermis.⁹⁶ This condition usually follows inflammation in which there is basal cell damage at the dermal–epidermal interface of the skin (eg, lichen planus, Riehl’s melanosis, lupus erythematosus, incontinentia pigmenti, and fixed-drug eruption).⁹⁶ This may lead to free melanin pigment, no longer in melanocytes or in keratinocytes, becoming deposited into the dermis (Figure 12A). The deposited melanin is then phagocytosed by dermal macrophages, known then as “melanophages.” These melanophages are usually found in the upper dermis but may descend to the level of the deep papillary vascular plexus where they may persist for months to years or even decades (Figure 12B). A characteristic slate-gray pigmentation of the skin is observed.⁹⁶

There are conflicting explanations for the mechanism of development of pigmentary incontinence, particularly as to the origin of the melanosomes observed within the macrophages and the process of transfer of melanosomes from the epidermis to the dermis. There are reports of melanosomes in melanocyte dendrites being taken up by macrophages in the dermis or of being discharged directly into the dermis.⁹⁶ Other studies have suggested that, in the inflammatory process, keratinocytes degenerate and are phagocytosed by macrophages, which have migrated into the epidermis from the dermis. The macrophages containing the phagocytosed dyskeratotic cells and their melanosomes then migrate back to the dermis.⁹⁶ Thus, there are a number of very interesting possibilities as to how the melanin found in the dermis during inflammatory processes is actually deposited there.

Other causes of hyperpigmentation

There are numerous other causes of hyperpigmentation and reflect much excellent research but are beyond the scope of this paper.

Conclusions

Deposition of melanin in the epidermis and its processing within the keratinocytes involves a number of steps critical for pigmentation in the skin. Of particular importance is the migration of melanoblasts from the neural crest to the dermis

during embryonic development, their subsequent development into melanocytes, and migration of the melanocytes into the epidermis. Cellular processes within the melanocyte lead to unique sorting and trafficking pathways involving synthesis of tyrosinase and other melanosomal proteins, formation of melanosomes, and development of a fully melanized melanosome. Interaction of the melanosome with cytoskeletal structures in the melanocyte, such as microtubules and actin filaments, is critical for its movement to the tip of a melanocyte dendrite and its incorporation into phagocytic vesicles important in the donation of melanin to the surrounding keratinocytes.

The interaction of a melanocyte with its surrounding group of keratinocytes to form an epidermal melanin unit and the transfer of melanosomes from melanocytes in this unit to the keratinocytes is distinctive to the skin pigmentary system and essential for melanin pigmentation in the skin. Levels of melanization of the skin can be influenced by a number of factors, including synthesis of melanin in melanosomes, melanosome size, transfer of melanosomes to the keratinocytes, and degree of degradation of melanosomes in the keratinocytes. These processes can be under genetic control, as is seen in racial differences in skin coloration, or under hormonal control as observed by the effects of MSH or ACTH on melanocytes.

Environmental factors can also play an important role as seen after exposure of skin to UV irradiation. Defects in any one of the fundamental processes given above, which are involved in melanin deposition and processing in the epidermis can lead to both skin hypo- and hyperpigmentary disorders. Knowledge of these processes and further studies on their mechanistic role in the development of these disorders is critical for obtaining a better understanding of methodologies for their treatment.

References

- Ostrowski SM, Fisher DE. Pigmentation and melanocyte biology. In: Kang S, Amagai M, Bruckner AL, Enk AH, et al, eds. *Fitzpatrick's Dermatology in General Medicine*. New York, NY: McGraw-Hill; 2019. p. 819-836.
- Bologna JL, Orlow SJ. Melanocyte biology. In: Bologna JL, Schaffer JV, Cerroni L, eds. *Dermatology*. 4th ed. Philadelphia, PA: Elsevier; 2018. p. 1075-1086.
- Nordlund JJ, Boissy RE, Hearing VJ, King RA, Oetting WS, Ortonne J-P, eds. *The Pigmentary System: Physiology and Pathophysiology*. 2nd. Oxford, England: Blackwell Scientific; 2006. p. 1205.
- Kondi T, Hearing VT. Update on the regulation of melanocyte function and skin pigmentation. *Expert Rev Dermatol* 2011;6:97-108.
- Yamaguchi Y, Brenner M, Hearing VJ. The regulation of skin pigmentation. *J Biol Chem* 2007;282:27557-27561.
- Costin GE, Hearing VJ. Human skin pigmentation: melanocytes modulate skin color in response to stress. *FASEB J* 2007;21:976-994.
- Cochran AJ. The incidence of melanocytes in normal human skin. *J Invest Dermatol* 1970;55:65-70.
- Cohen J, Szabo G. Study of pigment donation in vitro. *Exp Cell Res* 1968;50:418-434.
- Van Den Bossche K, Naeyaert JM, Lambert J. The quest for the mechanism of melanin transfer. *Traffic* 2006;7:769-778.
- Fitzpatrick TB, Breathnach AS. Das epidermale melanin einheit-system. *Dermatol Wochenschr* 1963;147:481-489.
- Jimbow K, Quevedo WC, Fitzpatrick TB, et al. Some aspects of melanin biology. *J Invest Dermatol* 1976;67:72-89.
- Wagner RY, Luciani F, Cario-Andre M, et al. Altered E-cadherin levels and distribution in melanocytes precede clinical manifestations of vitiligo. *J Invest Dermatol* 2015;135:1810-1819.
- Fitzpatrick TB, Ortonne JP. Normal skin color and general considerations of pigmentary disorders. In: Freedberg IM, Eisen AZ, Wolff K, eds. *Fitzpatrick's Dermatology in General Medicine*. New York, NY: McGraw-Hill; 2003. p. 819-836.
- Young AR. Acute effects of UVR on human eyes and skin. *Prog Biophys Mol Biol* 2006;92:80-85.
- Slominski A, Tobin DJ, Shibahara S, et al. Melanin pigmentation in mammalian skin and its hormonal regulation. *Physiol Rev* 2004;84:1155-1228.
- Zanetti R, Prota G, Napolitano A, et al. Development of an integrated method of skin phenotype measurement using the melanins. *Melanoma Res* 2001;11:551-557.
- Marks MS, Seabra MC. The melanosome: membrane dynamics in black and white. *Nat Rev Mol Cell Biol* 2001;2:1-11.
- Körner A, Pawelk J. Mammalian tyrosinase catalyzes three reactions in the biosynthesis of melanin. *Science* 1982;217:1163-1165.
- Tripathi RK, Hearing VJ, Urabe K, et al. Mutational mapping of the catalytic activities of human tyrosinase. *J Biol Chem* 1992;267:707-712.
- Chi A, Valencia JC, Hu ZZ, et al. Proteomic and bioinformatics characterization of the biogenesis and function of melanosomes. *J Proteome Res* 2006;5:3135-3144.
- Lerner AB, Hendee JR. A rapid histochemical test for mammalian tyrosinase. *J Invest Dermatol* 1973;60:16-19.
- Han R, Baden HF, Brissette JL, et al. Redefining the skin's pigmentary system with a novel tyrosinase assay. *Pigment Cell Res* 2001;15:290-297.
- Lambert J, Vancoillie G, Naeyaert JM. Molecular motors and their role in pigmentation. *Cell Mol Biol* 1999;45:905-918.
- Fukuda M, Kuroda TS, Mikoshiba K. Slac2-a/melanophilin, the missing link between Rab27 and myosin Va: implication of a tripartite protein complex for melanosome transport. *J Biol Chem* 2002;277:12432-12436.
- Nagashima K, Torii S, Yi M, et al. Melanophilin directly links Rab27a and myosin Va through its distinct coiled-coil regions. *FEBS Lett* 2002;517:233-238.
- Strom M, Hume AN, Tarafder AK, et al. Melanophilin links Rab27a and myosin Va function in melanosome transport. *J Biol Chem* 2002;277:25423-25430.
- Robinson CL, Evans RD, Sivarasa K, et al. The adaptor protein melanophilin regulates dynamic myosin-Va: cargo interaction and dendrite development in melanocytes. *Mol Biol Cell* 2019;30:mpc-E18.
- Yamaguchi Y, Hearing VJ. Melanocytes and their diseases. *Cold Spring Harb Perspect Med* 2014;4:a017046.
- Wu X, Hammer JA. Melanosome transfer: it is best to give and receive. *Curr Opin Cell Biol* 2014;29:1-7.
- Tadokoro R, Takahashi Y. Intercellular transfer of organelles during body pigmentation. *Curr Opin Genet Dev* 2017;45:132-138.
- Klaus SN. Pigment transfer in mammalian epidermis. *Arch Dermatol* 1969;100:756-762.
- Wiksw MA, Szabo G. Effects of cytochalasin B on mammalian melanocytes and keratinocytes. *J Invest Dermatol* 1972;59:163-169.
- Wiksw MA. Action of cyclic AMP on pigment donation between mammalian melanocytes and keratinocytes. *Yale J Biol Med* 1973;46:592-601.
- Wiksw MA, Szabo G. Studies on the interaction between melanocytes and keratinocytes with special reference to the role of microfilaments. *Pigment Cell* 1973;1:23-38.
- Okazaki K, Uzuka M, Morikawa F, et al. Transfer mechanism of melanosomes in epidermal cell culture. *J Invest Dermatol* 1976;67:541-547.
- Singh SK, Kurfurst R, Nizard C, et al. Melanin transfer in human skin cells is mediated by filopodia—a model for homotypic and heterotypic lysosome-related organelle transfer. *FASEB J* 2010;24:3756-3759.

37. Scott G, Leopardi S, Printup S, et al. Filopodia are conduits for melanosome transfer to keratinocytes. *J Cell Sci* 2002;115:1441-1451.
38. Ando H, Niki Y, Ito M, et al. Melanosomes are transferred from melanocytes to keratinocytes through the processes of packaging, release, uptake and dispersion. *J Invest Dermatol* 2012;132:1222-1229.
39. Wu XS, Masedunskas A, Weigert R, et al. Melanoregulin regulates a shedding mechanism that drives melanosome transfer from melanocytes to keratinocytes. *Proc Natl Acad Sci U S A* 2012;109:E2101-E2109.
40. Tarafder AK, Bolasco G, Correia MS, et al. Rab11b mediates melanin transfer between donor melanocytes and acceptor keratinocytes via coupled exo/endocytosis. *J Invest Dermatol* 2014;134:1056-1065.
41. Szabo G, Gerald AB, Pathak MA, et al. Racial differences in the fate of melanosomes in human epidermis. *Nature* 1969;222:1081-1082.
42. Konrad K, Wolff K. Hyperpigmentation, melanosome size, and distribution patterns of melanosomes. *Arch Dermatol* 1973;107:853-860.
43. Thong HY, Jee SH, Sun CC, et al. The patterns of melanosome distribution in keratinocytes of human skin as one determining factor of skin colour. *Br J Dermatol* 2003;149:498-505.
44. Minwalla L, Zhao Y, Le Poole IC, et al. Keratinocytes play a role in regulating distribution patterns of recipient melanosomes in vitro. *J Invest Dermatol* 2001;117:341-347.
45. Alaluf S, Atkins D, Barrett K, et al. Ethnic variation in melanin content and composition in photoexposed and photoprotected human skin. *Pigment Cell Res* 2002;15:112-118.
46. Miyamura Y, Coelho SG, Wolber R, et al. Regulation of human skin pigmentation and responses to ultraviolet radiation. *Pigment Cell Res* 2007;29:2-13.
47. D'Orazio JD, Jarrett S, Amaro-Ortiz A, et al. UV radiation and the skin. *Int J Mol Sci* 2013;14:12222-12248.
48. Routaboul C, Denis A, Vinche A. Immediate pigment darkening: description, kinetic and biological function. *Eur J Dermatol* 1999;9:95-99.
49. Szabo G. The number of melanocytes in human epidermis. *Br Med J* 1954;1:1016-1017.
50. Staricco RJ, Pinkus H. Quantitative and qualitative data on the pigment cells of adult human epidermis. *J Invest Dermatol* 1957;28:33-45.
51. Alaluf S, Barrett K, Blount M, et al. Ethnic variation in tyrosinase and TYRP1 expression in photoexposed and photoprotected human skin. *Pigment Cell Res* 2003;16:35-42.
52. Tadokoro T, Kobayashi N, Znudzka BZ, et al. UV-induced DNA damage and melanin content in human skin differing in racial/ethnic origin and photosensitivity. *FASEB J* 2003;17:1177-1179.
53. Rees JL. The melanocortin 1 receptor (MC1R): more than just red hair. *Pigment Cell Res* 2000;13:135-140.
54. García-Borrón JC, Sánchez-Laorden BL, Jimenez-Cervantes C. Melanocortin-1 receptor structure and functional regulation. *Pigment Cell Res* 2005;18:393-410.
55. Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. *Nature* 2007;445:843-850.
56. Schauer E, Trautinger F, Kock A, et al. Proopiomelanocortin-derived peptides are synthesized and released by human keratinocytes. *J Clin Invest* 1994;93:2258-2262.
57. Chakraborty AK, Funasaka Y, Slominski A, et al. Production and release of proopiomelanocortin (POMC) derived peptides by human melanocytes and keratinocytes in culture: regulation by ultraviolet B. *Biochem Biophys Acta* 1996;1313:130-138.
58. Rousseau K, Kauser S, Pritchard LE, et al. Proopiomelanocortin (POMC), the ACTH/melanocortin precursor, is secreted by human epidermal keratinocytes and melanocytes and stimulates melanogenesis. *FASEB J* 2008;22:1844-1856.
59. Thody AJ, Graham A. Does alpha-MSH have a role in regulating skin pigmentation in humans? *Pigment Cell Res* 1998;11:265-274.
60. Lu D, Chen W, Cone RD. Regulation of melanogenesis by MSH. In: Nordlund JJ, Boissy R, Hearing VJ, King RA, Ortonne JP, eds. *The Pigmentary System Physiology and Pathophysiology*. New York, NY: Oxford University Press; 1998. p. 183.
61. Virador VM, Muller J, Wu X, et al. Influence of α -melanocyte-stimulating hormone and of ultraviolet radiation on the transfer of melanosomes to keratinocytes. *FASEB J* 2002;16:105-107.
62. Gilchrist BA, Blog FB, Szabo G. Effects of aging and chronic sun exposure on melanocytes in human skin. *J Invest Dermatol* 1979;73:141-143.
63. Zbytek B, Wortsman J, Slominski A. Characterization of an ultraviolet B-induced corticotropin-releasing hormone-proopiomelanocortin system in human melanocytes. *Mol Endocrinol* 2006;20:2539-2547.
64. Chakraborty AK, Funasaka Y, Slominski A, et al. UV light and MSH receptors. *Ann N Y Acad Sci* 1999;885:100-116.
65. Taieb A. Intrinsic and extrinsic pathomechanisms in vitiligo. *Pigment Cell Res* 2000;13:41-47.
66. Taieb A, Picardo M. Clinical practice: vitiligo. *N Engl J Med* 2009;360:160-169.
67. Picardo M, Bastonini E. A new view of vitiligo: looking at normal-appearing skin. *J Invest Dermatol* 2015;135:1713-1714.
68. Passeron T, Ortonne JP. Physiopathology and genetics of vitiligo. *J Autoimmun* 2005;25:63-68.
69. Bystryn JC. Theories on the pathogenesis of depigmentation: immune hypothesis. In: Hann SK, Nordlund JJ, Lerner A, eds. *Vitiligo: A Monograph on the Basic and Clinical Science*. Hoboken, NJ: Blackwell Science; 2000. p. 123.
70. Passeron T, Ortonne JP. Vitiligo and other disorder of hypopigmentation. In: Bologna JL, Schaffer JV, Cerroni L, eds. *Dermatology*. 4th ed. Philadelphia, PA: Elsevier; 2018. p. 1087-1114.
71. Ezzedine K, Harris JE. Vitiligo. In: Kang S, Amagi M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS, eds. *Fitzpatrick's Dermatology in General Medicine*. New York, NY: McGraw Hill; 2019. p. 1330-1350.
72. Manga P, Elbuluk N, Orlow SJ. Recent advances in understanding vitiligo. *F1000Res* 2016;5:1-9.
73. Tobin DJ, Swanson NN, Pittelkow MR. Melanocytes are not absent in lesional skin of long duration vitiligo. *J Pathol* 2000;191:407-496.
74. Grimes PE. New insights and new therapies in vitiligo. *JAMA* 2005;293:730-737.
75. Harris JE, Harris TH, Weninger W, et al. A mouse model of vitiligo with focused epidermal depigmentation requires IFN- γ for autoreactive CD8+ T-cell accumulation in the skin. *J Invest Dermatol* 2012;132:1869-1876.
76. Bertolotti A, Boniface K, Vergier B, et al. Type 1 interferon signature in the initiation of the immune response in vitiligo. *Pigment Cell Melanoma Res* 2014;27:398-407.
77. Ortonne JP, Bahadoran P, Fitzpatrick TB, et al. Hypomelanoses and hypermelanoses. In: Wolff K, Johnson RA, eds. *Fitzpatrick's Color Atlas and Synopsis of Clinical Dermatology*. 6th ed. New York, NY: McGraw Hill; 2003. p. 185-207.
78. Oetting WS. The tyrosinase gene and oculocutaneous albinism type 1 (OCA1): a model for understanding the molecular biology of melanin formation. *Pigment Cell Res* 2000;13:320-325.
79. Hayashi M, Suzuki T. Albinism and other genetic disorders of pigmentation. In: Kang S, Amagai M, Bruckner AL, et al, eds. *Fitzpatrick's Dermatology in General Medicine*. New York, NY: McGraw Hill; 2000. p. 1309-1329.
80. Passeron T, Mantoux F, Ortonne JP. Genetic disorders of pigmentation. *Clin Dermatol* 2005;23:56-67.
81. Spitz RA. Piebaldism. Waardenburg syndrome, and related disorders of melanocyte development. *Semin Cutan Med Surg* 1997;16:15.
82. Oiso N, Fukai K, Kawada A, et al. Piebaldism. *J Dermatol* 2012;40:330-335.
83. Spritz RA. Molecular basis of human piebaldism. *J Invest Dermatol* 1994;103:137S-140S.
84. McAleer MA, O'Regan GM, Irvine ADS. Atopic dermatitis. In: Bologna JL, Schaffer JV, Cerroni L, eds. *Dermatology*. Philadelphia, PA: Elsevier; 2018. p. 208-227.
85. Huizing M, Anikster Y, Gahl WA. Hermansky-Pudlak syndrome and related disorders of organelle formation. *Traffic* 2000;1:823-835.

86. Morgan NV, Pasha S, Johnson CA, et al. A germline mutation in BLOC153/reduced pigmentation causes a novel variant of Hermansky-Pudlak syndrome (HPS8). *Am J Hum Genet* 2006;78:160-166.
87. Pastural E, Barret FJ, Dufourcq-Lagelouse R, et al. Griscelli disease maps to chromosome 15q21 and is associated with mutations in the myosin-Va gene. *Nat Genet* 1997;16:289-292.
88. Menasche G, Pastural E, Feldmann J, et al. Mutations in RAB27A cause Griscelli syndrome associated with hemophagocytic syndrome. *Nat Genet* 2000;25:173-176.
89. Menasche G, Ho CH, Sanal O, et al. Griscelli syndrome restricted to hypopigmentation results from a melanophilin defect (GS3) or a MYOSA-F exon deletion (GS1). *J Clin Invest* 2003;112:450-456.
90. Miazek N, Michalek I, Pawlowska-Kisiel M, et al. Pityriasis alba-common disease, enigmatic entity: up-to-date review of the literature. *Pediatr Dermatol* 2015;32:786-791.
91. Silpa-archa N, Kohli I, Chaowattanapanit S, et al. Postinflammatory hyperpigmentation: a comprehensive overview. *J Am Acad Dermatol* 2017;4:591-605.
92. Chang MW. Disorders of hyperpigmentation. In: Bologna JL, Schaffer JV, Cerroni L, eds. *Dermatology*. Philadelphia, PA: Elsevier; 2018. p. 1115-1143.
93. Rodrigues M, Pandya AG. Hypermelanoses. In: Kang S, Amagi M, Bruckner AL, et al, eds. *Fitzpatrick's Dermatology in General Medicine*. New York, NY: McGraw Hill; 2019. p. 1351-1389.
94. Tomita Y, Maeda K, Tagami H. Melanocyte-stimulating properties of arachidonic acid metabolites: possible role in postinflammatory pigmentation. *Pigment Cell Res* 1992;5:357-361.
95. Taylor S, Grimes P, Lim J, et al. Postinflammatory hyperpigmentation. *J Cutan Med Surg* 2009;13:183-191.
96. Kang WK, Yoon KH, Lee ES, et al. Melasma: histopathological characteristics in 56 Korean patients. *Br J Dermatol* 2002;146:228-237.
97. Grimes PE, Yamada N, Bhawan J. Light microscopic, immunohistochemical, and ultrastructural alteration in patients with melasma. *Am J Dermatopathol* 2005;27:96-101.
98. Lee AY. Recent progress in melasma pathogenesis. *Pigment Cell Melanoma Res* 2015;28:648-660.
99. Ten S, New M, Maclaren N. Addison's disease. *J Clin Endocrinol Metab* 2001;86:2909-2922.
100. Schachtel A, Kalus A. Diabetes and other endocrine diseases. In: Kang S, Amagi M, Bruckner AL, et al, eds. *Fitzpatrick's Dermatology in General Medicine*. New York, NY: McGraw Hill; 2019. p. 2492-2526.
101. Scott AP, Bloomfield GA, Lowry PJ, et al. Pituitary adrenocorticotrophin and the melanocyte stimulating hormones. In: Parsons JA, ed. *Peptide Hormones*. London, UK: Palgrave; 1976. p. 247-271.
102. Masu S, Seiji M. Pigmentary incontinence in fixed drug eruptions. *J Am Acad Dermatol* 1983;8:525-532.